Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Phytochemical Analysis and Insecticidal Activity of Flowers of *Gloriosa superba* L. against *Culex quinquefasciatus* Say, *Anopheles stephensi* L., *Aedes aegypti* L. and *Aedes albopictus* Skuse

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Received: 06 Feb 2020 Revised:08 Mar 2020 Accepted: 10 Apr 2020

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ABSTRACT

The nature provides enormous biological active materialsfrom plants to treat various dreadful communicable and non-communicable diseases. *Gloriosa superba* belongs to the family Liliaceae is an important known perennial herbal species and also called "Glory lily". The flowers of *G. superb* was extracted using chloroform and methanol and evaluated for phytochemicals and insecticidal activity against human vector mosquitoes of *Culex quinquefasciatus, Anopheles stephensi, Aedes aegypti* and *Aedes albopictus* at 1000, 500, 250, 125 and 62.5 ppm concentrations. The phytochemical analysis of chloroform extract of flowers from*G. Superb* showed lesser quantity of alkaloids, flavonoids, saponins, protein andsteroids. The methanol extract of flowers from *G. superb* showed higherquantity of alkaloids, flavonoids, tannins, phenols, saponins and steroids. The anthraquinons was absent in both the extracts of *G. superba.* The insecticidal activity of methanol extract showed 100% against *C. quinquefasciatus, A. stephensi* and 98 % against *A. aegypti* and 96 % against *A. albopictus*, at 1000 ppm concentration, respectively. The chloroform extract of *G. superb* pronounced 92 %, 84%, 80% and 78 % against *C.quinquefasciatus, A.stephensi, A. aegypti* and *A. albopictus,* respectively. The results suggested that methanol extract of flower from *G. superb* can be used as biopesticide to control diseases causing mosquitoes and various biological applications.

Key words: *Gloriosa superba*, Phytochemicals, *Culex quinquefasciatus*, *Anopheles stephensi*, *Aedes aegypti*, *Aedes albopictus*

INTRODUCTION

Gloriosa superba is a perennial, greenish, climbing herb and native of South Africa. It is widely cultivated throughout the world as an ornamental plant and grows naturally in many countries of tropical South-Eastern Asia such as Bangladesh, India, Srilanka, Malaysia and Myanmer. *Gloriosa superba* L. commonly known as superba lily, glory lily, tiger lily or tiger claws, isone of the important medicinal plant of Tropical countries such India, Sri Lanka, (1).It is commonly known species with different vernacular nameslike Agnishikha and Agnimukhi in Sanskrit and Bachnag Karihari in Hindi (2). In India, it is widely distributed in foot hills of Himalayan regions upto an altitude of 6000 feet, tropical andsub-tropical regions,Andaman Islands(3-4).*G. superba* also called as Mauve beauty, Purple prince, Fire lily, Orange gem andOrange glow due to its showy flowers and is used as anornamental plant worldwide (5). In Zimbabwe,*G. superba* is considered as national flower and also considered as a state flowerof Tamil Nadu, India.

*G. superba*is an annual climbing perennial herbaceous vine growing from 3.5 to 6.0 meters inlength (6). It has pointed dark green, glossy leaves inwhorls of 3 to 4. The tendril of *G. superba*c lings onto other plants for growth. Leaves were occurred opposite side oralternate side, with 6 to 20 cm of length and 1.5 to 4cm of wide. The tuber of *G. superba*is highly poisonous in nature. The flower of *G. superba* were attractive bornewith long stalks, six erect petals, colour from bright yellow to orange, red and sometimes yellow to deep pinkish-red. The capsuleof fruit was split open to release their soft red seeds with aspongy test. The whole plant of *G. superba* is toxic; especially the tubers are highly toxic due to the presence of alkaloid group of phytochemical Colchicine (7). Colchicine and colchicoside were alkaloids produced from its seeds and important principles components for drugs (2). The species also contains another toxic alkaloid called Gloriosine. These two alkaloids were present in every part of the plant.

The colchicine content presentin tubers is from 0.15 to 0.3% and in the seeds is 0.7 %to 0.9%. Colchicine plays an important role in the studyof cell division as it inhibits the process of mitosis by inhibiting the spindle formation and arresting the polymerization of β–tubulin proteins and thereby inducing polyploidy. It is also used as an anticancer agent (8-9). *Gloriosa* contains colchicocide, tannins, β-sitosterol and superbine but in smallamounts (10-11). *G. superb* had anti-inflammatory, bactericidal, fungicidal, anticancer activity, antithrombotic potential and other pharmacological activities (12-16) used for Snake bite (17); Antioxidant activity (18), Anthelmintic activity (19). It isused to treat patients suffering from gout whichhas been donefrom the *G. superb* leaf extract for seed germination (20), antibiofilm and antibacterial studies (21).

The methanol extracts of *Gloriosa superb* showed enormous occurrence of phytochemicals likephenols, alkaloids, flavonoids and tannins (22). The leaf samples of *Gloriosa* showed thepresence of proteins, phenols, tannins, starch,terpinoides (23). flowers, seeds and tubers samples of *G. superba* showed high content of flavonoids. Glycosides and alkaloids were found in maximum amount in seed and tubers inall samples, however, absent in leaves andflower parts (7). Saponins were found in moderate concentration in all the samples of seeds and tubers extracts but showed the absence in all the sample extracts of leaves (22). A high concentration of colchicine in the seeds of *G. superba*(24).

Over the last decades, deforestation, habitat invasion, over growth of population, climatic changes, and insecticide resistance have contributed to the re-emergence and outbreak of several vector-borne diseases. Mosquitoes are one of the voracious haematophagous dipteran arthropod and it transmits different types of pathogens and parasites to humans and animals. They serve as vector for many life-threatening diseases like dengue, dengue haemorrhagic fever, chikungunya, malaria, filariasis, Japanese encephalitis, etc. (25-27).

Among these diseases lymphatic filariasis infects 650 million Indian peoples were affected across the 21 states and union territory.165.5 million peoples were *Culex quinquefasciatus* is a major vector for bancroftian filariasis in tropical and subtropical countries. Lymphatic filariasis is caused by *Wucheria bancrofti* a thread like helminth worm that lives in the lymph glands of human beings. In India alone 25 million people were infected with harbour microfilaria (mf) and also 19 million peoples were suffered due to the infestation of filarial disease (28). *Anopheles stephensi* is one of the major vectors of malaria in India as well as in some of the West Asian countries and has been shown to be directly responsible for about 40 – 50% of the annual malarial incidence. Malaria alone kills 3 million each year, including 1 child every 30 seconds. Although mosquito borne diseases currently represent a greater health problem in tropical and subtropical climates, no part of the world is immune to this risk (29). *Aedesae gypti* and *Aedes albopictus* are responsible vector for dengue and chikungunya. In recent times the number of infected persons were increasing (30).

Continuous application of chemically synthesised insecticides *viz.,* organochlorides, organophosphates and pyrethroids such as temephos and fenthion and other insect growth inhibitors such as methoprene and diflubenzuron were regularly applied for the control of mosquito larvae in stagnant water bodies. Synthetic chemical insecticides have created a various of ecological imbalance like the development of resistance in vector insects, environmental problems and affects the mammals. Nowadays, mosquito control programs were failures due to the ever-increasing resistance against chemical insecticide (31). Recent literatures have inspired the isolation of insecticidal potential properties of phytoconstituents derived from plant origin and numerous medically important plant extracts have been evaluated for their toxicity againstthe larvae of human vector mosquito species (26).

The use of botanical derivatives in mosquito control as an alternative to synthetic insecticides offers a more environment friendly method of insect control than the use of synthetic chemicals. Large number essential oils from plant may be novelsources against mosquito larvae, since plants constitute a rich source of bioactive components. Essential oils extracted from neem (32) and *Tagetes patula*(33) killed the larvae of *Ae. aegypti*, *An. Stephensi* and *Cx. quinquefasciatus*.

MATERIALS AND METHODS

Collection of the plant materials

Gloriosa superba flowers were collected from cultivated field in and around Kanchipuram district of Tamil Nadu (Fig. 1). About 5kg of flowers were collected and authenticated by Dr.S. Mutheeswaran, Botanist, Centre for Biodiversity and Biotechnology, St Xavier's College,Palayamkottai, Tamil Nadu, India, and voucher specimen was stored in Herbarium of P.G. and ResearchDepartment of Botany, Arignar Anna Govt Arts College Cheyyar, Thiruvannamalai, Tamil Nadu, India. The flowers were dried to obtain 500gm of sample and extraction was done using soxhlet apparatus in Life Tech Research Centre, Chennai, Tamil Nadu, India for further analysis.

Preparation of plant extract

The *G. superba* flower material was cut into small pieces and dried in shade for seven days till it completely dried. Then it was pulverised into fine powder. The 500g of powered materials were packed in Soxhlet apparatus and successive extraction was performed using Chloroform and Methanol solvents. The solution of the extract was filtered through wattman filter paper No 1 and concentrated using rotary evaporator and dried under vacuum.

Phytochemical analysis

The phytochemical screening was performed by the procedure adapted by Raman (34) assess the qualitative chemical composition of different solvents (Chloroform and Methanol) extract using commonly employed precipitation and coloration reaction to identify the major secondary metabolites like Alkaloids, Glycosides, Flavonoids, Saponins, Tannins, Protein, Steroids, Phenols and Antheroquinones.

PHYTOCHEMICAL ANALYSIS

Test for Tannins

1ml of sample was taken, to that few drops of 0.1 % ferric chloride was added and observed for brownish green or blackish blue colour.

Test for Saponins

1 ml of sample was taken, to that 2 ml of water was added.The suspension was shaken in a graduated cylinder for 15 minutes. A layer of foam was formed that indicates the presence of saponins.

Test for Flavonoids

1 ml of sample was taken, to that add NaOH solution was added. Formation of intense yellow colour, which becomes colourless on further addition of diluted hydrochloric acid indicated the presence of flavonoid.

Test for Alkaloids

1 ml of sample was taken, to that few drops of dragendorffre agent was added. A prominent yellow precipitate indicates the test as positive.

Test for Protein

1 ml of sample was taken, to that few drops of Bradford reagent was added. A blue colour indicates the presence of Protein.

Test for Steroids

1 ml of sample was taken, to that two drops of 10% concentrated sulphuric acid was added and observed for brown colour.

Test for Phenol

1 ml of sample was taken, to that 3 ml of 10% lead acetate solution is added a bulky white precipitate indicates the presence of phenolic compounds.

Test for Anthraquinones

1 ml of sample was taken, to that aqueous ammonia was added and observed for change in colour. Pink, red, or violet colour in aqueous layer indicates the presence of anthraquinones.

Larvicidal activity

The chloroform and methanol extract of flowers from *G. superba* was tested against mosquito larvae of *Culex quinquefasciatus, Anopheles stephensi*, *Aedes aegypti* and *Aedes albopictu s*using WHO procedure with some minor modification (35). The various concentrations of chloroform and methanol extracts were evaluated with the concentrations of 1000, 500, 250, 125 and 62.5 ppm). The desired plant extract was dissolved in 1ml of DMSO was mixed with 99 ml of distilled water. Temephos was tested as positive control and DMSO was tested as negative

control. Ten numbers of mosquito larvae were released in all the concentrations and no food was offered during experimental period. Five replicates were maintained simultaneously for each test concentration. The mosquito larval mortality was noted after 24h of experimental period. The percentage of mortality rate was calculated using Abbott formula (36).

RESULTS

The phytochemical analysis of chloroform extract of flowers from *G. superb* showed lesser quantity of alkaloids, flavonoids, saponins, protein and steroids. The methanol extract of flowers from *G. superb* showed highest quantity of alkaloids, flavonoids, tannins, phenols, saponins and steroids. The anthraquinones was absent in both the extracts of *G. superb* (Table 1).

The mosquito larvicidal activity of methanol extract showed 100 % against *C. quinquefasciatus, A. stephensi* and 98 % against *A. aegypti* and 96 % against *A. albopictus*, at 1000 ppm concentration, respectively. In 500 ppm concentration showed 76%, 84 %, 80% and 82%; at 250 ppm concentration showed 56%, 56%, 58% and 58%; at 125 ppm concentration infected 48%, 44%, 46% and 42% at lowest concentration of 62.5 showed 28%, 32%, 30% and 32% against the larvae of *C. quinquefasciatus, A. stephensi, A. aegypti* and *A. albopictus,* respectively (Table 2). The chloroform extract of*G. superba* pronounced 92 %, 84%, 80% and 78 % against *C. quinquefasciatus, A. stephensi, A. aegypti* and *A. albopictus,* respectively. In 500 ppm concentration pronounced 68%, 68%, 68% and 62%; at 250 ppm concentration showed 52%, 52%, 42% and 44%; at 125 ppm concentration showed 44%, 36%, 24% and 30%; the lowest concentration of 62.5 ppm showed 18%, 20%, 16% and 12% against the larvae of *C. quinquefasciatus, A. stephensi, A. aegypti* and *A. albopictus,* respectively (Table 3).

Among the twoextracts methanol extract showed potential larvicidal activity against the larvae of *C. quinquefasciatus, A. stephensi, A. aegypti* and *A. albopictus,* respectively. No mortality was observed in negative control. The positive control showed 100% mortality against selected mosquito larvae. The larvicidal activity was due to the presence of highest quantity of alkaloids, phenols and other group of constituents.

DISCUSSION

Phytochemicals play an important role in the treatment of different types of diseases and disorders and are still used in both Traditional and Morden medicine. They are synthesized by specific biochemical pathways, for plant defence and adaptation to environmental stress. Bioactive compounds are generally accumulated as secondary metabolites range from medicinally useful agents to deadly poisons. Many of the secondary metabolites isolated from plants are used in phytochemical drug industry (37). The phytochemical analysis of tuber of *G. superba* confirmed that the presence of aromatic acid, alkaloids, terpenoids, saponins, tannins, flavonoids, reducing sugar, carbohydrate and proteins. No aminoacids were reported in shoots, tubers and flowers extracts (38).

G.superba was extracted and observed preliminary phytochemical investigation reported that alkaloids, glycosides, saponins and steroids (39). The phytochemical analysis of *G. superba* leaves was extracted with acetone, chloroform, dichloromethane and methanol (40). They reported that methanol extract showed highest quantity of flavonoids, phenols, glycosides and tannins and lowest quantity of steroids followed by acetone, chloroform and dichloromethane extracts. The tuber of *G. superba* extracted with acetone, chloroform, dichloromethane and methanol and reported that methanol extract having very highest quantity of alkaloids, saponins, phenols, glycosides and moderate quantity of flavonoids, steroids, tannins followed by Acetone, chloroform and dichloromethane extract.

According to earlier findings of the active constituents of alkaloids *viz*., colchicines, gloriosine, superbrine, chelidonic acid and salicylic acid were highly present in *Gloriosa superba*(9,41). Additionally, the tubers are highly poisonous

compared with other parts of the *G. superba*. The tuber of *G.superba* are highly toxic due to the presence of potentially active alkaloids colchicines compared with other parts of the plant (7). The methanol extract of *Gloriosa superba* showed enormous occurrence of phytochemicals like phenols, Alkaloids, Flavonoids and Tannins, the results are in agreements with those of Senthilkumar(22). The flower and seed consisthigh content of Alkaloids, Flavonoids, Saponins, Steroids and Phenol it is also agreed by many authors (7,23-24). The glycosides and Alkaloids were found in maximum amount in seed and tubers of all sample but they were absent in the leaves and flower extracts (22). Saponins were found in moderate concentration in all the samples of seeds and tubers extracts but showed the absence in all the sample extract of leaves.In the present study the results are clearly showed the flowers of *G.superba* have many phytochemical compounds in chloroform and methanol extracts.

The plant extracts and essential oils from plant pronounced good ovicidal, larvicidal, repellent, oviposition deterrent and adulticidal activity against disease causing vector mosquitoes such as *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus* etc (26, 32, 42, 43).The toxic effect of *Annona squamosa, Centella asiatica, Gloriosa superba, Mukia maderaspatensis, Pergularia daemia,* and *Phyllanthus emblica* leaves were extracted using organic solvents such as hexane, chloroform, ethyl acetate, acetone and methanol and tested against *Haemaphysalis bispinosa*, *Paramphistomum cervi*, 4th instar larvae of *Anopheles subpictus* and *Culex tritaeniorhynchus* at different concentrations (44). All tested plant extracts inflicted notable toxicity against pest insects after 24 hrs of exposure. However, the potential activity was recorded in hexane extract of *Annona squamosa,* methanolic extracts of *P. emblica* and *G. superba* on *H. bispinosa* with the LC_{50} values of 145.39, 256.08, 225.57 ppm. Similarly, methaolic extracts of *P. daemia, G. superba, C. asiatica,* and *P. emblica* against *P. cervi* exhibited the LC_{50} values were 59.61, 60.16, 77.61, and 60.60ppm. The extracts of ethyl acetate and acetone of *A. squamosa*, acetone extracts of *G. superba*, methanolic extract of *C. asiatica*, methanol, hexane and ethyl acetate extracts of *P. daemia* against *A. subpictus* with the LC_{50} values of 18.60, 17.48, 18.43, 26.62, 50.39, 13.63, and 34.06 ppm. The ethyl acetate extract of *P.daemia*, ethyl acetate, chloroform, extracts of *A. squamosa*, methanol and ethyl acetate extracts of *P. emblica* against *C.tritaeniorhynchus* with the LC_{50} values of 31.94, 60.01, 63.81, 54.82 and 69.09 ppm, respectively. They suggested that methanolic extracts of *G. superba, C. asiatica, P. emblica* and *P. daemia* extracts may prove vector insect control at crude extracts level.

The larvicidal and ovicidal potential of *Clerodendron phlomides* was tested against Aedes aegypti and recorded maximum efficacy rate in chloroform extract followed by hexane and methanol extracts on larvae and eggs. The chloroform extract of C. phlomides at 1000 ppm showed an ovicidal effect of 15.6±1.30, 33.2±3.64 and 45.6±5.49 % on 0-6, 6-12 and 12-18 hr old eggs. The LC_{50} values of chloroform extract were 10.21, 45.30, 235.06 and 335.51 ppm on 1st, 2nd, 3rd and 4thinstars of *Ae. aegypti* in 24hr on dose dependant manner (45). *Couroupita guianensis* leaves was extracted using hexane, chloroform and ethyl acetate and phytochemically analysed along with tested their toxicity on 2nd and 4thinstar larvae of *C. quinquefasciatus* and *A. aegypti (46).*They observed that hexane extract inflicted high activity compared with chloroform and ethyl acetate extract of *C.guianensis.*

The adulticidal and adult emergence inhibition effect of leaves from *Anisomeles malabarica, Euphorbia hirta, Ocimum basilicum, Ricinus communis, Solanum trilobatum, Tridax procumbens,* and seed from *G.superba* were extracted using acetone, ethyl acetate, chloroform and hexane was tested against *A.stephensi* with the concentration of 1000, 500, 250, 125, 62.5, and 31.25 µg/ml (47). The potential emergence inhibition activity was recorded in chloroform extracts of *S.trilobatum, O. basilicum,* Ethyl acetate extract of *A. malabarica*, acetone of extract of *T. procumbens, R. communis* and seed extract of *G. superba* with EI_{50} values of 157.87, 119.82, 143.12, 111.19, 139.39, and 134.85 µg/mL, respectively.The adulticidal activity was found in acetone, methanol extracts of *G. superba, R. communis, T. procumbens, S. trilobatum* and ethyl acetate extract of *O. basilicum* with LD_{50} values of 108.77, 120.17, 163.11, 127.22, 118.27, and 93.02µg/mL, respectively. The hexane fraction, dichloromethane fraction 2, dichloromethane fraction 1, and methanol fraction was derived from *G. superba* showed cytogenetic activity against *A. aegypti* (48).

The results suggested that methanol extract of flower from *G. superba* can be used as biopesticide to control diseases causing mosquitoes and various biological applications.

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Table 1. Qualitative analysis of Phytochemicals present in the Chloroform and Methanol extract of flowers of *Gloriosa superb*

S.No	Test	Chloroform	Methanol
1	ALKALOIDS	+	+++
2	FLAVONOIDS	+	+++
3	PROTEINS	+	+
4	TANNINS	_	+
5	SAPONINS	+	+++
6	STEROIDS	+	+++
7	PHENOL	_	+++
8	ANTHROQUINONES	-	-

(+) = Positive, (-) = Negative, (+++) = highly positive

Table 2. Larvicidal activity of methanol extract of flower from *Gloriosa superba* against *Culex quinquefasciatus, Anopheles stephensi, Aedes aegypti* and *Aedes albopictus*

Replication	Insect	Concentration (ppm)						
		1000	500	250	125	62.5	Control	Temephos (100)
1	*Culex quinquefasciatus*	10	8	6	5	3	0	10
2		10	7	5	5	2	0	10
3		10	8	6	5	3	0	10
4		10	8	6	4	3	0	10
5		10	7	5	5	3	0	10
Mean ± S.D.		10 ± 0	7.6 ± 0.54	5.6 ± 0.54	4.8 ± 0.44	2.8 ± 0.44	0	10 ± 0
% of Mortality		100	76	56	48	28	0	100
1	*Anopheles stephensi*	10	9	7	4	4	0	10
2		10	9	5	4	3	0	10
3		10	9	5	4	3	0	10
4		10	8	5	5	3	0	10
5		10	7	6	5	3	0	10
Mean ± S.D.		10 ± 0	8.4 ± 0.89	5.6 ± 0.89	4.4 ± 0.54	3.2 ± 0.44	0	10 ± 0
% of Mortality		100	84	56	44	32	0	100
1	*Aedes aegypti*	10	8	6	5	3	0	10
2		10	8	6	5	3	0	10
3		10	8	6	5	3	0	10
4		10	8	6	4	4	0	10
5		9	8	5	4	2	0	10
Mean ± S.D.		9.8 ± 0.44	8 ± 0	5.8 ± 0.44	4.6 ± 0.54	3 ± 0.70	0	10 ± 0
% of Mortality		98	80	58	46	30	0	100

1	*Aedes albopictus*	10	8	6	4	3	0	10
2		10	8	6	4	3	0	10
3		10	8	6	4	3	0	10
4		10	9	5	5	3	0	10
5		8	8	6	4	4	0	10
Mean ± S.D.		9.6 ± 0.89	8.2 ± 0.44	5.8 ± 0.44	4.2 ± 0.44	3.2 ± 0.44	0	10 ± 0
% of Mortality		96	82	58	42	32	0	100

Values are mean ± S.D. of five replicates.

Table 3. Larvicidal activity of chloroform extract of flower from *Gloriosa superba* against *Culex quinquefasciatus, Anopheles stephensi, Aedes aegypti* and *Aedes albopictus*

Replication	Insect	Concentration (ppm)						
		1000	500	250	125	62.5	Control	Temephos (100)
1	*Culex quinquefasciatus*	9	7	6	4	2	0	10
2		9	7	5	4	2	0	10
3		9	7	5	5	1	0	10
4		9	6	5	4	1	0	10
5		10	7	5	5	3	0	10
Mean ± S.D.		9.2 ± 0.44	6.8 ± 0.44	5.2 ± 0.44	4.4 ± 0.54	1.8 ± 0.83	0	10 ± 0
% of Mortality		92	68	52	44	18	0	100
1	*Anopheles stephensi*	9	7	5	5	2	0	10
2		9	7	5	4	3	0	10
3		8	7	6	3	1	0	10
4		8	6	5	3	1	0	10
5		8	7	5	3	3	0	10
Mean ± S.D.		8.4 ± 0.54	6.8 ± 0.44	5.2 ± 0.44	3.6 ± 0.89	2 ± 1.0	0	10 ± 0
% of Mortality		84	68	52	36	20	0	100
1	*Aedes aegypti*	8	7	5	2	1	0	10
2		8	7	5	2	1	0	10
3		8	8	4	2	3	0	10
4		7	6	4	3	1	0	10
5		9	6	3	3	2	0	10
Mean ± S.D.		8 ± 0.70	6.8 ± 0.83	4.2 ± 0.83	2.4 ± 0.54	1.6 ± 0.89	0	10 ± 0
% of Mortality		80	68	42	24	16	0	100
1	*Aedes albopictus*	8	6	5	3	1	0	10
2		8	6	5	3	1	0	10
3		8	6	4	3	1	0	10

4		7	7	4	2	1	0	10
5		8	7	4	4	2	0	10
Mean ± S.D.		7.8 ± 0.44	6.4 ± 0.54	4.4 ± 0.54	3 ± 0.70	1.2 ± 0.44	0	10 ± 0
% of Mortality		78	64	44	30	12	0	100

Values are mean ± S.D. of five replicates

Fig. 1. Flowers of *Gloriosa superb*

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

REVIEW ARTICLE

Comparative Analysis of Phytochemical and Antimicrobial Activity of *Butea monosperma* (Lam.) Taub. and *Pongamia pinnata* (L.) Pierre: A Review

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Received: 31 Jan 2020 Revised:02 Mar 2020 Accepted: 04 Apr 2020

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ABSTRACT

India holds the richest source of biodiversity. The various trees, shrubs, herbs, grasses, ferns, and mosses are the source of medicines since ancient times. According to the World Health Organisation half of the population in the world depend upon herbal medicine for their alignments. The present investigation on phytochemical and antimicrobial aspects of *Butea monosperma* (Lam.) Taub. and *Pongamia pinnata* (L.)Pierre, related to the concern for the number of attractive resources that get wasted or blown away. These two plants resulted in moderate to significant antibacterial and antifungal activities against the tested pathogens. Moreover, the phytochemical analysis further revealed the presence of bioactive constituents to be exploited to establish drug development procedures and explore the possibility of medical health care to serve the society. The conventional system of medicine contains both aromatherapy and folk medicines are playing continuously a significant role largely in the active care system of the population. The present paper details the different conventional and medicinal profits of the plant and exploit was made to collect information about the chemical composition and pharmacological and antimicrobial features of the plants.

Keywords: Antibacterial, Antifungal, Aromatherapy, Biodiversity, Phytochemical

INTRODUCTION

A review is a fundamental or scholarly paper that contains the present knowledge involving the research fields, theoretical data and methodological contribution to a specific topic. Reviews are the secondary facts which do not

have any kind of real experimental study. It is very much necessary to provide a proper direction to a researcher for fundamental research work to reach a successful and meaningful result. A review contains the history morphology, status and conservation of medicinal plants to get the product of benefit. The human world can't survive without nature. There are three fundamental indispensable things for humans is nutrients, dresses, and shields and now the fourth one is a healthy environment provided by the ecosystem of plants in our nature. Nature holds a golden mark and gives the storehouse of carminative to relieve all diseases of mankind. Plant kingdom entitled a rich house organic compound. Among them compounds have been used for medicinal purposes and could perform as a route for the development of novel agents having good efficiency in various pathological disorders in the future.

Herbs were always the important form of medicine present in India and presently they are becoming famous throughout the world, as people fight vigorously to stay healthy in the situation of chronic stress (a poor quality)and pollution and to treat diseases with medicines that work in the count with the body's immune system (Dewick,1996). From ancient times it is accepted that green medicines are sole purpose and more harmless or careful than synthetic ones. Mankind has a long story in the use of herbal medicines. Indians had a rich knowledge of the use of medicinal plants and records of the Assyrians, ancient Hebrews, Greeks and Chinese have all shown the extensive uses of a plant with medicinal properties. Insert of medicinal plants has fascinated the scientific community to design drug-using herbal products and develop new methods for healing disease (Magherini, 1998).

Taxonomical classification (Saxena and Brahmam, 1994-96)
Kingdom: Plantae
Phylum: Magnoliophyta
Order: Fabales
Family: Fabaceae
Genus: *Butea*
Species: *monosprma*
Botancal Name: *Butea monosperma* (Lam.) Taub.

Why We Consider *Butea monosprema*

Butea monosperma (Palasha) which belongs to the family Fabaceae grows widely in India and all over the world, popularly known as the "flame of the forest" (Patil et al., 2006). It is well known as bastard teak (Kirtikar et al., 1935). The leaves are mainly lanceolate with a good source for eye treatment. This plant which is used medicinally requires a detailed study before its use because therapeutic efficiency depends on the quality of the plant drug used. Different parts of this plant have been used for many of the diseases like anti-inflammatory and antibacterial etc. The first choice is the selection of leaves in our current research work because only scanty works have been done on leaves (Ahmed and Siddaraju, 2012).

Taxonomic classification (Saxena and Brahmam, 1994-96)
Kingdom: Plantae
Phylum: Magnoliophyta
Order: Fabales
Family: Fabaceae
Genus: *Pongamia*
Species: *pinnata*
Botanical name: *Pongamia pinnata* (L.) Pierre

Why We Consider *Pongamia pinnata*

Pongamia pinnata is a fast-growing small-sized plant commonly called as kanuga in Telgu. This plant is native in tropical and temperate regions of Asia. Since ancient times this plant has long been valuable utility in India and neighboring areas as a source of conventional medicines, animal nutrients, animal dung, timber, and fuel. Extracts of the plant possess a sufficient great role in anti-microbial fields. It has also an alternative source of energy. The seed powder of the plant is given an expectorant in the treatment of bronchitis. An inflammatory of *Pongamia* leaves is used to relieve rheumatism. In the literature survey of on an elite medicinal plants *P.pinnata* showed that it is a potential medicinal plant. *Pongamia pinnata* , the seed contains six compounds with Karang, pongaglbrone, and pongapin, pinnatin, and kanjone have been isolated from seeds.

Why the Phytochemical Analysis is Important?

The living ecosystem contains plant-kingdom acts as the sole source of therapeutic compounds that holds enormous applications in the pharmaceutical industry. Successful research into the biologically active substance or natural products from plants has made a great effort in various fields like biological and chemical science. In recent years, rapid attention has been deposited to the investigation of naturally occurring antioxidants and bioactive compound for antimicrobials (Nostro and Germano, 2000), because of the increasing number of fooder in the form of both animal and human being a demand for food products free from fabricated chemical intermixing compounds. This phytochemical analysis is studied to find out the phytochemicals, which are present naturally in the plants and shows biologically mediated great role by playing which is extremely important for plants to defend themselves against the disease-causing pathogens (Krishnaiah et al., 2007).

Phytochemicals may be known as primary or the secondary metabolites as it contains different classes of enzymes inside it. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds are terpenoids, flavonoids, glycosides, saponin, etc. Sometimes the phytochemicals can be harmful or be very helpful. The secretion of these compounds is permitted for limited health claims by the US Food and Drug Administration. So it is an important attempt to carry out the evaluation of leaf extracts of these two plants and to isolate active constituents and study the characterization of isolated compounds by modern technologies (Patil et al., 2006). The preliminary phytochemical analysis of *Butea monosperma* showed the presence of various active substances. The phytochemical analysis of the plant is very commercially important and has a great interest in pharmaceutical companies for the production of new drugs for the curing of various diseases (Divya, 2011). *Butea Monosperma* contains different bioactive compounds like alkaloids, flavonoids, animo acids, resin, saponin etc. (Table 1). The Phytochemical analysis of *Pongamia pinnata* showed various flavonoid derivates and several other phytochemicals like triterpenes, steroids, amino acids, fatty acid and ester (Limaye. 1925. *P. pinnata* also contains different phytoconsituents describe in table 2.

Why Antimicrobial Analysis is Important?

Infection causing pathogens and the disease caused by them has now become the principal problem of the world and almost 57 million people affected and killed because of this. The unexpected spread of disease and antibiotic resistance, as well as the discovery of new strains of disease-causing agents are of great concern to our world health organization.Operative treatment of disease involves the development of new pharmaceuticals or some hidden quality contains a source of drugs. The analysis refers to the process of killing or inhibiting the disease-causing microbes. 5,7-dihydroxy -3,6,4-trimethoxy flavone-7-O-α-L xylopyranosyl (1→3)-O-α-L-arabinopyranosyl-(1→4)-O-β-D galactopyranoside showed antimicrobial activity. Seed oil of *Butea monosperma* possess antimicrobial potential against pathogenic bacteria and fungi. So the oil has fungicidal and bactericidal properties (Gaurav et al., 2008). Antimicrobial (antifungal, antibacterial or antiviral) they all have different modes of action by which they act to

suppress the infection. A paper represents that the *Pongamia pnnata* leaves show the antimicrobial effect. It was interpreted that these leaf extracts have the ability that contains the defending system in the form of latent quality of antibacterial compounds against the inside present pathogens and that they can be used in the treatment of hazardously infecting diseases. It was expected that this study would reveal the discovery of some compounds used in formulating new and more potent antimicrobial drugs that contain the actions against the synthetic ones (Olusola et al., 2011). When antibacterial research is compared with antifungal it has been shown that small developmental progress has been made in the development of new antifungal agents, which has been justified by the low establishment of fungal infections. However, the current increase in the incidence of fungal infections has led to aggressive research on new antifungal agents as evidence by the rise in the number of publications since the 1960s (Manjunatha, 2004).Typically a long period of 8 to 10 years is required for antifungal to be approved for clinica1 use. New ideas regarding antifungal therapy a target identifications and traditional drugs technologies can significantly accelerate the process of new antifungal development, reducing the time to cure or providing better quality to patients.

CONCLUSION

Herbal medicines have been known to be useful for the human world from ancient times. The therapeutic efficiency of many originating plants for several disorders has been described by practitioners of traditional medicines. The phytochemical screening of various extracts of our target plant expected to reveal the presence of secondary metabolites. This review provides an outlook on various phytochemical and antimicrobial aspects that need to be done to carry forward the available information in developing suitable clinical therapeutics.

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Table 1. Different Phytochemical constituents of *Butea monosperma*

Sl. No.	Plant parts	Phytochemicals	References
1	Stem	Steroids: Stigmasterol-β D-glucopyranoside and nonacosanoic acid	Burli et al., 2007
2	Bark	Amino acid: Allophanic acid, butolic acid, butrain, histidine	Indurwade et al, 2005, Burli et al., 2007, Pandey et al., 2010, Tyagi et al., 2010
3	Leaves	Fatty acid: linoleic acid, lignoceric acid	Burli et al., 2007
4	Seeds	Enzymes: Polypeptidase, lipolytic enzyme Monospermosid (butrin 3-e-D-glucose)	Indurwade et al., 2005; Jawaharlal and Sabir, 1978; Rastogi and Mehrotra, 1979
5	Flowers	Triterpene: butrin, isobutrin, isomonospermoside, steroids	Kasture et al., 2011; Surin and Ananthaswamy, 2011; Gupta et al., 1970

Table 2. Different Phytochemical constituents of *Pongamia pinnata*

Sl. No.	Plant parts	Phytochemicals	References
1	Seeds	Sterols, fatty acid, β-sitosteryl acetate, galactoside, flavones: (2S)-(2″,3″:7,8) furanoflavanone	Li et al., 2006; Yadav et al., 2004
2	Leaves	Flavone, chalcone like Pongone, galnone, pongalabol	Li et al., 2006; Gandhidasan et al., 1987
3	Bark	Chromenoflavanone(-)-isoglabrachromene	Saha et al.,1991; Tanaka et al.,1992
4	Stem	Prenylated Chromenoflavanone, Chromenochalcone	Li et al., 2006; Carcache-Blanco et al., 2003

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Perception of Career Skills by Vietnamese Business Undergraduate Students

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Received: 14 Feb 2020 Revised:16 Mar 2020 Accepted: 18 Apr 2020

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ABSTRACT

Universities have been under growing pressure to deliver employable post-graduates in recent shifts in education and labor market politics. Career skills are an increasingly important element of higher education in business. This study analyzes the views of Vietnamese business undergraduate students to determine what skills they deem to be essential for their future careers. A group of 284 Vietnamese business undergraduate students (172 females and 112 males) from Ho Chi Minh City, Vietnam, participated in the survey. They completed a self-administered questionnaire, and it assessed ten career skills. The results showed that the level of students' perception in career skills had high. The average mean score from the questionnaire was from 3.41 to 4.20, and this has met the requirements for a future career. The results are explored in order to suggest developing curricula for business education in Vietnam.

Keywords: Business administration, student perception, career skills.

INTRODUCTION

The concept of employability skills can sometimes be referred to as career skills and can sometimes be regarded as necessary skills in the workplace or know-how in the workplace. (Hollenbeck, 1994). Career abilities in this paper apply to such executive competencies as communication (Hancock et al., 2009; Vo et al., 2020), social interaction (Selvadurai, Choy, & Maros, 2012), data analysis(Soule, Fanguy, Kleen, Giguette, & Rodrigue, 2017), analytic and problem-solving(Bratianu & Vatamanescu, 2017), computer literacy(Hungerford, Baxter, LeMay, & Helms, 2012), global awareness(White, 2017; Wye & Lim, 2009), business ethics (Sarfraz, Rajendran, Hewege, & Mohan, 2018; White, 2017), business(Bhamidimarri, 2016; Srivastava & Khare, 2012), leadership(Gault, Redington, & Schlager, 2000; Srivastava & Khare, 2012), self-management (Sarfraz et al., 2018). Such skills allow for the study of new

applications as those applications are more and more needed, which confirms that "graduate attributes" are more relevant than the research subject (Harvey, 2000). According to students were expected from a broader business perspective to develop skills and abilities in written and oral communication (Hancock et al., 2009).Vo et al. (2020) has shown that the level of communication skills of Vietnamese undergraduate business students is not high, that most students have communication skills at the ordinary level, and that this has not yet met the requirements for future studies and careers.Selvadurai et al. (2012) has reported that the public sector employers are pursuing some generic skills in the field of information and social interaction.

Soule et al. (2017) indicated that the challenge for educators is to balance a wide range of topics, such as statistics, math modeling, data management, computer programming, accounting, and marketing, which must be addressed in data analytics courses to provide students with useful skills in this area.Bratianu and Vatamanescu (2017) found that, when it comes to solving problems, graduates understand more than undergraduates that challenges in business can have both an economic and an emotional dimension.Hungerford et al. (2012) demonstrated that social media has little input into computer literacy programs, and even if covered, only a few apps are included. There are dozens of applications, many of which are widely used, particularly in large enterprises and multinational corporations. Students needed to know how to use social media for business purposes. Computers are typically taught by business colleges in microcomputers with the freshman or sophomore-level courses (Hungerford et al., 2012).Global awareness was one of the University's most developed skills(White, 2017; Wye & Lim, 2009).Ethical standards are always referred to as gaps in undergraduate skills in Australia(Hancock et al., 2009).

Therefore, White (2017)proposes three directions for graduate business schools to be more proactive in their teaching of ethics and corporate responsibility (ECR): (1) include stand-alone courses in the MBA curriculum emphasizing ethical behavior by business professionals; (2) integrate ECR issues into other core courses; and (3) offer elective courses in the area of social entrepreneurship, environmentally friendly.has shown that commercial awareness, financial and people management, and market awareness skills for sales and understanding have been established as business skills. Bhamidimarri (2016) has shown that commercial awareness, financial and people management, and market awareness skills for sales and understanding have been established as business skills.Understanding market mechanisms and being able to deal with the market forces for income enhancement is an area that needs special attention(Srivastava & Khare, 2012). Leadership has been defined as a process of social influence in which one person may contribute to a common task by helping and supporting others(Srivastava & Khare, 2012).To marketing recruiters to consumer goods companies, leadership skills (leadership/teamwork and partnership building) are paramount(Gault et al., 2000).Sarfraz et al. (2018) found thatself-management skills grouped nine skills, including time management, organization, planning, self-discipline, working under pressure, self-management, priority setting, multitasking, and load management skills. Overall, many research topics were discussed in terms of students' employability and work skills, but all subjects were not Vietnamese undergraduates, particularly finance and marketing students. There has been no scientific research topic on the employability and career skills of undergraduate students at the University of Finance – Marketing, Vietnam.In order to fill this void, the researchers were aimed at exploring students' career skills among Vietnamese students and at clarifying the theoretical basis and the status of Vietnamese students in general and the University of Finance students – Marketing, Vietnam.

METHODS

Participants

The convenience sampling method was used to recruit students who volunteered to help with the study and administer the survey. The survey instrument was distributed to 318 Vietnamese students of the University of Finance – Marketing, of which 284 questionnaires were returned, for an 89.31% return rate, which exceeds the 30% response rate most researchers require for analysis (Dillman, 2000). The sample of this study was drawn from 284

respondents who completed the survey instrument. There were more girls (60.6%) than boys (39.4%) among the 284 Vietnamese students who were surveyed. Of these, 48 (16.9%) freshmen, 82 (28.9%) sophomores, 114 (40.1%) juniors, and 40 (14.1%) seniors. Thirty-six of these students had a GPA of less than 6.5, 190 students had a GPA of6.5 to 7.5, and 58had a GPA of greater than 7.5. Table 1 shows the distribution of participants.

Measure

Questionnaires are designed to survey on Vietnamese students of the University of Finance – Marketing.At first, social-demographic items were introduced in the questionnaire. Then, perceptions of career skills of the University of Finance – Marketing 's undergraduate students of participants were measured by Student's Perception of Career Skills Questionnaire (SPCS), a total of 37 items. The questionnaire included ten career skills including (1) communication, (2) social interaction, (3) data analysis, (4) analytic and problem solving, (5) computer literacy, (6) global awareness, (7) business ethics, (8) business, (9) leadership, (10) self-management.The questionnaire questions were read carefully by all participants, and the answer was selected, which was best presented.The participants answered on a 5-point Likert scale at five different stages(Croasmun & Ostrom, 2011).The internal consistency reliability estimate for this sample was 0.67 for communication, 0.71 for social interaction, 0.87 for data analysis, 0.78 for analytic and problem solving, 0.87 for computer literacy, 0.88 for global awareness, 0.81 for business ethics, 0.80 for business, 0.86 for leadership, and 0.87 for self-management.

Analyses

Upon providing a summary of the study's purpose, all participants received informed consent. The research was approved by the ethics committee of the University of Finance – Marketing. For data processing, the Social Sciences Statistics Program (SPSS) version 16 was used.The coding procedure was performed as follow: 1 = Strongly Disagree, 2 = Disagree, 3 = Neutral, 4 = Agree, 5 = Strongly Agree.According to Narli (2010), the interval width of the 5-Likert scale should be computed in order to set up the group boundary value for result discussions. Interval Width = (Upper value – Lower value)/n = (5-1)/5 = 0.8. Group boundary values are built that help to discuss research results based on the above interval width, which are judged as below:

+ 1.00 - 1.80 = Totally Unnecessary/Strongly Disagree
+ 1.81 - 2.60 = Unnecessary/Disagree
+ 2.61 - 3.40 = Average/Optional/Neutral
+ 3.41 - 4.20 = Necessary/Agree
+ 4.21 - 5.00 = Totally Necessary/Strongly Agree

RESULTS

On the questionnaire, the participants scored in the average. The highest mean score of business undergraduate students' perception was 4.14 for the study on their career skills perceptions. The number of participants, mean, standard deviation values about these variables are shown in Table 3. Among the 37 items of perceptions of career skills of University of Finance – Marketing 's undergraduate students, the top ten indicators, listed from the highest to the lowest average point, are as below:Self-confidence (M = 4.14, SD = 1.07); Apply appropriate leadership styles (M = 3.99, SD = 1.15); Be the leader (M = 3.98, SD = 1.13); Time management (M = 3.94, SD = 1.06); Identify the main problems facing difficulties (M = 3.92, SD = 1.03); Understand the market economy (M = 3.90, SD = .96); Applying methods to make decisions (M = 3.89, SD = 1.00); Be willing to learn (M = 3.87, SD = 1.12); Organization (M = 3.83, SD = .99); Creativeness (M = 3.83, SD = 1.06). The five indicators with the lowest average point are as below: Marking assembly business (M = 3.52, SD = .93); Understand and apply quantitative techniques (M = 3.49, SD = .96); Using database programs (SPSS, Eviews, …) (M = 3.45, SD = 1.11); Write a summary of the report (M = 3.39, SD = .94).

DISCUSSION

This research found and measured students' perceptions of their value career skills from the set goals. Administration Business students are most aware of the value of career skills; the second is awareness of the value of professional knowledge and, lastly, awareness of the value of career attitudes. Descriptive statistics of the survey results reported that students are highly aware of the value of career skills leadership – executive, problem-solving – decision making and autonomy – self-control skills group. These are the essential, prerequisite, and important skill groups for Business Administration students studying majors. Next, students are aware of the value of a positive attitude in being proactive in silence and speaking when needed in their learning and future career activities. Finally, students assess the factors that affect the perception of career values, mainly from successful people in the business. Besides, students are also aware of the values of professional attitudes in handling the situations, tackling stress and business pressure. However, students are also lowly aware of the "macroscopic" level values of professional knowledge, such as economic management, business administration, etc. On the other hand, the research was carried out on a relatively small sample. Therefore, the analytical techniques were limited, and the authors only measured the perceptions of the students. In summary, students from the Faculty of Business Administration, University of Finance – Marketing are all aware of the career value that Business Administration offers. For the future, further research will expand the sample size so that more students in other fields, faculties, and schools are made aware and tested to establish a professional receipt measurement scale. Better analytical methods will generalize and make the career's value more effectively.

CONCLUSION

The research aimed at examining the understanding of Vietnamese business undergraduates of their career skills. Detailed quantitative study design and detailed methodology for the study have been used by the researchers. Students of business undergraduates from the University of Finance – Marketing, Vietnam, belong to career skills groups. Consequently, all ten skill groups must be impacted concurrently to develop the career skills of students.Currently, the applicant's job skills are of considerable significance as a basis for employers to choose the most appropriate applicant in the recruiting process in addition to their qualifications. Therefore, the university needs more subjects, and the number of classes, along with the number of seminars, allows the students to be more comfortable in their career skills. It is the first study to explore Vietnamese business undergraduates' perceptions of their career skills, which add to the small amount of research accessible to other institutions of higher education throughout Vietnam. Besides limited investigations of this dimension in Vietnam, the findings led to the provision of basic guidelines and foundations for developing career-enhancing strategies for students at the University of Finance and Marketing, Vietnam.

ACKNOWLEDGMENTS

This research is funded by the University of Finance – Marketing, Vietnam, under grant number CS.01.15.

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Table 1. An overview of survey participants

		n	%
Gender	Male	112	39.4
	Female	172	60.6
	Total	284	100
School Year	Freshman	48	16.9
	Sophomore	82	28.9
	Junior	114	40.1
	Senior	40	14.1
	Total	284	100
GPA	Less than 6.5	36	12.7
	6.5 to 7.5	190	66.9
	Greater than 7.5	58	20.4
	Total	284	100
n: Number of participants; %: Percentage; GPA: Grade Point Average (out of 10)			

Table 2. Observed variables measuring perceptions of career skills

Observed variables	Encryption
Communication (KNTT)	
Write a summary of the report	KNTT1
Negotiation	KNTT2
Communicate at an appropriate level	KNTT3
Social interaction (KNCN)	
Relations with people at many levels	KNCN1
Work, independent evaluation	KNCN2
Understand the difference between different people	KNCN3
Data analysis (KNPT)	
Accurate tool application for problems	KNPT1
Understand and apply quantitative techniques	KNPT2
Multi-dimensional perception	KNPT3
Determine the relationship between issues	KNPT4
Understand the accuracy and reliability of data	KNPT5
Analytic and problem solving (KNQĐ)	
Predict and offer alternative solutions	KNQĐ1
Identify the main problems facing difficulties	KNQĐ2
Applying methods to make decisions	KNQĐ3
Computer literacy (KNIT)	
Using spreadsheet programs (MS Excel...)	KNIT1
Using a word processing program (MS Word...)	KNIT2
Using database programs (SPSS, Views...)	KNIT3
Global awareness (KNTC)	
Understand the global economic environment	KNTC1
Understand the differences in culture	KNTC2
Understanding of the impact of other economic systems on the Vietnamese economy	KNTC3
Understand the differences between economies	KNTC4
Business ethics (KNĐĐ)	
Identify the conflict of business ethics	KNĐĐ1
Identify personal moral conflict	KNĐĐ2
Ethical decision making	KNĐĐ3
Business (KNKD)	
Knowledge of the dependence of business functions	KNKD1
Understand the market economy	KNKD2
Marking assembly business	KNKD3
Analyze branch tendency	KNKD4
Leadership (KNHĐ)	
Be the leader	KNHĐ1
Apply appropriate leadership styles	KNHĐ2
Time management	KNHĐ3
Self-planning the work	KNHĐ4
Organization	KNHĐ5
Self-management (KNTQ)	
Adapt to change	KNTQ1

Creativeness	KNTQ2
Be willing to learn	KNTQ3
Self-confidence	KNTQ4

Table 3. Vietnamese undergraduate business students' perception of their career skills

Observed variables	M	SD	Order
KNTT1	3.39	0.94	37
KNTT2	3.79	1.13	17
KNTT3	3.70	0.96	24
KNCN1	3.71	1.07	23
KNCN2	3.82	0.97	12
KNCN3	3.54	1.10	32
KNPT1	3.48	0.95	35
KNPT2	3.49	0.96	34
KNPT3	3.80	1.08	15
KNPT4	3.77	1.04	18
KNPT5	3.80	0.93	16
KNQĐ1	3.75	0.98	21
KNQĐ2	3.92	1.03	5
KNQĐ3	3.89	1.00	7
KNIT1	3.70	1.08	27
KNIT2	3.75	1.11	20
KNIT3	3.45	1.11	36
KNTC1	3.74	0.98	22
KNTC2	3.75	1.05	19
KNTC3	3.70	0.97	25
KNTC4	3.82	1.09	11
KNĐĐ1	3.56	0.96	31
KNĐĐ2	3.64	0.99	29
KNĐĐ3	3.70	0.98	26
KNKD1	3.59	0.92	30
KNKD2	3.90	0.96	6
KNKD3	3.52	0.93	33
KNKD4	3.67	1.03	28
KNHĐ1	3.98	1.13	3
KNHĐ2	3.99	1.15	2
KNHĐ3	3.94	1.06	4
KNHĐ4	3.81	0.98	13
KNHĐ5	3.83	0.99	9
KNTQ1	3.81	0.97	14
KNTQ2	3.83	1.06	10
KNTQ3	3.87	1.12	8
KNTQ4	4.14	1.07	1
M: Mean; SD: Standard deviation			

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Knowledge of Rare Diseases and its Management

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Received: 30 Jan 2020 Revised:01 Mar 2020 Accepted: 04 Apr 2020

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ABSTRACT

Health sector in Pakistan lacks the basic health facilities due to economic constraints and hence increasing the death rate among the patients with preventable diseases. There is a progressive trend in preventable disease outbreaks in Pakistan at nearly double pace from 38 outbreaks in 2015 to 70 in 2016. Increasing preventable deaths in Pakistan demand attention towards Primary Health concerns.The aim of this study was to investigate the knowledge and management skills of the physician's regarding the rare diseases. A cross-sectional study was conducted over a sample of 118 physicians serving at various government & private hospitals of Karachi, Pakistan. The data was collected from March to August 2014, all the physicians working at the registered clinics or hospitals with at least one-year work experience were enrolled in the study. Demographic characteristics and physician knowledge regarding rare disease was recorded. Moreover, the level of skills and awareness was rated from 1 (poor) to 5 (Excellent) by the enrolled physicians based on their perception and work experience. The study showed that there is considerable unawareness about rare diseases among the healthcare providers as indicated by the self-reported scores i.e. 62.7% physicians rated their level of knowledge as average or below average. Overall, 44.1% physicians suggested that delayed diagnosis is the major cause of mortality among rare disease patients followed by misdiagnoses or treatment unviability i.e. 27.1% each. Moreover, the specialized physicians were found to have greater knowledge of the rare diseasesas compared to the general practitioners (p-value = 0.036). As per the physicians rating, the general practitioners lack the basic knowledge and awareness of rare disease i.e. 49.2% gave a score 1 (poor) to general practitioners and 43.2% indicated it as substandard. Consultants are more aware about the rare diseases as compared to

the general practitioners. It is essential to employ strategic plans in order to identify the rare diseases in Pakistan, by increasing the knowledge and awareness among the healthcare professionals.

Keywords: Rare Diseases, Physicians knowledge, Skills, Awareness.

INTRODUCTION

According to the Center for Disease Control and Prevention (CDC), there has been a progressive increase in the outbreaks of the preventable diseases in Pakistan [1]. The incidence rate doubled, i.e. from 38 outbreaks reported in 2015 to 61 reported by the first half of 2017 [1]. Under such situation where the health care sector is grappling with the primary health concerns, discussion and management of the rare medical conditions is nearly scarce [1]. Among the western countries the reported incidence rate of rare disease is 1 per 5,000 births [2]. While, in Pakistan we barely have appropriate statistics indicating the incidence rate but it has been reported that the countries with high rate of 'intermarriages' are more likely to develop rare genetic disorders [2]. In view of the above theory, the rate of 'intermarriage' is 60% in Pakistan which might be an indication of high prevalence of rare diseases [2]. For instance, Qatar has the intermarriages rate of 40% and stand with the incidence rate of 1 per 1,300 births with rare diseases [2]. Progressive medical training of the healthcare providers (HCP's) in the field of genetics, immunology, neurology and pediatrics, etc. for the effective diagnosis and management of rare diseases is essential [3]. As the lack of awareness and knowledge regarding the rare diseases is one of the major reasons for delayed diagnosis or misdiagnosis [4]. General practitioners, pharmacists and pediatricians are usually the first level identifiers who are likely to diagnose all sorts of unusual or rare conditions [5]. Evidently, both education and awareness of the rare diseases play an important role in the enhancement of the diagnostic knowledge [6]. It is essential for appropriate timely management of the rare disease patient and in-time forwarding of the case to a specialized hospital for further treatment [6]. Apparently, lack of educational background and knowledge regarding the rare diseases among the medical doctors has greatly compromised the diagnostic and management criteria in the past [7]. Diagnostic evaluation of such rare conditions requires expertise and without proper medical institutes focusing on high quality and recent medical education, the chances of appropriate management and treatment are remote [8].

Globally, the representation of rare diseases in the classifications that are used to record health data is inadequate because still no general international solution for the routine coding of rare diseases exists [9]. As none of the pharmaceutical industries and health sector own the medicinal responsibility of these rare conditions worldwide, hence the medicinal drugs used for these conditions are termed as orphan drugs [10]. Due to decreased demand and rarity of the diseases, the underlining economic principles are inattentive towards the orphan drug manufacturing and regulation. Internationally some governments, researchers, scientists, and patient advocacy groups have contributed to improvethe rare diseases research, their efforts were mostly independent and resulted induplicative solutions [11]. Moreover, in a developing country like Pakistan, government barely invests in the pharmaceutical research and development sector. Therefore, the formulation and regulation of new or old drugs in association with rare diseases remains uncertain as we do not have any supporting local data or statistics [12].

Even if the diagnostic and treatment criteria's meet, financial sustainability is yet another obstacle for the Pakistani population. Many of the international countries like China, Canada, United States and European countries as well, have implemented the compulsory screening of the rare diseases among the newborns [13]. It is evident from the literature that nearly 50 different types of rare illnesses are being identified globally since the past 50 years but unfortunately <1% of the newborns in Pakistan are screened for such conditions [13]. As only a very few of the private hospital provide these facilities, the government should employ effective measures to offer these services throughout the country in all the healthcare public and private divisions [13]. Currently,we lack rare disease research and hence, the data presentation regarding the knowledge, skills and attitude of the HCP's is essential as they are the

foundation of identification and treatment of the disease. Therefore, this study aimed to assess the knowledge and management skills of the physician's regarding the rare disease.

METHODOLOGY

A cross-sectional study was conducted at various government & private hospitals of Karachi, Pakistan namely Civil Hospital Karachi, Jinnah Post graduate Medical Center, Memon Medical Institute, Dr. Ziauddin University Hospital, Abbasi Shaheed Hospital.A total of 118 physicians voluntarily participated in the study during March to August 2014. An independent institutional review committee approved the study proposal and written informed consent was obtained from each physician enrolled. The physicians working at registered clinics or hospitals and those having at least one-year work experience were included in the study. While the non-allopathic physicians, nursing & pharmacy interns and medical doctoron non-clinical job roles were excluded from the study. A survey questionnaire was designed with reference to previous studies conducted on rare disease [14,15]. The form consisted of 22 questions investigating physicians' demographics, resources in health care facility and the obstacles faced while treating a patient with rare disease.The physician's experiences with rare diseases was subjectively assessed. Level of knowledge, skills and awareness among different categories of the healthcare facilitators was rated from 1 (Poor) to 5 (Excellent) based on their perception and work experience. Moreover, a pilot study on 10% of the sample size was conducted prior to the study to assess the appropriateness of the questionnaire.The data was analyzed using SPSS version 17.0 and results were displayed as frequency and percentages. Chi-Square was used to measure the association between the variables, where p-value<0.05 was considered statistically significant.

RESULTS

Total 118 physicians were enrolled out of which 50.8% were males and 49.2% were females. Around 49.2% of the enrolled physicians were Bachelor of Medicine & Bachelor of Surgery (MBBS) doctors, 31.4% were having fellowship and 17.8% were diplomate. Overall, 39.8% of the enrolled physicians were general practitioners, 44.9% were providing services in private hospitals while 13.6% in government hospitals. The departmental information and work experience of the enrolled physicians is presented in table 1. The knowledge and skills of the physicians regarding rare diseases and orphan drugs was assessed and results indicated that 37.3% physicians self-reported their knowledge of rare diseases as good to excellent. Around 44.1% physicians witnessed and managed rare disease mortalities 1 to 2 times per year. Overall, 50.8% physicians were aware of some or other international social groups that are supporting the cause of rare disease management. While 11.9% were aware of local support group for rare diseases and their management plan (Table 2). 50.8% physicians reported that consultants have good knowledge of the rare diseases. For general practitioner majority of the physicians 49.2% rated their knowledge as poor and 43.2% as substandard (Table 3). As per the recommendations of the enrolled physicians, the knowledge and awareness of the rare diseases was comparatively higher among the specialized doctors as compared to the general practitioners (p=0.036).

DISCUSSION

This study with the aim to explore the knowledge and management skills of the physicians towards the rare diseases is unique, as not much research has been conducted so far both nationally and internationally for rare disease exploration and management. The physicians enrolled in the current study mostly believed that they have average or below average level of knowledge and skills of rare diseases 62.7% and 37.3% rated their knowledge as good to excellent (Table 2). Moreover, 44.1% physicians witnessed and managed the rare disease mortality at least once or twice every year. While, 9.3% of the enrolled physicians never managed such rare disease mortalities. This finding is supported by Reidenberg et al., Knight et al., & Moore et al., the knowledge of the rare diseases varies among

different parts of the world depending upon the resource settings and different states have categorized different management systems for such diseases [14-16]. The clinical experts are required for appropriate patient management and care [5], currently no national rare disease registry is available to provide accurate and complete data on the prevalence of rare disease in the region [5]. However, periodic review and up-gradation is required as there are certain rare diseases of past that have now become common and are no longer considered as rare currently [5]. The system needs to be well defined and aligned with the international standards and local healthcare infrastructure. Based on our findings, the enrolled physicians suggested that delayed diagnosis (44.1%) is the main cause of mortality among the rare disease patients, followed by misdiagnosis and medicine unavailability (27.1%). The diagnosis of rare disease is usually hindered by the misdiagnosis and unidentified etiological factors [17]. Hence, the delayed diagnosis leads to inappropriate management and treatment which in turn increases the morbidity and mortality risk [17, 18]. Although, it is known that the accurate early diagnosis is the key for the confrontment of the rare disease challenges but it has been observed that the single attempt appropriate diagnosis and correct treatment takes years of experience [19-21].

More than half of the physicians (50.8%) were aware of international support groups that provide assistance through experiences, to aid in easing the diagnostic and treatment problems in management of such rare disease patients. Moreover, 88.1% physicians were also unaware of the local support groups working for rare disease managment. Several national and international support groups have initiated campagins for rare disease awareness and are serving to support rare disease management [22]. National Organization of Rare Disease (NORD) recently carried out a program to enhance rare disease awareness, with the aim to pave ways for rare disease patient care [23]. Moreover, for research development the Australian association for the Children Wellbeing in Healthcare and the Association of Genetic Support provided the list of support groups for the management of rare disease research [23]. In Pakistan, Rare Disease Day is celebrated every year since 2012 by several non-profit and healthcare organizations [24]. But we still lack rare disease associations and support groups to put forward this intiative.

Around 55.9% of the physicians out source resources for diagnosis and treatment of rare disease patient's due to lack of awareness regarding the local support groups (Table 2). Also, supported by another study, suggesting that even with the diagnosed rare disease cases the physicians are usually left with no clues for the management and treatment of the disease [23]. The physicians mostly agree with the idea of Rare Diseases and Drug Information Center to create some effective route of information for doctors encountering any such rare disease cases [23]. Besides, individual knowledge, these physicians were asked to rate the knowledge and awareness of rare diseases among different healthcare categories. As per the inferences drawn, the general practitioners, paramedics and administrator mostly had poor knowledge while the consultants, registrar and medical officers are having good to excellent knowledge of rare diseases as per the opinion of the enrolled physicians (Table 3). Moreover, the medical doctors are known to have significantly better knowledge and understanding of the rare diseases as compared to the general practitioners ($p<0.05$). Similarly, a study by Engel et al., also denoted that 86% of the general practitioners had poor knowledge while 80% medical doctor indicated good knowledge of rare diseases [20]. A self-reported assessment of knowledge of physicians about rare disease in Belgium showed that 86% of the general practitioners rated their knowledge as substandard or poor and 72% pediatricians rated their knowledge as average [5].

In a country with insignificant doctor to patient ratio; with un-authoritative, un-ethical and illegal medical practices; the awareness level of the HCPs points towards the neglected responsibilities of the healthcare system. Medicine is upgrading with quick research and development across the globe. But locally our physicians barely invest their time for such upgradations which is one the major reasons for the lack of improvisation of the health standards and managements practices. Although the study was conducted through a dedicated and systematic approach to assess the awareness status of physicians about rare diseases and their management practices in Karachi, there are certain limitations to be considered. The study was cross-sectional and only captured the responses at one point in time. Moreover, it was not possible to correlate every significant factor (both qualitative and quantitative) that would gauge the awareness level of the physicians about rare diseases. The contributory factors or predictors of

unawareness must be explored to estimate the precise influence of these variables on the awareness level and management skills of the physicians. Furthermore, the small sample size was also one of the major limitations of the current study.

CONCLUSION

Physicians in the hospital setting are more confronted to the rare disease patients during their professional experience as compared to the general practitioners. Although, the general practitioners are the first source for identifying the rare disease patients before consultation with the specialized medical doctors. It has been identified that the awareness level is poor among the general practitioners as compared to the medical doctors. The awareness and knowledge of general practitioners is essential for the timely and apt diagnosis, management and treatment of these rare conditions. Specific information centers with accurate and reliable information must be available and easy accessibility must be assured for the upgradation of physician's knowledge regarding rare diseases. Updated and validated information on screening, differential diagnosis, prevention and patient referral to the appropriate medical facility is essential for all the physicians working under such situations where under and misdiagnosis is common.

RECOMMENDATIONS

Since there is no active classification system for rare diseases in Pakistan, there has to be an epidemiological well-established system that synchronizes well with the World Health Organization (WHO) standards of rare diseases. In addition, the health regulators should tempt to define some criteria to enumerate any disease as rare disease in terms of its prevalence. There has to be some system to admit patients with rare diseases and some authority should be taking care of their diagnostic and treatment expenses.Pakistan Medical & Dental Council (PMDC) should keep a track of the awareness level of physicians and there has to be some vigilant checking criteria for general practitioners as they had been lacking in knowledge upgradation. International social support groups like European Organization for Rare Diseases (EURORDIS), NORDS and Bin Adam Foundation (locally) should be encouraged to promote awareness regarding rare diseases.

CONFLICTS OF INTEREST

The Author(s) have no conflicts of Interest.

ACKNOWLEDGMENT

Authors would like to acknowledge Mirza Izhar Hussain for guidance and the assistance of Getz Pharma Medical Affairs department in manuscript editing and formatting.

SOURCE OF FUNDING

None.

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Table 1. Sociodemographic characteristics of the study population (n=118)

Variables		n(%)
Gender	*Male*	60(50.8)
	Female	58(49.2)
Age Groups (Years)	*25-34*	35(29.7)
	35-44	41(34.7)
	45-54	22(18.6)
	≥55	20(16.9)
Qualification	*MBBS*	58(49.2)
	Fellowship or equivalent	37(31.4)
	Diploma or equivalent	21(17.8)
	Others	2(1.7)
Salary (PKR)	*10,000-20,000*	9(7.6)
	20,001-30,000	37(31.4)
	30,001-40,000	25(21.2)
	40,001-50,000	22(18.6)
	>50,000	25(21.2)
Practice Type	*General Practice*	47(39.8)
	Private Hospital Job	53(44.9)
	Govt. Hospital Job	16(13.6)
	Other	2(1.7)
Department	*Emergency Room*	31(26.3)
	Intensive/ Critical Care Unit	23(19.5)
	Surgery	14(11.9)
	Pediatrics	7(5.9)
	Medicine	1(0.8)
	Oncology	7(5.9)
	Gynecology	3(2.5)
	Other	32(27.1)
Work Experience (Years)	*<5*	40(33.9)
	5-10	54(45.8)
	>10	24(20.3)

Table 2: Awareness & practices of the Rare Disease experts

Variables		n%
Knowledge and skills regarding rare disease	Good to Excellent	44(37.3)
	Average & Below average	74(62.7)
Managed Rare Disease Mortalities	Never	11(9.3)
	1-2 times in entire life	44(37.3)
	1-2 times in a year	52(44.1)
	1-2 times in a month	11(9.3)
Frequent Factor to Death	Delayed Diagnosis	52(44.1)
	Misdiagnosis	32(27.1)
	Delayed Treatment	2(1.7)
	Unavailability of Medicines	32(27.1)

Information Access at healthcare setting	Yes	80(67.8)
	No	38(32.2)
Seek Technological Support	Yes	43(36.4)
	No	75(63.6)
Mortality Meetings	Yes	28(23.7)
	No	90(76.3)
Need for Information Centre	Strongly Disagree	1(0.8)
	Disagree	1(0.8)
	Indifferent	18(15.3)
	Agree	43(36.4)
	Strongly Agree	55(46.6)
Most Needed Information	Diagnostic Tests	15(12.7)
	Treatment Plan	12(10.2)
	Medicine Prices	11(9.3)
	Support Structure	20(16.9)
	All of these	60(50.8)
Out-Sourcing Resources	Yes	66(55.9)
	No	52(44.1)
International Support Groups	Yes	60(50.8)
	No	58(49.2)
Local Support Groups	Yes	14(11.9)
	No	104(88.1)

Table 3: Physicians' response rating for the level of knowledge, skills & attitude regarding rare disease among different categories of the healthcare facilitators p value

Categories	**Response Rating (1 to 5)**				
	Excellent	**Good**	**Average**	**Substandard**	**Poor**
Consultant	34(28.8)	60(50.8)	22(18.6)	2(1.7)	-
Registrar	19(16.1)	45(38.1)	31(26.3)	13(11.0)	10(8.5)
Medical Officer	-	7(5.9)	21(17.8)	44(37.3)	46(39.0)
Paramedic	-	2(1.7)	7(5.9)	22(18.6)	87(73.7)
Administrator	-	-	2(1.7)	17(14.4)	99(83.9)
General Practitioner	2(1.7)	1(0.8)	6(5.1)	51(43.2)	58(49.2)

**Values are given as n(%)*

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

REVIEW ARTICLE

Ethnobotanical Studies on Medicinal Plants of Odisha (India): A Review

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Received: 28 Jan 2020 Revised:02 Mar 2020 Accepted: 04 Apr 2020

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ABSTRACT

Ethnobotany is defined as the study of the plants used by aboriginals for food, medicine, shelter, textiles, fabrics, ornaments etc. The science of ethnobotany has recently received much attention in certain parts of the world among the botanists, anthropologists, phytochemists, pharmacologists, foresters, archaeologists, paleobotanists, linguists, folklorists, herbalists, vaidyas, hakims, barefoot doctors etc. The interest is developing mainly to serve the growing needs of agro-industries, especially the herbal drug industry, conservation and development of plant genetic resources. Plants and plant-based medicines have been used since long for prolonging life span of man by combating various diseases. Ancient ethnic communities around the world had learnt to utilize their neighbourhood herbal wealth for curative as well as offensive purposes. Different parts of medicinal plants such as root, stem, bark, leaf, flower, fruit and seed are taken in different forms such as powder, juice, paste, decoction etc. In several areas of the world people still rely on herbal remedies for treatment of various common disease including cold, influenza, malaria, jaundice, diarrhoea, dysentery, diabetes, women diseases, venereal and skin diseases. Results show persistence of herbal medicine practices to alleviate different kinds of human as well as domestic animal ailments and this medication play vital role in the life of tribal as well as rural inhabitants. The brief description about the historical background of ethnomedicinal studies in India and in the state of Odisha is reviewed and presented herein. An outline of researches related to the documentation of ethnomedicinal plants of Odisha and their biological evaluation are also discussed.

Keywords: Ethnomedicine, Ailments, Biological activity, Odisha.

INTRODUCTION

Plants and plant-based medicaments have been employed since times immemorial for prolonging life of man by combating various ailments. Ancient ethnic communities around the world had learnt to utilize their neighbourhood herbal wealth for curative as well as offensive purposes. Due to lack of literacy, their knowledge of plants developed often at the cost of their dear life through centuries old experience could not be perfectly documented and it had rather descended from one generation to another as a domestic cultural heritage. As the ethnic groups migrated from place to place in search of their livelihood, their folklore knowledge also became fragmented and travelled with them often with 'additions and deletions'. Their findings in course of time have become basic leads for chemical, pharmacological, clinical and biochemical investigations, which ultimately gave birth to drug discovery. The approach to new drug discovery involves a collection programme for medicinal plants with primary emphasis on the use of plants by the aboriginals in the tropical and sub-tropical regions of the world. This approach integrates a philosophy of looking plant leads that had already been proved effective in tribal societies where experiments were done on human beings directly. This in short goes under 'Ethnobotany'.

Etbnobotany is defined as the study of association and inter-relationship of human societies, especially primitive human societies like tribals and aboriginal communities, with the surrounding flora. Powers (1874) [1] used the term 'aboriginal botany' which meant a study of the plants used by aboriginals for food, medicine, shelter, textiles, fabrics, ornaments etc. The term 'Ethnobotany' was coined by J.W Hershberger in 1985 [2] to indicate plants used by the aboriginals from ethno study of people and botanical study of plants. Robbins et al. (1916) [3] gave a broad definition of the area of ethonobotany as the investigation and evaluation of the knowledge of all phases of life amongst the primitive societies and the effects of the plant environment upon life, customs, beliefs and history of the tribal people. Vestal and Schultes (1939) [4] considered ethnobotany as a part of economic botany. Jones (1941) [5] confined it to the study of the interrelations of primitive man and plants. Schultes (1962) [6] defined ethnobotany as the study of the relationship which exists between people of primitive societies and their plant environment. In India, there is a tremendous scope for study of literature for ethnobotonical exploration because of the vast heritage of 'Vedic' literature which was dated back to 2000 to 800 B.C. The first attempt to classify the medicinal plants was made at the time of Charaka and Sushruta (1s' millennium B.C.). In these treatises, attempts were made to classify according to their Pharmacological application.

In the last three decades the studies of ethnobotany in the world is greatly emphasized, particularly in the under developed and developing countries, where small or large portions of populations still depend on natural resources in practically indigenous condition and the impact of modern systems of medicine has not reached them. Unfortunately today the situation is quite complicated. The tribal traditions are fast disappearing due to urbanisation, rapid industrialization and changes in sustenance economy. The successful implementation of a number of welfare schemes launched for the upliftment of tribal societies by social welfare departments in Odisha resulted in the loss of their primitive habits. Therefore, it is required to gather as much information as possible in shortest possible time from these tribal people. This can be achieved only if there is research on cross-cultural lines including, Anthropology, Botany, Chemistry and Pharmacology. In India, several premier institutions such as Council of Scientific and Industrial Research (CSIR), Central Institute of Medical and Aromatic Plants (CIMAP), Central Drug Research Institute (CDRI), Botanical Survey of India (BSI), Tropical Botanical Garden and Research Institute (TBGRI) have put in considerable effort to gather information on medicinal plants from different parts of the country.

Odisha is considered as one of the richest biodiversity region in south East Asia. Odisha's unique location in peninsular India has been blessed with an interesting assemblage of flora and fauna diversity. Out of the total geographical area of 155,707 sq. km. the state records 52,472 sq. km. (33%) of forest area or may be less according to forest survey of India. Though Odisha harbours a diversified floristic composition six forest pockets were found as

richest greeneries of biodiversity. The important biodiversity zones located in different parts of Odisha are Similipal national park, Gandhamardan hill ranges, Mahendragiri hill ranges, Deomali hill ranges, Niyamagiri hill ranges and Saptasajya-Kapilash hill ranges. Saxena and Brahmam (1994-1996) [7] reported 2727 species of plants under 228 families along with 750 species of medicinal plants.

In India the Scheduled Tribes belonging to 550 tribal communities of 227 ethnic groups as per the classification made by anthropologists on linguistic basis. They inhabit about 5000 forested villages. Predominant tribal areas cover about 15% of the total geographical area of the country. There are about 106 different languages and 227 subsidiary dialects spoken by tribals in India. Odisha with 62 tribes and 60 other small tribes has a tribal population of 9,590,756 (according to 2011 census) which constitutes 22.5% of the of the state's total population. Population of Kandhas, Santalas and Sauras are the predominant tribal groups in the state of Odisha. Each tribe has its own habitat and environmental setup, dialect, socio-cultural traditions and historical way of life. The tribals and rural people of the state are found to be the repository of accumulated experience and knowledge of indigenous flora. Living close to nature, rural folks are familiar with thousands of wild plants and animals. By empirical reasoning and trial or error, tribals and rural folks have screened and developed a highly complex and very specific knowledge on the local flora. Therefore, the ethnobotanical study may lead to find new information on unexploited plant resources and new uses on existing resources as source medicine.

MATERIALS AND METHODS

Several extensive ethnomedicinal surveys had been done in several districts of Odisha. Indigenous traditional herbal practitioners, chief of local community, knowledgeable old men and women of the community and patients were interviewed to gather information regarding ethnomedicinal uses of plants. The views given by them were recorded as data. With the help of tribal knowledgeable aged people and tribal medicine men voucher plant specimens were collected. Plants were identified by using 'The Botany of Bihar and Orissa'] (Haines 1921-1925) [8, 'Supplement to the Botany of Bihar and Orissa' (Mooney, 1950) [9] and 'The Flora of Odisha' (Saxena and Brahmam, 1994-1996) [7]. Main objective of this investigation was to tap these entire knowledge systems before they are wiped out or lost. These studies would also help to document the traditional skill and craft of the tribes of Odisha and rural communities in utilizing plant resources of their surroundings for healthcare-practices.

Different Approaches of Medicinal Plant Research

From thousands of years ago man has known about the benefit of drug from nature. The drugs used by the ancient civilization were extracts of plant or animal products with a few organic salts. Though several approaches are envisaged, field recording of plant uses directly from the ethnic people and tribal healers has priority and most reliable. Scanning of field notes on the old 'Herbarium Sheets' and Museum Sculptures of antiquity, data retrieval from ancient literature etc. are the other roots which have been found to be very effective and followed throughout the world with reasonable success. The following account gives in brief the whole gamut of medicinal plants that lead to the generation of data on plant uses since dawn of civilization.

Archaeological Sculptures

Archaeological Sculptures of antiquity play a vital role in giving away the clues of medicinal plants. Some of the countries still protect and preserve the archaeological remains. India has a rich treasure of archaeological sculptures of antiquity which can be of great value in tracing the plants which were used during early civilization. In India, from the basis of relief's of the Great stupa at 'Sanchi' and from the railing of 'Bharkut' stupa belonging to first and second century B.C. respectively, Sitholey (1979) [10] described 40 plants. In the book 'Buddhist Art of Gandhara' by Sir J. Marshall, a picture was reproduced from archaeological sculptures wherein Lord Buddha was presented with a

bundle of herbs and it was identified as "Soma" (*Ephedra*) of antiquity by Mahdihasan (1967) [11]. The quantum of work carried out in this direction is very scanty and it has large scope. Archaeological sculptures will also help in knowing the period in which they were in use.

Ancient Literature

Every country harbours ancient literature on herbal healings including *Materia-Medica* that might contain valuable information on uses of plants such as folklore, recipes, prescriptions, drug preparations, mode of administrations, precautions to be taken etc. With critical study and identification of plants, animals or minerals that go into the drug, one can easily get the leads for carrying research in the light of modern equipments and animal models. The ancient literature of India can also be included for information on medicinal plants. Of the four Vedas: *Rig-vedam*, *Yajur-vedam*, *Sama-vedam* and *Atharva-vedam* which were supposed to have been written somewhere during 4000-5000 B.C. in India, the 1st and the 4th i.e. *Rig-vedam* and *Atharva-vedam* are known to contain valuable information on medicinal uses of nearly 2000 plants and recently Sharma (1992) brought out a list of 248 botanical drugs mentioned in these 'Vedas' along with the botanical nomenclature. Singh and Chunekar (1972) [12] published a glossary of medicinal plants contained in the ancient works of *Charak Samhita*, *Susruta Samhita* and Vagbhatta's *Ashtanga Hridiya Samhita*.

As the linguistic knowledge of erstwhile classical literature is fast dwindling, there is an urgent need for active R & D (Research and Development) programme in this direction. Our present knowledge of Indian *Materia Medica* accounts for nearly 3,500 species under various crude drugs, both of indigenous and exotic origin. 'A catalogue of Indian medical plants' by John Fleming (1810) [13], *Materia medica* of Hindoostan by Ainslie (1813) [14], *Pharmacographia* by Fluckiger and Handbury (1879) [15] and *Indigenous Drugs of India* by Chopra et al (1956, 1958) [16, 17] are some of the treatises worth mentioning in this direction. Many treatises started appearing as a result of intensive work from different parts of the globe.

Herbaria and Musea

Herbarium sheets and field notebooks are also good source of ethnobotanical data. The information recorded on the Herbarium sheets and Museum samples by the field botanists are believed to be the most reliable, as these are the first hand information and attached to the specimen or sample itself. A systematic screening of such information from herbarium and museum samples is another area of research which is going to pay rich dividends in the modern drug development programme. If the earlier identification proves to be wrong, it can be rectified and the authenticity of the information noted on the sheet need not be doubted. The contributions of a few dedicated workers like Altschul (1973) [18] from United States of America have opened new vistas in this direction. Similar works were also attempted in India in the Industrial Section of the Indian Museum (BSIS), Calcutta and in the 'Kanjilal Herbarium' (Assam) at Shillong.

Field Survey

Field surveys in tribal inhabited forest areas and amongst primitive human societies is an important and critical area of research because the habitat and the environment where the tribes and primitive people live and learn are more fruitful and rewarding. Various forest areas rich in tribal population are to be identified, survey trips conducted at regular intervals in different seasons and the tribal uses of plants be studied *in situ* by establishing close intimacy with the tribal healers. Care is taken to prioritize vulnerable areas for immediate attention. Though attempts have been made all over the world since beginning of the 20th century to carry out the medicinal plants studies, the output is very meager. An experienced taxonomist, an anthropologist, a linguistic and a medical man can form an ideal team to study, identify, collect, document and prepare inventories on plants used by earlier races for food, shelter, clothing, medicine, craft, religious ceremonies, crops, agricultural patterns, music and entertainment.

Late Dr. E.K. Janaki Ammal initiated some studies in India on subsistence of foods. A number of institutions are now engaged in researches in various fields about the study of medicinal plants. Some of them are Botanical Survey of India, Central Council for Research in Ayurveda & Siddha, Central Council for Research in Unani Medicine, National Botanical Research Institute, Lucknow, Central Institute of Medicinal and Aromatic Plants, Lucknow, Regional Research Laboratories at Jammu, Jorhat and Bhubaneswar, FRLHT, Bangalore, M.S. Swaminathan's Foundation, Universities, Colleges, NGOs etc. Although flora of Odisha has been compiled by Saxena and Brahmam (1994-1996) [7] but detailed information about their medicinal properties are lacking. The present review article highlights the occurrence, distribution and utilities of ethnomedicinal plants reported from different regions of Odisha and their authentication by scientific evaluation.

Observations and Discussion

Ethnobotany is not only a common but also a familiar word to ancient Hindus. Kirtikar and Basu (1987) [19] stated that the ancient 'Hindus' should be given the credit for cultivating what is now called ethnobotany. It has been realized all over the world that much valuable knowledge about the use of plants including medicinal uses is still endemic among many tribal society. The Ayurvedic system of medicine not only provides cure for a large number of general and chronic diseases but also improve the inner body strength. Generally wasteland plants are called as weeds and said to be unwanted. But 'Ayurveda' suggested 'no plants of the world is useless'. In Ayurvedic system of medicine a large number of ethnomedicinal plants employed for the treatment of several ailments.

Studies on Ethnomedicinal Plants of Odisha

As a result of intensive and extensive ethnobotanical field explorations and interactions with tribal healers, village medicine men and senior women folk who practice native phytotherapy in different districts of Odisha, it became possible to generate enormous amount of data. After identifying the plant specimens in the Herbarium and scrutinising the data, it was found that some of the information were quite interesting and worth pursuing for their efficacy. Over 1000 species of medicinal plants have been widely used by the tribal population of Odisha are rural inhabitant. The tribes as well as the rural communities in the state are found quite dependant on their surrounding vegetation for the treatment of their common ailments. The floristic surveys on ethnomedicinal plants in different districts of Odisha were conducted by several researchers [20-49] to document the plant resources used in treating different diseases.

Ethnobotany of Bhumij tribe of Sunabera plateau in Koraput district in Orissa has been studied by Aminuddin and Girach (1993) [20]. Bal (1942) [21] enumerated some useful plants of Mayurbhanj district of Odisha. Brahmam and Saxena (1990) [22] reported 77 interesting medicinal plant species from Gandhamardan hills of Orissa. Studies on the less known medicinal uses of some plants in the tribal areas of Odisha have been described by Chaudhuri et al. (1985) [23]. Some ethnobotanical notes from tribal belt of Koraput (Odisha) were reported by Das and Kant (1998) [24]. Nawarangpur district ethnobotanical survey conducted by Dhal et al. (2015) [25] reported 51 plants used for common ailments. An account of 44 ethnomedicinal plants reported to be used by tribal communities of Sundergarh district (Girach et al. 1998) [26]. Hemadri and Rao (1989) [27] recorded 201 plants from folk-lore claims of Koraput and Phulbani districts of Orissa State. The magico-religious beliefs about plants among the tribals of Orissa were studied by Jain (1971) [28]. Medicinal claims of 60 plants used by local healers and herbalists of Kendrapara district of Odisha have been studied by Jena and Satapathy (2015) [29]. Forty six plant species of ethnobotanical value occurring in Angul district of Odisha were enumerated by Mahalik et al. (2014) [30]. Ethnobotonical studies of some plants used for curing urinary disorders in Dhenkanal district of Odisha were conducted by Mahalik et al. (2015) [31]. Mishra and Satapathy (2004) [32] gave valuable information about the use of some plants as life supporting food and food-drink amongst tribal population of Kandhamal district of Odisha. The medicinal plants (70 species) which are being used by the village folk of Jajpur district in Odisha for dental care reported by Mohapatra and Satapathy

(2004) [33]. Mukhrjee and Namhata (1990) [34] reported ethnobotanical information concerning 22 plants, collected from tribals namely, Oraon. Munda, Bhaiyan, Gond, Dhanuar and Routia of Sundergarh district, Orissa. Folk medicines used for snake bite in different forest areas of Kalahandi district of Odisha, along with details regarding the plants used, their parts used and mode of administration was discussed by Mund and Satapathy (2011) [35]. The medico-botanical study of 131 invasive species of Dhenkanal district of Odisha was published by Nayak and Satapathy (2015) [36] with details on locality of occurrence along with local name of these plants. Panda and Padhy (2008) [37] reported 111 ethnomedicinal plants in Kalahandi district. Rath and Satapathy (2013) [38] in one of their ethnobotanical studies, have reported 63 tribal and other folk-lore medicines used against women healthcare gathered from the aboriginals of Keonjhar district of Odisha. Forty six herbal folk medicine in Keonjhar district of Odisha for lactation in nursing mother was given by Rath et al. (2014) [39]. Ethnomedicinal studies in the state of Odisha revealed 34 plant species used against different diseases of Bondo tribes in Malkanagiri (Rout et al. 2014) [40].

Satapathy (2008) [41] studied 50 medicinal plants useful in the health care of Juang, Kohla and Munda tribals of Keonjhar district of Odisha. Over 88 plant species used as veterinary medicine by the tribal and rural folk in Jajpur district, for different diseases of their domestic animals were enumerated by Satapathy (2010) [42]. Satapathy (2015) [43] reported 50 species used by hill communities of Sukinda valley in Jajpur district of Odisha for curing 30 different types of diseases. Sixty different ethnomedicinal drug plants of Sundergarh areas of Odisha were studied by Satapathy and Brahmam (1996) [44]. An ethnobotanical exploration in tribal belts of Sundergarh district was also carried out by Satapathy and Panda (1992) [45]. Satapathy et al. (2001) [46] have reported the folk-lore use of anti-diabetic plant species occurring in Odisha along with the method of preparation and dose of administration of crude drugs as suggested by tribal and non-tribal herbalists. Ethnomedicinal plants used by the tribals of Odisha were presented by Saxena and Dutta (1975) [47]. Folklore medicinal plants of district Phulbani (Odisha) have been described by Subudhi and Choudhury (1985) [48]. Tribedi et al. (1982) [49] studied the distribution, local names and folk uses of plants commonly used by the tribal people of Mayurbhanj district of Odisha.

Phytochemical Investigation and Biological Activity Study

Phytochemical investigations on selected ethnomedicinal plants were done to screen different secondary metabolites. Ethnobotanical plants were also screened to find out their biological activity in combating various diseases at different research centres (Table 1) [50-114]. The tribals those depend on forest wealth are the real custodians that safeguard the medicinal plants till now. Generally the folk people are well acquainted with the medicinal properties of their surrounding vegetation. Majority of the population (rurals and tribals) of the state still depend upon plant resources of their neighbourhood for their daily needs. General uses of plants such as edible, domestic needs, industrial applications etc. are comparatively easy to record as large section of the population were either seen consuming or sold in the local weekly markets. However, more difficulty arose when recording was done from the traditional healers. What the majority of the investigators including the authors of this article found during their long association with different tribal communities is that the tribal medicine men or herbal practitioners are very possessive of their knowledge and they do not divulge any information in the presence of their own people. But, they cooperate and reveal to a larger extent privately in the absence of their own clown. They fear that their status and supremacy in the society is largely due to their knowledge and they do not want to create a competitor in their society. The valuable knowledge acquired through centuries old experience is still alive in smaller pockets but the size and number of pockets are becoming smaller and smaller day by day.

CONCLUSION

Medicinal plants have great importance in providing health care to above 80% of population of India. Due to easy availability, low cost and less side effect, the use of medicinal plants is increasing day by day. But due to lack of systematic dissemination of knowledge concerning these popular plant remedies it is feared that little of this

knowledge will survive when the older generation passed away. The present review article highlights important ethnobotanical information especially about the uses of plants or plant parts against various common human ailments by the tribals of Odisha. Hence, proper documentation of plant materials used in traditional medicine could benefit general health care and promote forest conservation as well as ecological research. The present article also describes the investigations of a good number of medicinal plants with an established ethnobotanical relevance for their phytochemcial and biological studies might perhaps pave the way for further scientific and clinical studies. The findings of the above mentioned studies also could promote the evaluation of new phytochemical drugs for future generation.

ACKNOWLEDGMENT

The authors are thankful to the authorities of Centurion University of Technology and Management, Odisha for providing necessary facilities during the investigation.

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Table 1: Biological activities evaluation of some selected medicinal plants used by the tribes of Odisha

Medicinal Plants & Family	Plant Part (s) Used	Extract Type	Experimental Organism	Biological Activity	Reference
Adenanthera pavonia L.	Bark	n-hexane	*Shigella flexneri*	Antidiarrhoeal	[52]
Aegle marmelos (L.) Correa (Rutaceae)	Fruit/ leaf	Methanol	Rat	Antidiarrhoeal	[53]
Allium cepa L. (Liliaceae)	Bulb	Ether & Ethanol	Guinea pig	Anti-asthmatic	[54]
Ampelopteris prolifera (Retz.) Copel (Thelypteridaceae)	Whole plant	Aqueous, Ethanol, Methanol	*Streptococcus mitis*	Antibacterial (Meningitis)	[55]
Annona squamosa L. (Annonaceae)	1. Seed 2. Leaf	Aqueous	1. Rat 2. Bacteria	1. Antitumor 2. Antibacterial	[56]
Aristolochia indica L. (Aristolochiaceae)	Aerial part	Chloroform	Mice	Hypoglycemic, anti-diabetic	[57]
Artocarpus heterophyllus Lam. (Moraceae)	Leaf	Aqueous ethanol (10%)	*Aspergillus* sp. *E. coli*	Antimicrobial	[58]
Asparagus racemosus Willd. (Asparagaceae)	Root	1. Alcohol 2. Aqueous	Rat	1. Hepato-protective 2. Promotes lactation	[59]
Averrhoa carambola L. (Averrhoaceae)	Leaf	1. Aqueous 2. Petroleum ether	1. *Salmonella typhi* 2. *E. coli*	Antimicrobial	[60]
Azadiracta indica A.Juss (Meliaceae)	Leaf	Aqueous	Rat	Reduce blood glucose level	[61]
Barringtonia acutangula (L.) Gaertn. (Barringtoniaceae)	Leaf	Aqueous methanol chloroform	Rat	Antidiarrhoeal	[62]
Boerhaavia diffusa L. (Nyctaginaceae)	Root	Methanol	Mice	Anticonvulsant	[63]
Butea monosperma (Lam.) Taub. (Fabaceae)	Flower Bark	Methanol	Rat	Liver disorder & wound healing	[64]
Calotropis gigantea R.Br. (Asclepiadaceae)	Root	Aqueous	*E. coli*	Antibacterial	[65]
Careya arborea Roxb. (Lecythidaceae)	Bark	Ethanol	Albino rat	Anti-ulcer	[66]
Capparis zeylanica L. (Capparidaceae)	1. Root 2. Leaf	1. Methanol & ethanol 2. Ethanol	- Mice	1. Antioxidant 2. Immuno modulatory	[67]
Clerodendrum inerme (L.) Gaertn. (Verbenaceae)	Aerial part	Methanol	Wistar albino rat	Antidiabetic, Reduction in blood sugar	[68]
Clerodendrum philippinum Schauer (Verbenaceae)	Leaf	Methanol	Wistar albino rat	Antidiabetic	[69]

Clerodendrum serratum L. (Verbenaceae)	1. Leaf 2. Leaf	1. Petroleum ether 2. Methanol	1. *E. coli* 2. Wistar albino rat	1. Antimicrobial 2. Antidiabetic	1. [70] 2. [71]
Clerodendrum viscosum Vent. (Verbenaceae)	1. Leaf 2. Leaf	1. Methanol 2. Methanol	1. *Klebsiella pneumoniae* 2. Rat	1. Antimicrobial 2. Antidiabetic	1. [70] 2. [72]
Cryptolepis buchananii Roem. & Schult. (Periplocaceae)	Root	Methanol	Mice	Analgesic	[73]
Cuscuta reflexa Roxb. (Cuscutaceae)	Whole plant	Ethanol	Rat	Reduce high blood pressure	[74]
Cynodon dactylon (L.)Pers. (Poaceae)	Aerial parts	Ethanol	Mice	Anticonvulsive property	[75]
Eucalyptus globulus Labill. (Myrtaceae)	Leaf	n-hexane	*Micrococcus luteus*	Antibacterial	[76]
Euphorbia hirta L. (Euphorbiaceae)	Leaf	Methanol	*E. coli*	Antimicrobial	[77]
Ficus benghalensis L. (Moraceae)	Root	Methanol	Rat	Immune modulatory	[78]
Gloriosa superba L. (Liliaceae)	Tuber	Ethanol & Aqueous	Indian earthworm	Anthelmintic	[79]
Holarrhena pubescens (Buch.-Ham.) Wall. ex G.Don (Apocynaceae)	Seed	Ethanol	Rat	Antidiarrhoeal	[80]
Justicia adhatoda L. (Acanthaceae)	Leaf	Methanol	Fungi	Antifungal	[81]
Lawsonia inermis L. (Lythraceae)	1. Bark 2. Leaf	1. Ethanol 2. Petroleum ether	1. Rat 2. *Salmonella typhi*	1. Antibacterial 2. Antimicrobial	1. [82] 2. [83]
Mangifera indica L. (Anacardicaceae)	1. Leaf 2. Leaf	1. Ethanol 2. Petroleum ether	1. Rat 2. *Pseudomonas aeruginosa*	1. Antidiabetic 2. Antibacterial	1. [84] 2. [85]
Madhuca indica Gmel (Sapotaceae)	Leaf	Aqueous	Rat	Antiulcer	[86]
Melia azedarach L. (Meliaceae)	Bark	1. Methanol 2. n-hexane 3. n-hexane	1. *Streptococcus mitis* 2. *Shigella flexneri* 3. *Candida krusei*	Antimicrobial	1. [87] 2. [87] 3. [88]
Mikania micrantha Kunth (Asteraceae)	Whole plant	n-hexane	*Pseudomonas aeruginosa*	Antibacterial	[89]
Morinda citrifolia L. (Rubiaceae)	Leaf	Aqueous ethanol	*Candida albicans*	Antimicrobial	[90]
Naringi crenulata (Roxb.) Nicolson (Rutaceae)	Leaf	Methanol	Wistar albino rat	Antidiabetic	[91]
Nyctanthes arbor-tristis L. (Oleaceae)	Leaf	Alcohol	Guinea pig	Tranquilizing & Antihistaminic	[92]

Ocimum sanctum L. (Lamiaceae)	Leaf	1. Aqueous & Alcohol 2. Ethanol	1. *E. coli* 2. Mice	1. Antibacterial 2. Anti-cancer	[93]
Oxalis corniculata L. (Oxalidaceae)	Whole plant	Methanol	Rat	Antidiabetic	[94]
Phyllanthus emblica L. (Euphorbiaceae)	Fruit	Ethanol & Aqueous	Rat	Antioxidant	[95]
Polyalthia longifolia (Sonn.)Thw. (Annonaceae)	Bark	Methanol	Rat	Antidiabetic	[96]
Pongamia pinnata L. (Fabaceae)	Leaf	n-hexane	*Streptococcus mitis*	Antibacterial	[97]
Scindapsus officinalis (Roxb.)Schott (Araceae)	Root	Methanol	Rat	Analgesic Anti-inflammatory	[98]
Seseli diffusum (Roxb. ex Sm.) Sant. & Wagh. (Apiaceae)	Whole plant	n-hexane	*Shigella flexneri*	Antibacterial Antioxidant	[99]
Sida acuta Burm.f. (Malvaceae)	1. Leaf 2. Root	1. Ethanol 2. Aqueous	1. *Aspergillus niger* 2. Rat	1. Antifungal 2. Antidiabetic	1. [100] 2. [101]
Sida rhombifolia L. (Malvaceae)	Root	Methanol	-	Antidiarrhoeal	[102]
Sonchus asper (L.) Hill (Asteraceae)	Whole plant	Methanol	*Staphylococcus aureus*	Antibacterial	[99]
Sterculia foetida L. (Sterculiaceae)	Bark	1. Methanol 2. n-hexane	1. *Salmonella enteric ser typhi* 2. *Rhizopus oryzae*	1. Anibacterial Antioxidant 2. Antifungal	1. [103] 2. [104]
Streblus asper Lour. (Moraceae)	Leaf	Ethanol	-	Inhibition of sub-gingival bio-film formation	[105]
Syzigium cumini (L.) Skeels (Myrtaceae)	Bark	Ethanol	Rat	Antidiarrhoeal	[106]
Tectona grandis L.f. (Verbenaceae)	Leaf	Aqueous ethanol	*Klebsiella pneumoniae*	Antimicrobial	[58]
Terminalia arjuna (Roxb. ex DC.) Wight & Arn. (Combretaceae)	Bark	Alcohol	Dog & Cat	Decrease blood pressure & heart beat rate	[107]
Terminalia bellirica (Gaertn.) Roxb. (Combretaceae)	Fruit	Ethanol	Rat	Antiulcer	[108]
Terminalia catappa L. (Combretaceae)	1. Fruit 2. Leaf	1. Petroleum ether & Methanol 2. Aqueous ethanol	1. Rat 2. *Klebsiella pneumoniae*	1. Antidiabetic 2. Antimicrobial	1. [109] 2. [90]
Terminalia chebula Retz. (Combretaceae)	Fruit	Aqueous	Rat	Antioxidant & radioprotective	[110]

Thespesia populnea (L.) Sol. ex Corr. (Malvaceae)	Bark	1. n-hexane 2. Methanol	*1. E. coli 2. Shigella flexneri*	Antidysenteric, Antidiarrhoeal	[111]
Tinospora cordifolia (Willd.) Hook.f. & Thoms. (Menispermaceae)	Stem & Leaf	Aqueous	Rat	Immune modulatory & cytotoxic effect	[112]
Toddalia asiatica (L.) Lam. (Rutaceae)	Leaf	Methanol	Wistar albino rat	Reduction in blood glucose level	[113]
Trigonella foenum-graecum L. (Fabaceae)	Seed	Dry powder	Rat	Improves blood sugar level & Antioxidant	[114]
Vetiveria zizanioides (L.) Nash (Poaceae)	1. Root 2. Stem	1. Oil 2. Decoction	-	1. Aphrodisiac 2. Urinary tract infection	[115]
Zingiber officinale Rosc. (Zingiberaceae)	Rhizome	Aqueous	Rat	Reduce total serum lipids & Total serum cholesterol	[116]

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Seasonal Study on Population of House Sparrow (*Passer domesticus*) with Reference to Urban Areas of District Kupwara from Jammu and Kashmir, India

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Received: 10 Feb 2020 Revised:14 Mar 2020 Accepted: 16 Apr 2020

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ABSTRACT

The house sparrow is one of the ubiquitous birds around us and is mainly connected with human habituation. It is typically absent from deserts, grasslands and extensive woodlands. The aim of the study was to access the population status of house sparrow in urban areas in different seasons. For this purpose regular field visits on monthly basis were carried out from May 2017 to April 2019 in three urban areas Viz. Kupwara, Salkoot and Branwari in Kupwara district, Jammu and Kashmir, India. For counting the numbers of house sparrow Point count method was used. From the results it was observed that the population of house sparrow was found to be maximum in Salkoot and Branwari in both summer and monsoon seasons as compared to the winter season. In comparison to the other urban sites the minimum population of house sparrow was found in kupwara in all the three seasons i.e. summer, monsoon and winter. Therefore, it is concluded that alteration of built-up structure and intensive management of greens spaces by humans within the study region is prompting to a differential change in the habitat for house sparrows.

Key words: Seasonal study, urban areas, Kupwara District, House sparrow, Population.

INTRODUCTION

House sparrow locally known as chidya that belongs to family Passeridae. It is a little bird having a mass of 24-39.5 g with a typical length of 16 cm. In most parts of the world the house sparrow has been highly successful where it has

been introduced which is mainly due to its adaptability to a wider range of conditions and its early adaptation to living with humans. The house sparrow mostly feeds on the ground but it frequently flocks on trees and bushes. Nests are most frequently built in eaves and other cervices of houses, though cavities are preferred as well. A drastic decline of house sparrow population in recent years has been reported in India and ornithologists have observed a sharp decline in house sparrow population across Bangalore, Haryana, Delhi, Rajasthan and West Bengal [1, 2, 3 and 4]. A high reduction of house sparrow population has been reported in Hamburg (77%), Glasgow (99%) and London (60%) [5]. The primary causes of this decline have not been ascertained yet. But lack of weed seeds, insect food due to rapid use of insecticides and competition for food with other living birds are the potential factors of house sparrow decline [6]. Hence, the present study was carried out to assess the population status of house sparrow in different seasons in urban areas of district Kupwara, Jammu and Kashmir, India.

METHODOLOGY

Kupwara is an active district which is located in the Indian state of Jammu & Kashmir with an approximate distance of 90 kms away from the capital of Srinagar and an average altitude of 5300 feet from the mean sea level. The Latitudinal range of the district is 34.4319º N and Longitudinal extension is 74.1240º E. Mainly the east and northern areas of the district are hilly while southern areas are plain and the district is having a total geographical area of 2,379 square kilometres. The study was conducted for the period of May 2017 to April 2019 in three urban areas Viz. Salkoot, Branwari and Kupwara. The study period was divided into three different seasons namely summer (March-June), monsoon (July-October) and winter (November-February). Early morning i.e. from 6.00 A.M to 9.00 A.M was chosen as an ideal time for counting of birds because during this time they are most active and in the evening from 5.00 to 7.00 P.M. Point count method [7] was used for counting the numbers of house sparrows. The collected data was later statistically analysed to estimate the population of house sparrow on seasonal basis.

RESULTS

The present investigation was carried out from May 2017 to April 2019 in three urban areas I.e. Kupwara, Branwari and Salkoot in district Kupwara, Jammu and Kashmir, India. The results on population of house sparrow in different seasons are shown in Table 1. During the seasonally study period I.e. from May 2017 to April 2019, the average number of house sparrow population in Salkoot was found to be maximum in summer season (12.5 ±1.29 to 10.5±1.29) followed by monsoon season (11±1.41 to 10±3.65) and minimum in winter season (7.5±1.29 to 5.5±2.6) (Table 1). Similarly, in Branwari the average number of house sparrow population was found to be maximum in summer season (11.75±3.94 to 9.5±5.80) followed by monsoon season (9.5±1.29 to 8.5±1.73) and minimum in winter season (6.25±1.5 to 4.25±1.70) (Table 1). Also in Kupwara the average number of house sparrows was found to be minimum in summer (8.75±2.5 7.25±2.21) followed by monsoon season (6.5±1.29 to 6.25±1.70) and winter season (5.5±1.29 to 3.25±0.95) (Table 1).

DISCUSSION

The highest number of House sparrow population in Salkoot and Branwari during summer and monsoon seasons may be due to the availability of rich diversity of home gardens where different types of shrubs, bushes and weeds were present which provide them different varieties of invertebrate food, these invertebrates form an important part of sparrows nestling diet during the breeding season as they use them for rearing their young chicks [8]. In these areas the agricultural practices were found to be high because of which there was the availability of different variety of foods such as grains, rice, pulses, etc. Same observations were observed by Kurkhade *et al.*, [9] in Parner tehsil, who reported that the maximum population of house sparrow is due to the farming potential as it supplies different varieties of greeny vegetables to super cities of Maharashtra. Along with green vegetables, Jawar, Bajra, sugarcane, Wheat and Pulses are the significant accident harvests of the zone that support the house sparrow population.

Monika [10] stated that house sparrow population was found to be more in the areas indulging in large hectares of agriculture in the Haridwar of Uttarkhand when compared to the highly developed residential areas. In comparison to other urban sites the lowest number of house sparrow population was found in Kupwara during both summer and monsoon seasons may due to loss of green shrubby areas. The absence of grassy vegetation results in nonavaliability of insects because of which house sparrows face a shortage of nestling diet [11]. The agricultural practices were found to be poor in Kupwara due to unavailability of water to cultivation and as a result there was scarcity of food grains for the house sparrows. Hole *et al* [12] reported similar kind of findings who stated that the main reason for population decline of house sparrow in an area was due to spiralling of farming practices which reduced the food supply for house sparrows. Another reason might be due to the impact of urbanization and various types of development activities such as construction of shopping complexes, apartments, etc for which different types of plants as well as trees are being cut down due to which the roosting sites of house sparrows got completely destroyed .Similar kind of findings were supported by Singh *et al* [13] in urban and suburban regions of Jammu and Kashmir who stated that extensive urbanization and development activities which completely leads to loss of greeny vegetation and hence the roosting sites of house sparrows were replaced by concrete buildings, playgrounds, etc. In comparison to other urban sites the buildings in Kupwara were mostly made of new architectural style that lack suitable holes because of which house sparrows were unable to build their nests. Similar findings were reported by Smith [14] who stated that unavailability of suitable nesting sites in modern concrete type of buildings in the urban areas result in the population decline of house sparrows in London.

During the study period the lowest number of house sparrow population in winter season was observed in all the three urban sites (Salkoot, Branwari & Kupwara) might be due to impact of severe weather conditions particularly low temperature and snow cover which caused stress and affect the movement and distribution patterns [15]. Easterbrook [16] also recorded more or less continuous decline of sparrow population during winter season.

CONCLUSION

The present study elucidated that the study sites like Salkoot and Branwari even though being urban areas a high density of house sparrows was observed in both summer and monsoon seasons due to the availability of ideal habitat for its living as well as abundant food resources due to great potential of agricultural practices in these areas. In Kupwara, which is highly urbanized area in comparison to other study sites the population density of house sparrows was found to be low in all the three seasons (summer, monsoon & winter) which is mainly due to impact of urbanization processes and various developmental activities that has resulted in the loss of habitat for house sparrows. Therefore, the present study suggests that for construction and renovation of buildings enforce best practices and develop appropriate polices, cutting down of trees and plants especially medium sized trees should be avoided, plantation of more and more trees should be done, use of insecticides and pesticides in parks, gardens, etc should be minimized and use of bio fertilizers should be encouraged.

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Table 1. Seasonal occurrence of House sparrow (*Passer domesticus*) in three urban sites during the study period May 2017-April 2019

Periodic visits	Name of urban sites	Mean± SD number of Adults in different Seasons		
		Summer	Monsoon	Winter
From May 2017 to April 2018	SALKOOT	12.5±1.29	11±1.41	7.5±1.29
	BRANWARI	11.75±3.94	9.5±1.29	6.25±1.5
	KUPWARA	8.75±2.5	6.5±1.29	5.5±1.29
From May 2018 to April 2019	SALKOOT	10.5±1.29	10±3.65	5.5±2.64
	BRANWARI	9.5±5.80	8.5±1.73	4.25±1.70
	KUPWARA	7.25±2.21	6.25±1.70	3.25±0.95

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Prediction of Allergic Response of Goat Lactoperoxidase through AlgPred

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Received: 14 Feb 2020 Revised:16 Mar 2020 Accepted: 18 Apr 2020

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ABSTRACT

Allergy is a type of hypersensitive reaction which commonly induces the IgE (Immuno globulin E) antibody response. Allergens are small antigens in the form of pollen, foods or drugs that trigger the IgE antibody response. Goat (*Capra hircus*) is an important source of milk and milk products for human consumption. It contains many bio-compounds that could be useful in patients suffering from a variety of long standing diseases such as immunological reaction (allergy), metabolic disorders, cardiovascular disease, and neurological degeneration as well as promoting health of the intestine. It has also shown chemo-preventive properties for cancer. But before the use of goat milk, it is necessary to check an allergic response of the milk proteins. AlgPred is sophisticated bioinformatics software to predict allergic response through *in-silico* studies. For the present study, AlgPred was used to predict the allergic response of milk protein (enzyme) i.e. goat lactoperoxidase (GLPO) and compare it with cow lactoperoxidase (CLPO). The result was based on mapping of IgE epitopes, MAST, amino acid composition, dipeptide composition, allergen representative peptide and overall allergic response checked by hybrid approach. After observation of results, it was found that goat lactoperoxidase do not contain IgE epitopes, thus not an allergen as cow lactoperoxidase and have equally nutritive value as cow milk and could be used as an alternative source for the production of dairy foods in food industries in place of cow milk.

Key words: Allergy, AlgPred, Cow lactoperoxidase (CLPO), Epitopes, Goat lactoperoxidase (GLPO), IgE.

INTRODUCTION

Allergy or hypersensitivity is a series of complex reactions. Many types of external and internal factors contribute to the development of the allergy and involve in triggering the symptoms. Most common allergy (Type I hypersensitiity) is induced by certain types of compounds or chemicals known as allergens. Allergens are the emerging problem of modern society and related to body metabolism [1-4]. Homologous allergens come from different extrinsic and intrinsic sources and bind to specific IgE antibodies [5]. In immunology, epitope is a part of antigen or allergen which is bound to specific part of antibody (paratope) and responsible for allergic reaction. The major problem in case of allergen based research, is finding the specific epitope or responsible sequence which interact with IgE antibody in hypersensitivity reaction [6].

Currently, the findings of new epitopes are becoming more important due to increased cases of allergy and excessive use of genetically modified (GM) crops or biopharmaceutical products. Consumption of healthy food such as milk and milk products are increasing in demand. Due to intolerance and allergies against cow milk, people have increasing interest in consumption of goat milk as a substitute or alternative of cow milk. In developing countries due to shortage of cow milk, goat milk and products derived from it play a valuable daily food source which is rich with calcium, protein and phosphate [7]. Due to health benefits of goat milk, markets around the world are based on home utilization as well as medical needs [8]. More than 20 proteins or allergens are found in milk of cow that can cause allergic or hypersensitive reactions in human. Casein and lactoglobulin (lg) are the most common milk proteins found in cow milk which work as allergens. Human milk is free of lactoglobulin which is reported identical to camel milk. Milk protein caseins have different amino acid composition, fraction number and peptide mapping in different species. It is reported that casein is the major portion in goat milk and have similarity to human casein but not identical from casein of cow milk. Peptide mapping of goat lactoglobulin is completely different from the cow milk. Allergies or hypersensitive reaction due to milk proteins have been reported by many researchers due to cross-reactivity reactions. Although it is also reported in goat and sheep milk caseins. Allergy due to milk or milk products are based on cross-reactivity and level of symptoms can be diagnosed by skin test as well as blood tests. Heat and many enzymatic treatments are used to reduce some degree of allergenicity caused by cow milk protein. Hypersensitive or allergic reaction also depends on genetic polymorphisms of milk proteins. Several researches have accounted real and considerable advantages to using alternative sources of milk in case of cow milk allergy such as goat milk, camel milk, mare milk as well as soy milk and it can be reported hypoallergenic in many researches. However, it is also reported that therapeutic benefits depend on degree of seriousness of the allergic reaction [9].

In 1578, goat milk suggested as a tonic for the digestive system and many growth factors of goat milk have been described in many medical texts [10]. In one study, goat milk was considered to be possible nutraceutical for many types of gastro-intestinal disorders. Other than nutritional advantages, demand of goat milk is increasing because of easy digestibility than cow's milk and may have many therapeutic values other than gastrointestinal ailments such as hypoallergic properties, Anticarcinogenic activity, Lactoferrin Antimicrobial activity, Anti-thrombotic activity [11-13]. According to Park and Haenlein [8], milk of goat is differing from human and cow milk because of following characters that is highly nutritious for human immune system, easy digestibility and have therapeutic values equal to medicine. Many hospitals and medical practitioners suggested goat milk and milk product to patients. Lopez-Aliaga reported many benefits of goat milk comparative to cow milk in their study. He reported that contribution of goat's milk in regular diet in place of milk of cow; enhances the release of bile and help to decrease the concentration of plasma cholesterol. [14].

Milk and milk product is always high in demand and goat gets third rank in production of milk and have great contribution in supplying milk and milk products. It plays a remarkable role in the development of rural health and increase the economy. Goat milk contain higher amount of nutrient such as calcium, phosphors and magnesium

when compared to milk of cow and human. It contains high quantity of protein, unique fatty acid i.e. medium chain triglycerides (lipid) and interesting characteristics at the levels of flavor, taste and aroma. Because of this quality, goat's milk and milk products are reported beneficial for human health in many disease conditions such as gastrointestinal disturbances and ulcers in adult. It also plays an important role in prevention of allergy, cardiovascular disease and used to increase the immunity of infants and old people. Goat milk is also used to prepare soft curd which is reported beneficial by many researchers for human health and have been used in certain regions of the world. [15-16].

The nutritional value of milk protein depends on concentration of essential amino acids for any species. A few differences present between milk of different species at the level of essential amino acids, which mostly affect the total protein content [17-18]. It is reported that goat milk contains higher amount of essential amino acids as compared to cow milk such as phenylalanine, cystine, threonine, tyrosine, lysine, valine, leucine as well as nonessential glutamic acid and proline.[19]. The presence of Antibody (Ab) or Immunoglobulins (Ig) and other proteins such as lactoperoxidase, lactoferrin, and lysozyme are responsible for antimicrobial activity of milk. It is commonly accounted that the antibacterial effect of goat milk is greater than the individual contributions of defensive proteins that is immunoglobulin and non-immunoglobulin. Naturally present proteins and peptides in milk of different species works synergistically with active peptides that are released after the digestion of precursor molecules [20]. It has been reported by many researchers that β- casein present in large quantity of total casein of goat milk. Although it is found that alpha S- 2 (α-S2) casein is relatively higher in milk of goat than α-S1 content of cow milk. This kind of difference could help in explaining the characters of goat milk such as asoft curd formation, good digestibility and low allergic (hypersensitivity) reactions in children [21]. It is also accounted that antimicrobial activity in goat milk is also due to α-S2 casein after digestion through gastrointestinal pepsin enzyme [22].

Many studies showed that a complex network between cells of immune system i.e. interactions between B-lymphocytes, T- lymphocytes, dendritic cell and monocytes may be involved in hypertension [23]. The decrease level of this type of immune response increases the level of angiotensin II which simulate and promote hypertension by affecting dysfunction of vascular system. As a result, it is reported that hypertension is also associated with immune system and inflammatory system but it has many functional differences from other immunological diseases [24-25]. The importance of goat milk, milk products and goat meat around the world has been reported by many researchers [15]. The purpose of this research is to focus on the uniqueness and importance of goat milk and milk product as an alternative source of cow milk mainly in term of its non-allergenicity.

MATERIAL AND METHODS

The aim of present research is to check the hypersensitivity response of lactoperoxidase present in goat milk, which has attracted the interest of researchers worldwide.Dataset: The dataset used in this study were obtained from RCSB protein data bank. Two ID were selected from protein data bank. The selected PDB ID is as follows:

1. 2EFB: It is for goat lactoperoxidase [26].
2. 4NT3: It is for cow lactoperxidase [27].

AlgPred method: AlgPred is a bioinformatics tool used for test of allergic peptides present in food products and drugs [28]. The result was based on mapping of IgE epitopes, MAST based analysis, amino acid composition, dipeptide composition, allergen representative peptide and overall allergic response is checked by hybrid approach. Both proteins ID 2EFB and 4 NT3 were used to check allergic response of goat lactoperoxidase and cow lactoperoxidase respectively though Algpred.

RESULTS

Lactoperoxidase is naturally present enzyme in milk. Abacteriostatic effect is the specific biological function of lactoperoxidase in the presence of thiocyanate and hydrogen peroxide.The aim of this study was to predict allergenic response of goat milk lactoperoxidase particularly as food allergens. According to the guidelines of FAO/WHO 2003, multiple tests are recommended for predicting the allergenic characters of the particular proteins or enzyme present in food products and drugs rather than single test before release in market for human consumption [29-31]. AlgPred [28], plays an important role in pharmaceutical industry to check the allergenicity of any drug or enzyme which is used against any particular disease. Their allergenicity nature can be identified with the help of bioinformatics tool AlgPred. In AlgPred bioinformatics tools allergenicity is checked on the basis of following criteria:

- Reactivity with the mast cell
- Mapping of IgE epitopes
- Composition of amino acid
- BLAST
- Presence of dipeptides.

Based on result obtained goat lactoperoxidase (2EFB) is found to be non-allergen as compared to cow lactoperoxidase (4NT3). The allergic response of milk protein (enzyme) i.e. goat lactoperoxidase (GLPO) was compared with cow lactoperoxidase (CLPO) using AlgPred. The result was based on mapping of IgE epitopes which was found to be not present in goat milk, no hits found for MAST hence it was considered as non-allergen. On the basis of amino acid composition, goat lactoperoxidase was found to be non-allergen and score was 1.121522 and threshold value was -0.4. Analysis of dipeptide composition also showed non allergen with score of 1.3059997 and threshold value was -0.2. At the level of allergen representative peptide, no hits found means non-allergen properties and overall allergic response is checked by hybrid approach which also showed that goat lactoperoxidase is a non allergen. After observation of all results, it was found that goat lactoperoxidase was not an allergen as cow lactoperoxidase. It has equal nutritive characters as cow milk and could be used as an alternative source of production of dairy foods such as soft curd, probiotics and prebiotics in food industries. (Table 1)

DISCUSSION

It is frequently reported that cow milk is one of the most common food allergies found in children. Although most children overcome the problem of cow milk allergy but some retain the allergy for life time. Hence, there is continuous search for alternative milk with novel characteristics for industrial application [28]. Goat milk is a rich source of nutrients. Earlier it was reported by many researchers that goat milk has minimum allergenicity [32-33]. Apart from nutritional advantages of goat milk, it is also showed significant effects against colic, asthma, eczema and minor digestive disorders [34]. Thus used for a medicinal purpose. It is also used in place of cow milk for those people who have allergy from the cow milk and resolves 30-40% of childhood cow milk allergy. [12, 35]**.** It has evoked an attention in goat milk as an alternative functional healthy food. Today, goat milk is one of the healthy consuming foods in developed countries [36]. Numerous studies and many researchers suggested that cow milk contain more than 20 milk protein that cause allergy whereas milk of goat is less allergenic compared to cow milk hence it may a good alternative due to difference in protein structure mainly casein [37]. A number of factors directly or indirectly affect the immune system and nutrients present in any food items are the main factor or determinates to check the immune response of the body. Many types of cells are participates in immune system (innate and adaptive immune response) in which Natural Killer (NK) cells, T-lymphocytes (T-cells) and B-lymphocytes (B-cells) are the main components. Although immunoglobulin's (Ig) are similar in structure with a variety of biological properties, it is found that IgG and IgA have a maximum serum concentration of total immunoglobulin. Goat milk plays an important involvement in maximum immunological reactions and draw anti-

inflammatory and antioxidant effects in the immune system of the body. This is important because inflammatory response is one of the characters of the innate immunity against first time infections. Furthermore, it is essential to maintain healthy intestinal microflora with the help of food supplements such as probiotics and prebiotics manufactured from milk of goat. It was reported that probiotics and prebiotics are essential food supplements for protecting against the infection i.e. allergy caused by pathogenic organism [38].

On the other hand, milk of goat contains a higher content of variety of lipids including short and medium chain fatty acid as well as mono and polyunsaturated fatty acids compared to milk of cow. It is more digestible than the other bovine milk because in goat milk the globules of lipid are significantly smaller diameter than the cow milk. Concentration, composition and size of total fat globules affect the viscosity of milk and play an important role in processing and manufacturing of milk products [39]. From an immunological point of view, goat's milk has the capability to activate both immune responses (innate and adaptive) in the human system and also inhibited the endotoxin-induced activation of monocytes. Finally, goat milk has their own microbiota; some may balance the intestinal microbiota of human and shown protective effects at intestinal mucosal sites through activating intestinal T regulatory cells. Goat's milk should be recommended as a dietary supplement for patients who are suffering from inflammation and allergic conditions, including elder people. [40]. It was accounted that anti-bacterial and antiviral activity of goat milk is due to higher concentration of medium-chain fatty acids. It inhibits the development of cholesterol, helps in the break-down of cholesterol deposits and easily absorbed by the intestine [39, 41-42].

It is reported that milk of goat is commonly well tolerated by persons who suffer from an allergic or hypersensitive reaction against milk of cow due to its minimum levels of a protein called α S1-casein. There are many versions of casein protein found in milk of all species in which α S1-casein alleles is the main version present in mammalian milk. It depends on the versions of a gene in particular animal. α S1-casein is the main protein present in the milk of cow, but in case of goat's milk, β-casein is present that is different version of casein protein. Still, it was reported that goat's milk has widely varying levels of α S1-casein. Digested particles from alpha S1-casein protein passes through the gut wall of intestine and interact to IgE. This type of interaction triggers the symptoms of milk allergy or hypersensitivity because IgE is one of the antibodies that are involved in hypersensitivity [43].

Immunoglobulin and other proteins such as lactoperoxidase, lactoferrin and lysozyme are responsible for the antimicrobial activity of milk. It is commonly reported that the total antimicrobial properties of whole milk is greater than the individual effect of immunoglobulin and non-immunoglobulin defensive proteins [20]. It is also proven that the natural protein present in goat's milk work as precursor for antimicrobial activity, which is enhancing the capacity of natural defenses of milk protein to eliminate invading pathogenic microbes. As a result, natural proteins found in foods, can be contemplated as a component of nutritional immunity [44]. The peptide mappings of goat protein are totally different from cow milk [45]. According to some researches, goat's milk could be consumed by an individual who is suffering allergy from cow milk without negative effects. This is the main reason which promotes the benefits of goat milk in the market. However, some symptoms of allergy to goat milk appeared in late age [46]. It might be advantageous for infants who are completely dependent on milk as their primary source of nutrients.

Apart from this research, it is reported that cross-allergenicity between milk proteins of both i.e. cow milk and goat milk which can lead to serious complications. They also suggested that individuals who are suffering from allergy to cow's milk protein should avoid consumption of goat milk and products [47]. Allergy-related symptoms may develop in people consuming goat milk who have already shown an allergy to cow milk. Bevilacqua and co workers suggested that the reduced allergenicity of goat milk might be directly related to lower levels of α S1- casein. [32].
Results of some studies also indicate that oligosaccharides present in milk of goat, play an essential role in intestine safeguard and perform repairing mechanisms after destruction induce by colitis. [48].

CONCLUSION

Goat milk and products are considered as the dairy products with greatest medicinal values and marketing potential. It has many nutritional, therapeutic and medicinal values for human health and immune system and has many beneficial effects on many immunological disorders. Some proteins of goat milk have immunological cross-reactivity with proteins present in cow milk but there is no allergic reaction seems between lactoperoxidase of both species. Production of goat milk and milk product is very important in developing countries to support health and economic status of rural people, which is the major part of the population. The major advantage of AlgPred approach is that it allows the prediction of allergenic proteins with high sensitivity or specificity, where the user can select protein based on his requirement. If a protein predicted to be an non-allergen by these approaches has a high probability that it is an non-allergen. Further confirmation of allergic response and therapeutic significance based on clinical and nutritional trials on human subjects should be conducted.

ACKNOWLEDGEMENT

The authors are gratefully acknowledged to Amity Institute of Biotechnology, Amity University Madhya Pradesh for providing facilities during the work.

Conflict of Interest

The authors declare no competing or conflicts of interest.

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Table 1. Comparison between Goat and Cow Lactoperoxidase

S.No.	Properties	Goat Lactoperoxidase	Cow Lactoperoxidase
1.	Name of sequence	2EFB	4NT3
2.	Length of sequence	1186	595
3.	Prediction by mapping of IgE epitope	Not Contain	Not Contain
4.	MAST result	No hits found Non Allergen	No hits found Non Allergen
5.	Prediction by SVM method based on amino acid composition	Non Allergen a) Score - 1.121522 b) Threshold value - -0.4 c) Positive predictive value-15.19% d) Negative predictive value-94.18 %	Non Allergen Score - 1.1007437 Threshold value- - 0.4 Positive predictive value-15.19 Negative predictive value-94.18
6.	Prediction based on SVM method (dipeptide composition)	Non Allergen Score - 1.3059997 Threshold- -0.2	Non Allergen Score - 1.1749075 Threshold- -0.2
7.	Blast result for ARPS	No hits found Non Allergen	No hits found Non Allergen
8.	Prediction by hybrid approach	Non Allergen	Non Allergen

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Plants used for Beauty Care by the Tribals and Rural Folks of Odisha, India

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Received: 02 Feb 2020 Revised:04 Mar 2020 Accepted: 06 Apr 2020

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ABSTRACT

Over one hundred different plant species were identified in beauty-care practices among the Munda, Santal, Saora, Kondha, Juang and Kolha tribes inhabiting the Sundergarh, Dhenkanal, Angul, Jajpur, Balasore, Mayurbhanj, Gajapati, Keonjhar, Kandhamal and Koraput districts of Odisha. Some usages of plant parts or produce as hair-wash, hair dyeing, hair oils, as well as in scalp & skin-care and tooth & nail-care mentioned herein are already in practice in the modern society. A few others are, however, new and interesting and these may find application towards development of novel beautician preparations.

Key words: Plant resources, Herbal cosmetics, Tribes, Odisha

INTRODUCTION

Beauty is the quality present in a thing or person that gives intense pleasure or deep satisfaction to the mind or the senses, especially the sight. Every human in the world has a great eagerness to be looking beautiful. Some are naturally beautiful and some are made self beautiful by artificially. Beauty is not only limited in the women but also spread among the men. Men are also more aware towards their beautification [1]. Beauty increases confidence and proud in some extent. Both men and women are using many beauty products or cosmetics for enhancing their beauty and looking young and attractive. Cosmetics are the substances applied to the body with the intention of beautifying it. The word cosmetics exist from ancient mankind and civilization. From long time, plant parts are used as the source of food and medicine and also used for the preparation of cosmetics. In 'Ayurveda', the idea of using herbs for beautification is well defined. In India, the cosmetics preparations are used for worship and sensual enjoyment since 'Vedic' period. The external application of 'kajala', 'tilaka', 'aguru', 'chandana', 'haridra' etc. to God and Goddess are seen in many rituals of India [2]. Cosmetics drugs are classified as Kustagna, Vamya, Kandungna, Vayasthapak, Keshya etc. by 'Charaka Samhita' and many a lepam are described in 'Susruta Samhita' and 'Astanga Hridaya' [3]. Ayurveda is one of the most ancient traditional systems of medicine in India and South Asian countries. Total 1773 number of plants are indicated in Ayurveda in which main classics of Ayurveda viz. Charak samhita,

Susruta samhita and Astang Sangrah have detailed description of about 700 herbs [4]. Several types of herbs mentioned in Ayurveda for management of the aging and to obtain healthy skin. The literature of the Ayurveda described over 200 herbs, numbers of minerals and fats to maintain and intensify the healthy and beauty of the skin [5]. The Primitive ayurvedic system of medicine mainly depends upon the plant based materials. Herbs have been an essential part of the skin treatment and beautification of the external part of the body. 'Solah shringar', the concept found in Indian literature in which 16 modes of beautification from head to toe is mentioned. For this purpose single herb or herbal mixture is used [6].

Several herbs have been used for conserving and increasing the beauty of human since ancient time. Herbal cosmetics are also known as natural cosmetics. Herbal cosmetics products are formulated, using various permissible cosmetics ingredients to form the base in which one or more herbal ingredients used to provide defined cosmetics benefits only, shall be called as "Herbal Cosmetics" [7]. Due to less side effects and the skin friendliness nature of these herbal medicine, its demand is growing rapidly in the world market. The herbal cosmetics are generally made up of herbs and shrubs so they are found in pure form. The natural content in the herbs does not have any side effect on the human body with nutrients and other useful minerals [8]. The plants contain huge and complex arsenal of active elements (phytochemicals) that are able not only to calm or smooth the skin but actively restore, heal and protect the skin. Cosmeceuticals are topical cosmetics-pharmaceutical hybrids deliberated to increase the health and beauty through the constituents that influence the skin's biological function [9].

A well-groomed hair style coupled with a healthy skin luster and symmetric tooth profile enhances face value and admired in all ages. The history of skin care, hair dressing and application of essential oils to the scalp dates back perhaps to 3,500 B.C. The use of essential oils and combs on the scalp are a common practice in India. Body massages with essential oils and other plant produce for better complexion and health of the skin has been in vogue among the tribal and atribal people in India since prehistoric period. They employ a number of herbal cosmetics for the maintenance of fine luster of the skin and scalp protection for luxuriant hair growth. Besides, they also use some plants or their produce against certain skin and scalp infections causing diseases such as leucoderma, psoriasis, alopecia and the appearance of pimples, acne, black-head, white spots on face, stye, boils, warts and corns. Preventive herbal cures are also in practice among some ethnic population of the state for a number of very common disorders of the skin, hair and teeth to keep them healthy. Unfortunately, these important practices among the rural folks have not been properly recorded. Documentation is especially poor when it concerns the ethnic communities. Thus, an in-depth investigation is warranted to document and verify the efficacy of these valuable ethno-cosmetic prescriptions. Further, the verified herbal cosmetics could be considered as a viable and safe alternative to synthetic cosmetics, the former being free of known associated adverse effects.

Although a number of reports are available on ethnomedicine of different parts of Odisha [10-40], there is not a single published account on the ethnic uses of herbs in beauty care.The present communication deals with over one hundred different plant species which are used in preparations for skin-care, tooth & nail-care, hair & scalp-care by the tribal people of Odisha. The requisite information was obtained through personal interviews with Munda, Santal, Saora, Kondha, Juang and Kolha tribes, who live in the plateau of the hills and the forest areas of Sundergarh, Dhenkanal, Angul, Jajpur, Balasore, Mayurbhanj, Gajapati, Keonjhar, Kandhamal and Koraput districts of Odisha. The author studied the plants used by aboriginals for their food, medicine, shelter and other purposes during their ethnobotanical field work in Odisha (41-48). Along with these studies, plants used exclusively for hair and scalp preparations as well as for skin, tooth and nail-care have also been recorded. As vernacular names of the plants, which are so employed, varied from place to place, correct botanical nomenclature to tally with the morphological features of these plants were ascertained following the standard flora books (49-51). Of interest, this is the first ethnobotanical report concerning the use of herbal-cosmetics in the State of Odisha.

METHODOLOGY

Studies on tribal communities inhabiting the forest areas of Sundergarh, Dhenkanal, Angul, Jajpur, Balasore, Mayurbhanj, Gajapati, Keonjhar, Kandhamal and Koraput districts of Odisha were carried out to find out medicinal and cosmetic herbals used among them. Information was gathered through enquiries made with their village heads, medicine-men, old women and other tribal people during field trips. Tribal resource persons were taken to the forests as guide-cum-informant for collection of voucher specimens. Repeated queries were made to get the information confirmed. Information presented here includes the name of ailments, plant part(s) used, their botanical names and vernacular names and the mode of usage. Ailments covered include graying of hair, alopecia, hair fall, scalp sores, pimples, acne, warts, corns, rashes, leucoderma, psoriasis, boils, burns etc. Plants or their products which are used to maintain skin health have also been included. Voucher specimens were preserved and deposited in the Post Graduate Department of Botany, Utkal University, Vani Vihar, Bhubaneswar. An enumeration is presented in which the species have been arranged alphabetically with their correct botanical name, popular synonym (if any), name of the family to which it belongs, vernacular (local) name, with the mode of uses etc.

Abbreviations

B-Bhuyan, E-English, J-Juang, K-Kolha, Kondh-Kondha, M-Munda, O-Oram, Or. Oriya, S-Santal, Sans-Sanskrit, Sao-Saora

ENUMERATION

Skin care

***Abrus precatorius* L. [FABACEAE]**
Crab's eye/Indian liquorices (E); Gunja (Sans) Kaincha/Runja (Or); Gujjbai (Sao); Kawet(S); Karjani (K). Roots along with those of 'chitraka' (*Plumbago zeylanica*) are made into a paste and applied to cure leucoderma.

***Acorus calamus* L. [ARACEAE]**
Sweet flag (E); Vacha (Sans); Bacha (Or).
The rhizome (approx. 20 g) along with three black peppers (*Piper nigrum*) is applied on the affected part to remove rashes.

***Alangium salvifolium* (L.f.) Wang. [ALANGIACEAE]**
(*A. lamarkii* Thw.).
Sage-leaved alangium (E); Ankolah (Sans); Dhalanku/Ankula/Ankapali (Or); Akarkanta/Akel (Kondh); Dhela(S). Leaf paste mixed with cow's butter (2:1) is applied locally for ringworm-scars and erysipelas.

***Allium cepa* L. [LILIACEAE]**
Onion (E); Palanduh (Sans); Piyaja (Or)
The bulb is sliced and rubbed on the skin twice in a day for six months to cure white spots including leucoderma.

***Allium sativum* L. [LILIACEAE]**
Garlic (E); Lasunah (Sans); Rasuna (Or)
The clove is cut into half and the cut end is rubbed on the face to cure pimples. The juice of the clove is also applied externally on the affected part of the face to clear the white spots, pimples and boils.

***Aloe vera* (L.)Burm.f. [LILIACEAE]**
Indian aloe/Barbados aloe (E); Ghrita-kumari/ Kumari (Sans); Ghee-kuanri (Or); Ghee-kumar(S)
Leaf juice is applied on the face to remove pimples and scars and also used by some tribal community for facial make-up.

***Anacardium occidentale* L. [ANACARDIACEAE]**
Cashew-nut (E); Venamrah (Sans.); Lanka-amba/Kajubadam (Or)
Tar from seed coat and bark is applied on corns and warts for their disappearance.

***Alstonia scholaris* (L.) R.Br. [APOCYNACEAE]**
Devil's tree (E); Saptaparnah (Sans.); Chhatiana/Chhanchana (Or); Kanumung (K); Chatnia (S)
Bark poultice mixed in 'kanji' (fermented rice water) is applied externally to cure skin eruptions, pruritus and ringworm-scars. An infusion of the bark is given in approx. 25 to 50 ml doses twice or thrice a day for skin diseases such as eczema, acne and ringworm. The latex is also used externally to remove acne and pimples.

***Argemone mexicana* L. [PAPAVERACEAE]**
Mexican poppy, prickly poppy (E); Svarnakshiri/Brahmadanti (Sans.); Agara/Kantakusuma (Or); Sundi Satkeu (Kondh); Gokhula janum (S); Kari-kanta (K); Nyadudid (Sao)
The yellow latex of this plant is applied to cure blisters and lip ulcer.

***Barleria prionitis* L. [ACANTHACEAE]**
Sahachara/Saireyaka (Sans); Daskeranta (Or); Kanta-phul (S); Tamresa (Sao)
Leaf-paste is warmed and applied to cure pimples and boils**.**

***Bombax ceiba* L. [BOMBACACEAE]**
Red silk cotton tree (E); Salmali (Sans.); Simuli (Or); Edel (K, S); Leka/Dakang (Kondh)
The roots along with equal quantity of seeds of 'Apamarga' (*Achyranthes aspera*) is made into a paste and used to cure leucoderma. A paste made out of the prickles is applied for restoring skin colour especially on the face. The gum and bark paste is used to treat acne, pimples, boils, burns, facial twitching and also to improve the texture and vigour of the skin.

***Buchanania lanzan* Spreng. [ANACARDIACEAE]**
(*B. latifolia* Roxb.)
Almondette (E); Priyalah (Sans.); Char (Or); Tarub (K); Tarop (S)
Seed oil is used to remove the scars on the body. .

***Calotropis gigantea* (L.) R.Br. ex Ait. [ASCLEPIADACEAE]**
Swallow wort/ Giant milk weed (E); Arka (Sans.); Arakha (Or); Akaona (S); Pak (Sao); Akond (Bh)
Flower paste is applied over the acne and pimples for the cure.

***Carica papaya* L. [CARICACEAE]**
Papaya (E); Erandakarkati (Sans.); Amruta-bhanda (Or); Jhoda (K)
Ripe fruit is made into a paste and applied on the affected part before one hour of bath to remove black spots. This paste can be applied all over the body in the same manner to remove any spot or scar due to skin diseases especially ringworm and psoriasis.

***Cassia senna* L. [CAESALPINIACEAE]**
(*Cassia angustifolia* Vahl)
Indian senna (E); Svarnapatri (Sans.); Sunamukhi (Or);
The leaves are made into a paste and are used as a beauty aid in improving colour and texture of the skin.

***Ceiba pentandra* (L.) Gaertn. [BOMBACACEAE]**
[*Eriodendron pentandrum* (L.) Kurz.]
White silk cotton (E); Sveta salmali (Sans.); Dhala simili/Bilati simili (Or)
The unripe fruit & leaf juice is applied to improve the texture and colour of the skin.

***Centella asiatica* (L.) Urb. [APIACEAE]**
Indian pennywort (E); Mandukaparni / Brahmi (Sans); Thalkudi/Ghodatapua Brahmi (Or); Thalkuni (K); Dua-sag (S)
The entire plant juice is used externally as well as internally in the treatment of leprosy and psoriasis.

***Chenopodium album* L. [CHENOPODIACEAE]**
Lamb's quarters/Wild spinach (E); Vastukah (Sans); Bathua sag (Or); Bhatua-arak (S)
Leaf juice is applied over the affected parts twice a day for 15 days or till the disappearance of the white spot or leucoderma.

***Chrozophora rottleri* (Geis.) A. Juss [EUPHORBIACEAE]**
Bichutia (S)
The leaf paste is applied for removal of pimples and acne.

***Cicer arietinum* L. [FABACEAE]**
Bengal gram/Chick pea (E); Chanakah (Sans); Buta/Sola (Or); Maraijan (K); But (S)
The seed powder (approx.100 g) is mixed with turmeric powder (approx. 20 g) and mustard oil (approx. 50 ml) to prepare a semisolid paste. This paste is applied on the face for enhancing skin glow. It can be used on hands and legs also for the said purpose.

***Cinnamomum verum* Presl. [LAURACEAE]**
(*C. zeylanicum* Bl.)
Cinnamon (E); Tvak, Darusita (Sans.); Dalchini (Or); Daru-chini (S, Bath)
Paste of cinnamon powder prepared with a few drops of fresh lime juice is applied to remove pimples and black heads and to restore normal skin colour on the face.

***Cissampelos pareira* L. [MENISPERMACEAE]**
Patha/Ambashta (Sans); Akanabindhi (Or, S); Akanamuli (B, M), Pitu-sing (K); Telomalla (Sao); Tamalo (Kondh); Tejomela (S)
The roots are boiled in water and the boiled water is used for bathing the young girls to cure urticaria and pimples.

***Citrus medica* L. [RUTACEAE]**
Citron (E); Matulungah (Sans.); Lembu (Or); Nimbu (S)
The juice of the fruit mixed with equal amount of cow's milk is massaged on the face for a fair complexion and rosy cheek.

***Citrus aurantifolia* (Christm.) Swingle [RUTACEAE]**
(*C. aurantium* var. bigaradia Hook.f.)
Sour orange (E); Brihat jambirah (Sans); Bara nimbu (Sao)
The rind of the fruits is sun-dried, powdered and mixed with water to prepare an ointment and applied on the affected part of the face to remove white and black spots.

***Cleome viscosa* L. [CAPPARIDACEAE]**
Wild mustard/Sticky cleome (E); Pasugandha (Sans); Anasorisha (Or); Chamani (K); Harhara (S)
Decoction of powdered plant is applied in the treatment of pimples and boils.

***Clerodendrum serratum* (L.) Moon [VERBENACEAE]**
Bharngi (Sans.); Kharkhari/Kanjakra (Or), Samarkani (S), Saram lutur (M, K), Neta (Sao)
Root paste mixed with warmed castor oil is massaged on the breast against breast abscess.

***Clitoria ternatea* L. [FABACEAE]**
Clitoria (E); Aparajita/Girikarnika (Sans); Aparajita (Or)
About 0.5 g ash of whole plant is given with equal amount of butter (cow's) once a day for one month against acne on the face.

***Coriandrum sativum* L. [APIACEAE]**
Coriander (E); Dhanyakam (Sans); Dhania (Or)
A teaspoon of coriander leaf juice, mixed with a pinch of turmeric powder, is an effective remedy for pimples, blackheads, erysipelas and dry skin. The mixture should be applied to the face, after washing it thoroughly, every night before retiring. The leaf juice is massaged on the lips for soft and rosy colour.

***Crataeva magna* (Lour.)DC. [CAPPARACEAE]**
(*C. reloigiosa* sensu Dunn.)
Three-leaved caper (E); Varunah (Sans); Baruna (Or, S), Rairangi (Ho)
Bark paste mixed with goat milk is used against skin eruptions and also as a facial cream.

***Cucumis sativus* L. [CUCURBITACEAE]**
Cucumber (E); Trapusah/Sukasa (Sans); Kakudi (Or); Kira (M)
Fruit paste is applied as a face pack or eye pack for removal of dark lines.

***Curcuma longa* L. [ZINGIBERACEAE]**
Turmeric (E); Haridra (Sans.); Haladi (Or); Holdi (Kondh); Haldi (Sao)
Turmeric powder alone or combined with the pulp of 'neem' leaves is used in skin diseases such as eczema, ringworm, pruritus etc.

***Dillenia indica* L. [DILLENIACEAE]**
Bhavya (Sans); Oou/Rai (Or); Korkotta (K, S); Korkot (S)
The slimy juice of the carpels (about 1 table spoonful) mixed with black pepper powder (1 g) is massaged gently on the face to cure pimples and boils.

***Eclipta prostrata* (L.)L. [ASTERACEAE]**
Trailing eclipta (E); Bhringarajah (Sans); Kesadura/Kesuta (Or); Kamri (Kondh)
Leaf juice mixed with the leaf juice of *Dolichos lablab* is used for tattering hand and body of the tribal women.

***Euphorbia antiquorum* L. [EUPHORBIACEAE]**
Triangular spurge (E); Vajrakantakah/Tridharah (Sans); Dokan-siju (Or); Etke (S)
The plant juice is used against heel cracks (a condition known as 'Kibes') and warts.

***Euphorbia hirta* L. [EUPHORBIACEAE]**
(*E. pilulifera* auct. non L.)
Dugdhika (Sans.); Chitakutei/Hariharika (Or); Pusitoa (K, S)
The latex of the plant is used in the treatment of warts and corns. The milky juice of the plant is applied on cracked / chapped of lips, nipple and tongue.

***Euphorbia nivulia* Buch-Ham [EUPHORBIACEAE]**
Nivulia (Sans); Kala siju (Or, B);
The latex is warmed and massaged on skin to sooth cracks due to dryness.

***Ficus benghalensis* L. [MORACEAE]**
Banyan (E); Vatah (Sans); Bara (Or); Bare (M, S); Bia (K); Bandang (Kondh)
The crack of the heels is filled with the sap of the tree especially during winter season as a remedial measure. The latex is also applied to cure bruises and erysipelas.

***Hemidesmus indicus* (L.) R.Br. [PERIPLOCACEAE]**
Indian sarsaparilla/Country sarsaparilla (E); Anantamulah/Sariba (Sans); Anantamula/Sugandhi-lai/Chemari-baramula (Or); God mela (K); Gargeri (Sao); Dudhilibutta (Bondo)
The root is made into a paste with cow's ghee and applied on the affected part to cure pimples. The root-paste, root-powder and root decoction are also used against pimple and acne. The root paste with cow's butter is applied to the face as a beauty aid for improving colour and texture.

***Holoptelea integrifolia* (Roxb.) Planch. [ULMACEAE]**
Indian elm/ Kanju (E); Putikaranjah/ Chirabilva (Sans.); Dhau-ranga/Pittala (Or); Chorla (M); Charha (S)
Latex in diluted form is applied to remove hairs on the face of some girls developed due to hormonal imbalance.

***Jasminum sambac* (L.) Ait. var. sambac [OLEACEAE]**
Arabian jasmine (E); Mallika (Sans); Malli (Or); Moghru (S)
Leaf coated ventrally with butter kept on the eye overnight to cure stye.

***Lawsonia inermis* L. [LYTHRACEAE]**
(*L. alba* Lam.)
Henna, Egyptiana priven (E); Medhini, Madayantika (Sans); Manjuati (Or); Bind/Mindi (M)
The leaf paste is applied for staining nails, foot and palm and hair of ladies especially during wedding. Leaf juice is used as prophylactic against scabies and leucoderma.

***Linum usitatissimum* L. [LINACEAE]**
Linseed/Common flax (E); Atasi (Sans); Alasi/Phesi (Or, Bath); Unchi (K)
An equal part of linseed oil and lime water mixed together is used for removing blemishes from the face.

***Madhuca indica* Gmel [SAPOTACEAE]**
(*Bassia latifolia* Roxb.)
Butter tree/Mohwa tree (E); Madhukah (Sans); Mahula/Tola-gachha (Or); Natikam (K); Aba (Sao); Mahua (Kondh)
The seed oil is applied as ointments to prevent crack in the skin during winter. The seed paste is massaged on the body one hour before bath to improve the texture and vigour of the skin.

***Murraya koenigii* (L.) Spreng. [RUTACEAE]**
Curry leaf tree (E); Kaidaryah/Surabhi-nimba (Sans); Bhurusunga/Merasinga (Or); Puspa (Bond); Mirsinga (Kondh)
Leaf poultice applied over the affected areas to treat burns, bruises, and pruritus and skin eruptions.

***Musa paradisiaca* L. [MUSACEAE]**
(*M. sapientum* L.)
Kadali, Plantain (E); Kadali (Sans, Or); Kadal (Sao)
A ripe banana is crushed and the mash is applied on the face and neck for fading away the tan colour of the face and neck and is used as a skin whitener.

***Oxalis corniculata* L. [OXALIDACEAE]**
Indian sorrel (E); Changeri/Amlika (Sans); Ambiliti/Kumari (Or); Tandi/Chatam arak (S)
Leaf juice is locally applied to cure warts, corns and other excrescences of the skin. The extract of the whole plant mixed with onion juice (1:1) is also applied to remove warts. The juice mixed with black pepper powder and ghee gives relief from red spots and eruptions on the skin caused by biliousness.

***Pandanus fascicularis* Lam. [PANDANACEAE]**
(*P. tectorius* auct. non Sol. ex Parkin.)
Screw pine/Umbrella tree (E); Ketakah (Sans); Kiya (Or, M)
The flower paste is applied locally to cure skin eruptions, pruritus and leucoderma.

***Phyllanthus emblica* L. [EUPHORBIACEAE]**
(*Emblica officinalis* Gaertn.)
Emblic myrobalan/Indian gooseberry (E); Amalaki/Dhatri (Sans); Dhatri-anla/Anla/Aenla (Or); Meral(S); Ener (Sao)
A paste made from fruits of this tree and seeds of groundnut (*Arachis hypogea*) along with lemon juice and petals of rose is applied as a lotion against dry skin.

***Pongamia pinnata* (L.) Pierre [FABACEAE]**
(*P. glabra* Vent.)
India beech/Pongam oil tree/Karanj (E); Karanjah (Sans); Karanja (Or); Kamu (Sao); Karonjo (Kondh); Kuruin (S)
Leaf juice is used against acne, pimples, skin eruptions and rashes.

***Raphanus sativus* L. [BRASSICACEAE]**
Radish (E); Mulika (Sans); Mula (Or); Morai (K)
The seeds are made into a paste with curd and applied to cure leucoderma.

***Santalum album* L. [SANTALACEAE]**
Sandal wood (E); Chandanah (Sans); Chandan (Or); Gondassaro(S)
Sandal oil mixed with twice its quantity of mustard oil is used for removing pimples. The paste of the sandal wood along with human saliva is applied on the affected part to cure sty and pimples. The wood paste is also applied locally to cure herpes.

***Saraca asoca* (Roxb.) de Wilde. [CAESALPINIACEAE]**
(*S. indica* L.)
Asoka (E); Ashoka (Sans); Asoka (Or, S)
Flowers and young fruits are made into a paste and applied over the body to improve the texture of the skin.

***Schleichera oleosa* (Lour.) Oken [SAPINDACEAE]**
(*S. trijuga* Willd.)
Lac tree/Ceylon oak (E); Raktamrah/Mukulakah (Sans); Kusum (Or, B, S); Baru (K, S); Kolumu (Kondh); Kosangi (Sao)
The seed oil is used for skin diseases especially for eczema, acne and scald.

***Sesamum indicum* L. [PEDALIACEAE]**
(*S. orientale* auct. non L.)
Sesame/Gingely (E); Tilah (Sans); Tila/Khasa (Or); Tilming (K); Pitilme (Kondh)
The seeds with equal amount of seeds of 'masur' (*Lens culinaris*) are made into a paste with fresh cow's milk and applied over the pimples for its cure. Oil is applied on affected parts with dryness of the skin and leucoderma.

***Solanum nigrum* L. [SOLANACEAE]**
Black night-shade (E); Kakamachi (Sans); Nunununia (Or); Phut-phutia (S)
The juice can be applied locally on the affected parts in chronic skin diseases such as acne, eczema and psoriasis. A juice or poultice of leaves can be effectively applied on eruptive skin diseases, whitlow and burns. The decoction of the berries and flowers is applied against erysipelas.

***Symplocos racemosa* Roxb. [SYMPLOCACEAE]**
Lodh tree (E); Lodhrah (Sans); Lodha (Or); Ludum (K); Lodam (S)
Leaves are made into a paste with equal amount of the rhizome of turmeric and juice of a lemon and are applied on the face for 10 to 15 days to remove black spots.

***Terminalia arjuna* (Roxb. ex DC) Wt. & Arn. [COMBRETACEAE]**
White murdah/Arjun tree (E); Arjunah (Sans.); Arjuna/Akha-gachha (Or); Kowa (K); Kahua (S)
An ointment made by mixing the bark powder and honey is applied over the affected area to treat acne.

***Terminalia chebula* Retz. [COMBRETACEAE]**
Black myrobalan/Chebulik myrobalan (E); Haritaki/Abhaya (Sans); Harida (Or); Rola (K, S); Kakra (Sao)
The fruit paste is applied to cure boils, pimples and acne.

***Urginea indica* (Roxb.) Kunth. [LILIACEAE]**
Indian Squill (E); Vanapalandu (Sans); Bana-piaja/Bana-uli (Or); Ban uli (S)
The powder of the bulb is applied locally on the affected parts to remove warts and corns.

***Vanda tessellata* (Roxb.)Don. [ORCHIDACEAE]**
(*V. roxburghii* R.Br.)
Rasna (Sans, Or); Malanga (Or); Dare sanki (S); Japa (M); Gaccho janaya (K)
The root paste is applied on the affected part to remove white spots (locally known as 'Bayasa-chhau') on the face caused due to tono virus infections.

***Ziziphus mauritiana* Lam. [RHAMNACEAE]**
[*Z. jujuba* (L.) Gaertn. non Mill.]
Indian jujube (E); Badarah (Sans); Bara koli (Or); Janumjan (K); Bodari (M); Dedaori janum (S); Koli (Sao)
A paste of the leaves and twigs is applied on painful boils of the face and stye for the immediate cure.

Scalp & Hair Care

***Abrus precatorius* L. [FABACEAE]**
Crab's eye/Indian liquorices (E); Gunja (Sans) Kaincha/Runja (Or); Gujjbai (Sao); Kawet(S); Karjani (K)
Roots along with those of 'chitraka' (*Plumbago zeylanica*) made into a paste and applied to promote hair growth in areas of scattered hair fall.

***Acacia sinuata* (Lour) Merril. [MIMOSACEAE]**
(*A. concinna* (Willd.) DC.)
Soap nut-acacia (E); Saptala (Sans); Bon-ritha gacha (S); Sikakai, Sikaya, Badiphula (Or, M)
The fruit paste is used to wash hair as anti-dandruff and hair conditioner. A paste of sun-dried fruits, 'methi' (*Trigonella foenum-graecum*) seeds along with root powder of 'khaskhas' (*Vetiveria zizanioides*) is used as a hair-wash to remove dandruff and making hair soft.

***Argemone mexicana* L. [PAPAVERACEAE]**
Mexican poppy/prickly poppy (E); Svarnakshiri/Brahmadanti (Sans); Agara/Kantakusuma (Or); Sundi-satkeu (Kondh); Gokhula janum (S); Odasamari/Kari-kanta (K); Nyadudid (Sao)
The latex is massaged on the head to get rid off dandruff.

***Azadirachta indica* A.Juss [MELIACEAE]**
Neem tree/Margosa (E); Nimbah (Sans); Limba/Nimba (Or, S); Ninba (Sao, Kondh, S)
Sun dried fruits are powdered and made to a paste with sesame oil (*Sesamum indicum*) which is massaged on bald head for hair regeneration.

***Bauhinia vahlii* (Wt. & Arn.) Benth [CAESALPINIACEAE]**
Shiali (Or); Sehari (Sao, Kondh); Gairtula (Kondh); Jom-lar (S)
Mucilage obtained after peeling of fresh bark is used as an adhesive by tribal-women for fixing the hair.

***Buchanania lanzan* Spreng. [ANACARDIACEAE]**
(*B. latifolia* Roxb.)
Almondette (E); Priyalah (Sans); Chara (Or); Tarub (K); Tarop (S)
The seed oil is massaged on the scalp to promote hair growth on bald head and prevent baldness.

***Cassia occidentalis* L. [CAESALPINIACEAE]**
(*Senna occidentalis* Roxb.)
Stinking weed/ Eastern senna/Foetid cassia (E); Kasamardah (Sans); Bana-chakunda/Chhota-chakunda/Kala chakunda (Or); Kasinda/Kurtasakunda (Sao); Goru (Kondh); Chakodara (K)
Mixture of leaf and seed pastes applied to cure scalp sores.

***Centella asiatica* (L.) Urb. [APIACEAE]**
Thalkudi (Or, B, J); Brahmi (S, M);
Leaves (3-4 nos.) are boiled in sesame (*Sesamum indicum*) oil (about 20 ml) and rubbed on scalp for better hair growth.

***Citrus medica* L. [RUTACEAE]**
Citron (E); Matulungah (Sans); Lembu (Or); Nimbu (S)
Seed paste is applied on head to check hair fall.

***Dillenia indica* L. [DILLENIACEAE]**
Bhavya (Sans); Oou/Rai (Or); Korkotta (K, S); Korkot (S)
The mucilage of the carpels with fresh turmeric (2:1) is applied as a paste to clean dandruff and lice and also for the luxuriant hair growth.

***Bacopa monnieri* (L.) Penn. [SCROPHULARIACEAE]**
[*Herpestis monniera* (L.) Kunth.]
Thymed leaved gratiola (E); Brahmi (Sans); Brahmi, Panikundi (Or)
Leaf juice is massaged regularly on the head one hour before bath for strengthening and blackening the hair.

***Cocculus hirsutus* (L.) Diels [MENISPERMACEAE]**
Broom creeper/Ink berry (E); Patalagarudah/Chilihindah (Sans); Musakani /Dahidayia (Or); Kamane-maradari (Kondh)
The green curd like leaf-extract is applied on scalp for curing eczema and softening the hair.

***Curcuma longa* L. [ZINGIBERACEAE]**
Turmeric (E); Haridra (Sans.); Haladi (Or); Holdi (Kondh); Haldi (Sao)
A paste of fresh rhizome is applied on scalp for hair growth.

***Eclipta prostrata* (L.) L. [ASTERACEAE]**
[*E. alba* (L.) Hassak.]
Trailing eclipta (E); Bhringarajah (Sans); Kesadura/Kesuta (Or); Kamri (Kondh)
Leaf juice mixed with castor oil is applied on scalp for strengthening of hair and also used as a preventive measure against premature graying of hair and alopecia. A preparation of warm sesame oil with plant paste (2:1) is applied to promote luster and growth of hair.

***Enhydra fluctuans* Lour. [ASTERACEAE]**
Hilamocha (Sans); Hidimichi (Or); Harakuch (S)
Leaf juice mixed with mustard oil is rubbed gently on scalp daily for a month to delay graying of hair and prevent hair fall.

***Gloriosa superba* L. [LILIACEAE]**
Malabar glory lily (E); Langali/Visalya (Sans); Pancha-angulia/Khandaphula/ Langalangulia (Or); Jagara (M); Lauri-kuli (Kondh); Bunumki-chung (K); Som (S)
A preparation is made of dried tuber powder preserved in 'kusum oil' and applied on scalp for removing dandruff and preventing hair fall. The tuber is made in to a paste with cow's urine and applied one hour before bath against alopecia.

***Gmelina arborea* Roxb. [VERBENACEAE]**
White teak/Comb tree/Gumbar tree (E); Gambhari (Sans); Gambhari/Bhadraparni (Or); Gumna (Kondh); Kasmar (K, S); Gumher (K)
The fruit pulp fried in coconut oil is applied on the head for better hair growth.

***Hibiscus rosa-sinensis* L. [MALVACEAE]**
China-rose/Shoe flower/Chinese hibiscus (E); Japa/Ondrapuspi (Sans); Mandara (Or); Joba-gacha (Sao, Kondh)
The flower paste is massaged on the head to postpone graying of the hair and to check hair fall due to high fever during typhoid and malaria.

***Jatropha curcas* L. [EUPHORBIACEAE]**
Purging nut/Physic nut (E); Dravanti (Sans); Baigaba (Or); Jaradumba (Kondh); Kula jara (K); Bhernda (S); Tokabindi (M)
The latex is applied in several scalp diseases.

***Laportea interrupta* (L.) Chew [URTICACEAE]**
[*Fleurya interrupta* (L.) Gaud.]
Ghoda-bichhuati (Or); Pita-marai (Kondh)
Crushed fresh leaves applied with 'neem' oil on the scalp to arrest falling of hair.

***Lawsonia inermis* L. [LYTHRACEAE]**
Henna/Egyptiana priven (E); Medhini/Madayantika (Sans); Manjuati (Or); Bind/Mindi (M)
Tribal women use leaf extract on fore head and for hair dyeing on religious and festive occasions. Root and leaf-paste are also applied for promoting lustrous hair growth and preventing premature graying.

***Linum usitatissimum* L. [LINACEAE]**
Linseed/Common flax (E); Atasi (Sans); Alasi/Phesi (Or, Bath); Unchi (K)
Seed oil is applied for luxuriant growth of hair.

***Madhuca indica* Gmel. [SAPOTACEAE]**
(*Bassia latifolia* Roxb.)
Butter tree/Mohwa tree (E); Madhukah (Sans); Mahula/Tola-gachha (Or); Natikam (K); Aba (Sao); Mahua (Kondh)
Decoction of dried flowers is applied on hair for a blackening and strengthening effect.

***Murraya koenigii* (L.) Spreng. [RUTACEAE]**
Curry leaf tree (E); Kaidaryah/Surabhi-nimba (Sans); Bhurusunga/Merasinga (Or); Puspa (Bond); Mirsinga (Kondh)
About 2-3 curry leaves in boiled coconut oil (approx.100 ml) is used as an excellent hair tonic to retain natural pigmentation and stimulate hair growth. Leaves are also prescribed in the shape of chutney or juice once in a day for one month to promote hair growth and prevent premature graying.

***Musa paradisiaca* L. [MUSACEAE]**
(*M. sapientum* L.)
Plantain (E); Kadali (Or, S, M, K).
Burnt leaf ash is applied to the head before bath to kill lice.

***Nicotiana tabacum* L. [SOLANACEAE]**
Tobacco (E); Tamakhuh (Sans); Tamakhu/Dokta (Or); Tamuk (Kondh, Sao)
Decoction of the dried leaf powder is applied on scalp to kill lice and dandruff.

***Phyllanthus emblica* L. [EUPHORBIACEAE]**
(*Emblica officinalis* Gaertn.)
Emblic myrobalan/Indian gooseberry (E); Amalaki/Dhatri (Sans); Dhatri-anla/Anla/Aenla (Or); Merel(S); Ener (Sao)
Fruit-rind is sun-dried, powdered and mixed with sesame oil (1:2) to be used as a hair growth promoter and as a hair stainer.

***Pongamia pinnata* (L.) Merr. [FABACEAE]**
(*P. glabra* Vent.)
India beech/Pongam oil tree/Karanj (E); Karanjah (Sans); Karanja (Or); Kamu (Sao); Karonjo (Kondh); Kuruin (S)
The seed oil is recommended against any type of scalp infection.

***Ricinus communis* L. [EUPHORBIACEAE]**
Castor (E); Erandah (Sans); Jada/Gaba (Or); Banda (Sao, Kondh)
The oil extract from the seeds is used as hair tonic to ensure dark, strong and lustrous hair.

***Rubia cordifolia* L. [RUBIACEAE]**
Indian Madder (E); Manjistha (Sans, O); Katasingi (Or); Kuramadu (S)
The seed oil is massaged to prevent early graying of hair and also to provide extra strength and shine to the hair.

***Sesamum indicum* L. [PEDALIACEAE]**
(*S. orientale* auct. non L.)
Sesame, Gingely (E); Tilah (Sans); Tila/Khasa (Or); Tilming (K); Pitilme (Kondh)
The seed oil is used for promoting hair growth. Decoction of leaves and roots are used as a hair-wash.

***Solanum violaceum* Orteg [SOLANACEAE]**
(*S. indicum* auct. non L.)
Poison berry (E); Brhati/Simhi (Sans); Denga-bheji/ Labenkudi (Or); Anjed (K)
The fruit and root paste are used against alopecia**.**

***Strychnos nux-vomica* L. [LOGANIACEAE]**
Nux-vomica/Snake wood (E); Karaskarah/Visha-mushti (Sans); Kochila (Or, S); Kara (M)
Decoction of dried seed powder is applied on the scalp against dandruff and premature hair drop.

***Terminalia bellirica* (Gaertn.) Roxb. [COMBRETACEAE]**
Belleric myrobalan (E); Bibhitakah (Sans); Bahada (Or, S); Dera (Ma); Lupurg (K); Lopong (S)
The fruit juice mixed with the sesame oil is applied regularly for 1-2 months to delay graying of hair.

***Tragia involucrata* L. [EUPHORBIACEAE]**
Indian stinging-nettle (E); Vrischikali/Dusparsa (Sans); Bichhuati (Or); Sengelsing (S, K); Janagatar (Sao); Jipenda (Ho)
The roots are made into a paste with country liquor and applied for thick hair growth and also arrest hair loss.

***Trichosanthes tricuspidata* Lour. [CUCURBITACEAE]**
[T. bracteata (Lam.) Voigt.]
Kakanasa/Mahankala (Sans); Mahakala (Or); Kaubutki (K)
The oil extract of seeds is used to stop early graying of hair.

***Tridax procumbens* L. [ASTERACEAE]**
Bisalyakarani (Or); Basal gacha (Kondh); Kulal puduga (M)
The leaf juice is massaged as a lotion on the shaved head for luxuriant hair growth.

***Vitex negundo* L. [VERBENACEAE]**
Five-leaved chaste tree (E); Nirgundi (Sans); Begunia/Nirgundi (Or); Languni (Sao); Sinduari (S, K); Sursing (Ho); Huri (M)
The decoction of the leaf and bark is used for washing the sore on the scalp.

***Ziziphus mauritiana* Lam. [RHAMNACEAE]**
[*Z. jujuba* (L.) Gaertn. non Mill.]
Indian jujube (E); Badarah (Sans); Bara koli (Or); Janumjan (K); Bodari (M); Dedaori janum (S); Koli (Sao)
About 10-15 leaves are ground with the young fruits of lemon (*Citrus medic*a) and this paste is massaged on the head to prevent premature hair fall.

Tooth & Nail Care

***Acacia nilotica* (L.) Delile [MIMOSACEAE]**
[*A. arabica* auct. non (Lam.) Willd.]
Indian gum Arabic tree/Black Babultree (E); Barburah (Sans); Baburi/Babur (Or, S); Babla/Gabla (S, K)
Fresh bark is regularly chewed for effective cleaning of dirty teeth. This also helps to strengthen loose teeth and arrest bleeding from gum.

***Citrus medica* L. [RUTACEAE]**
Citron (E); Matulungah (Sans); Lembu (Or); Nimbu (S)

Nails of the toes and fingers are soaked in fruit juices once in every month for enhancing their glossiness and strength.

***Ficus benghalensis* L. [MORACEAE]**
Banyan (E); Vatah (Sans); Bara (Or); Bare (M, S); Bia (K); Bandang (Kondh)
Aerial roots are used for preventing teeth and gum disorder. Chewing the root-stick and brushing cleanses and strengthens the teeth and gum.

***Jatropha gossypifolia* L. [EUPHORBIACEAE]**
Purging nut (E); Baigaba/Chhota-lakajada (O)
Stem and petiole sap is applied on the affected part of the nail against fungal infection.

***Mimusops elengi* L. [SAPOTACEAE]**
Bullet-wood tree (E); Bakulah (Sans); Baula/Bakula (O); Pal (Sao)
Bark powder is massaged on the gum or the twig is used as a tooth brush to strengthen the gum as well as the teeth.

***Ocimum sanctum* L. [LAMIACEAE]**
Holy basil/Sacred basil (E); Tulasi (Sans, Or)
Sun-dried leaves in powder form are used for brushing teeth. It can also be used as a tooth paste when mixed with mustard oil and is proven against pyrrhoea and other dental disorder.

***Solanum virginianum* L. [SOLANACEAE]**
(*S. xanthocarpum* Schrad & Wendl.)
Xanthocarpum/Yellow berried Nightshade (E); Kantakari (Sans); Ankaranti/Bheji-baigana (O); Rangani janum (S)
Fruit decoction is used for gargling against diseases of gums and teeth.

CONCLUSION

The study reveals the uses of over 100 different plant species in beauty-care among tribal communities living in the ten selected districts of Odisha. Most of these plants possess curative properties addressed to restore and enhance beautifying factors related to hair, face, skin, teeth and nail. It is interesting to note that some of these were not reported earlier as to their use neither in indigenous phytotherapy nor in modern beauty-care. The findings embodied in this paper indicated that the age-old knowledge of primitive people on cosmetic use of plant parts or extracts could provide a good deal of scope or clue in discovering new or less known sources of cosmetic ingredients towards development of novel beautician preparations. Needless to overemphasize that there has been a growing consciousness worldwide especially in the developed countries and, of late, in India for use of plant-based cosmetics with little associated adverse effects. It would not be surprising, therefore, to predict that in the forthcoming days herbal cosmetics will be the global choice by which the synthetic chemical-based cosmetics would eventually be replaced.

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Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Nephrotoxicity and its Management using Herbal Drugs

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Received: 13 Feb 2020 Revised:15 Mar 2020 Accepted: 18 Apr 2020

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ABSTRACT

The nephrotoxicity is the common kidney problems like to similar that the other organ toxicities like hepatic, lung and gastritis. If certain chemicals and drugs has been administered at over dose or routinely administered which can cause the nephropathy, hyperuricaemia and other problems. The drug and the chemicals mainly produce the nephrotoxicity for the elimination function of kidney has been injured the nephron cells and the renal tubules. Drugs such as antineoplastic and antibiotics are highly causing neprotoxic. Some medicinal herbs and it extracts has been used for the nephroprotective agent they have overcome the nephrotoxicities. It plays different mechanism of the drug, inducing nephrotoxicity. In this review we explain various medicinal plants are having a nephroprotective activity against various nephroprotoxins.

Keywords: Nephroprotective, Kidney, Creatinine, Nephrotoxins, Medicinal Plants.

INTRODUCTION

The kidney is the vital organ of human which plays a major role in the metabolism and excretion function in human endocrine, blood pressure and acid base balance. It also serves a major function in the urine formation, enzymes, hormones, water and electrolyte balance. [1] Kidney maintains hormones, vitamin-D, erythropoietin and vitamin D and some other main functions in our body. Nephrotoxicity is the common kidney problem functionally decrease the urine concentrating capacity, lowering the glomerular filtration rate and increase the creatinine level of urine and blood.[2] Avoiding the nephrotoxicity by administering the non-nephrotoxic drug and adjusting the dose level and by monitoring the renal functions. The various compounds will expose the kidney such as environmental pollutants, Reactive Oxygen Species (ROS) and Carbon Tetrachloride (CCl_4). [3]

Physiology of kidney

Pair of kidneys are reddish pea-nut shaped organs around 10-12cm length, 5-7cm wide, 3 cm thick and 135-150g in weight. [4] The kidneys lie on the rear abdominal wall, one on either side of vertebral column, below the peritoneum and diaphragm below. They stretch from the 12th thoracic vertebrae to 3rd lumbar vertebra. [5] At the concave border base, there is a deep vertical fissure called the renal hilum, from which the ureter and blood arteries, lymphatic vessels and nerves arise from the kidney.
Kidney has consist of millions of nephron cells, these are

- Bowman capsule
- Proximal convoluted tube (PCT)
- Loop of henle
- Distal convoluted tube

Functions

In average adult kidney receives 1.2-1.3 litres blood /mins in average adult glomerular filtration rate is 120ml/min in collecting duct the blood was ultra filtrated and it's maintain the body electrolyte balance by anti diuretic hormone. Hormones are restored and maintained. It has been eliminate the metabolites and toxic substance in kidney to maintain the body health. [6]

Types of Nephrotoxicities

- Acute renal failure (ARF)
- Chronic renal failure (CRF)

Acute renal failure (ARF)

It refers to sudden reversible loss of renal function which develops over a week or days. The various causes will induce the acute renal failure such as the acute tubular necrosis which occurs at ischemic and toxins. The toxins are exogenous or endogenous like cyclosporines, antibiotics and chemotherapeutic drugs.

Pre renal failure
It's due to under perfusion of kidney affected for 21% of ARF. It can be recovered after taking an appropriate therapy.

Post renal failure
The post renal failure was caused by the obstruction of urinary tract. It is accounted or 10% of cases.

Intrinsic renal failure
It is caused by diseased parenchyma cells. And it is accounted for 69% among the renal causes is acute tubular necrosis ATN occurs due to either ischemic or toxins. Toxins are exogenous and endogenous substance like, diabetes mellitus,[7, 8] anti hypertensive drugs and illegal abortifacients.

Chronic renal failure (CRF)

It is an irreversible loss of excretory and metabolic functions of endocrine. It will develop over a period of year.[9] Various causes of renal failure have been developed such as the diabetes mellitus and hypertension.[10] Some anti-

neoplastic agents like cyclosporine, vincristine and cisplatin [11] etc. Decline the kidney function for 3 months or more to the evidence of kidney damage has been reducing the glomerular filtration[12] rate ≤ 60mL/1.73m^2.

Nephrotoxicity Causing Agents

The drugs and chemicals are the well known nephrotoxic agents. Nephropathy causing different mechanism: The chemicals, drugs, diagnostic agents will causing the nephrotoxicity while using long term or high dose were shown in Table 1.

Common Causing Agents

Gentamicin Toxicity

It is a bactericidal and is active against many Gram-negative and Gram-positive pathogens including specious like's staphylococcus aureus. Gentamicin inducing renal toxicity is characterized by tubular necrosis. [19] Gentamicin produced hydrogen peroxide in rats and it also increases the production of ROS. More number of ROS productions has injured the macro molecules such as cellular damage, necrosis, and lipid peroxidation of membrane lipids, protein denaturation and DNA damage. [20] Gentamicin act as chelator to form iron chelating complex is a potent radical generator. [21]

Acetaminophen toxicity

Acetaminophen is inducing liver necrosis [22] has been reported extensively, approximately 1-2% of the patient producing nephrotoxiciy with over dose of acetaminophen, the mechanism of renal function is attributed for cytochrome P-450 mixed function of iso-enzymes present in the kidney, [23] Glutathione is an important element which involves in the detox of acetaminophen[24,25] and its element implicated formation of nephrotoxicity .

Cisplatin Toxicity

The cisplatin is the potent anti tumour drug but limited for its nephrotoxicity inducing property. Cisplatin is very effective in metastatic testicular and ovarian carcinoma. It is widely used for many solid tumors like lung, gastric hepatic and neck carcinoma. Cisplatin reduce the antioxidants [26] and antioxidant enzymes which leads to increase lipid peroxidation and reactive oxygen metabolites productions. Cisplatin has highly emetic drug. Antiemetic will are routinely administered before infusing the most important toxicity is renal toxicity it can be reduced by the maintaining the good hydration. The production of reactive metabolites which strongly binds to tissues macromolecules. The nephrotoxic effect also caused by sulphydryl binding of metal ions. [27-28]

Nephroprotectives

The Nephroprotectives prevents the cellular damages of the kidney from the chemical and environmental pollutants and prevents the exposure of kidney cells. Various plants and drugs are used as the nephroprotectives for their chemical constituents such as flavonoids, flavonol, alkaloids, glycosides, steroids and saponins. Each constituent have their own different medicinal values. These herbals are safe and without any side effects. Some common nephroprotective plants were shown in Table 2.

CONCLUSION

In this review we have discussed the renal functions, different types of renal failure and various toxicities injured the renal and its specifying mechanisms have been discussed. Many herbal plants containing rich in sources of chemical constituents which used for nephroprotective activity against the some nephrotoxins. In these studies we conclude that these plants are having the nephroprotective activity, it can be used against the nephrotoxicants and it has been proofed at the various journals, newsletters and pubmed sources.

ACKNOWLEDGMENT

Nil

Financial Support

None

Conflict of Interest

All authors declare that there is no conflict of interest

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Table 1. Drugs and Chemicals causes nephrotoxicity

S.No	Drugs & Chemicals	Examples	References
1.	Heavy metals	Arsenic, mercury, lead and bismuth	[13]
2.	Anti-neoplastic agents	Cyclosporines, Cisplatin, Vincristin, Methotrxate, 6-thioguanine, 5-fluoro urocil, Carmustine, Lomustine and Streptozocin.	[14]
	Antitumor antibiotics	Mitomycin, Mithramycin, Doxorubicin	[15]
3.	Anti-Microbial Agents	Tetracycline, Sulphadiazine, Amphotericin-B, Rifampicin, Acyclovir and Trimethoprin.	[16]
4.	Miscellaneous	NSAIDs like Ibuprofen, Indomethacin, Aspirin and Radiocontrast agents	[17]
5.	Aminoglycosides	Gentamicin, Amikacin, Kanamycin and Streptomycin	[18]

Table 2. Some common nephroprotective plants

S.No	Plant name	Parts used	Screening methods	References
1.	*Aerva lanata*	Whole plant	Gentamicin induced	[29]
2.	*Abutilon indicum*	Whole plant	Gentamicin induced	[30]
3.	*Boerhavia diffusa*	Root	Acetaminophen induced	[31]
4.	*Indicofera barber*	Whole plant	Acetaminophen induced	[32]
5.	*Punicagranatum*	Fruit peel	Ferric nitrilo tri acetate induced	[33]
6.	*Tectongrantis*	Bark	Alloxan induced	[34]
7.	*Aerva javanica*	Fresh roots	Cisplatin induced	[35]
8.	*Euphorbia nerinjifolia,*	Leaves	N-nitroso dimethyl amine induced	[36]
9.	*Carica papaya*	Seed	Cisplatin induced	[37]
10.	*Acorus calamus*	Aerial parts	Acetaminophen induced	[38]
11.	*Ficus Religeosa*	Latex	Cisplatin induced	[39]
12.	*Gingo bilaba*	Whole plant	Streptozotocin induced	[40]
13.	*Rubia cardifolia*	Root	Ethylene glycol	[41]
14.	*Eruca sativa*	Seeds	Mercuric chloride induced	[42]
15.	*Tamarindus Indica*	Fruit pulp	Fluoride induced	[43]
16.	*Drynaria fortune*	Whole plant	Acetaminophen induced	[44]
17.	*Curcuma longa*	Rhizome	Cadmium induced	[45]
18.	*Orthosiphon stamin*	Whole plant	Gentamycin induced	[46]

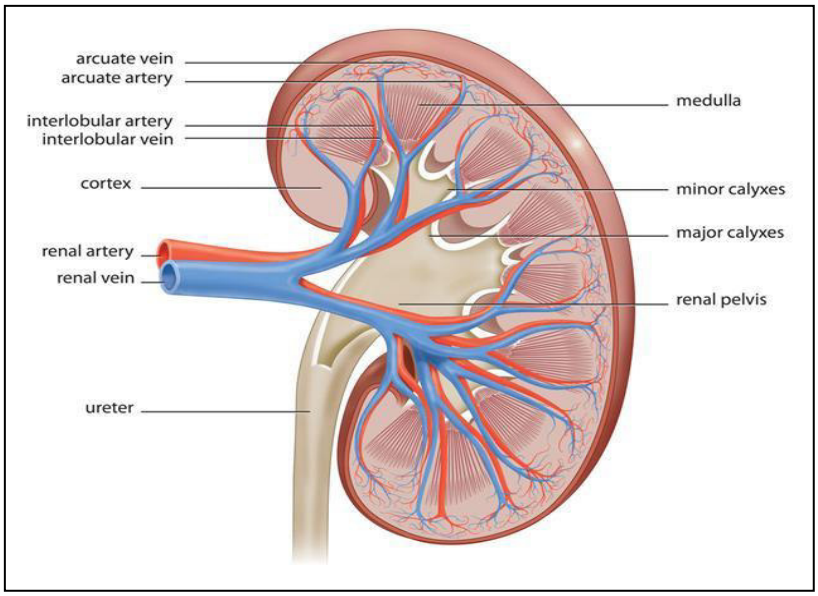

Figure 1. Cross section of kidney

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Emulgel: Amplifying the Application of Topical Drug Delivery

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Received: 10 Feb 2020 Revised:14 Mar 2020 Accepted: 18 Apr 2020

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ABSTRACT

Gels are the semisolid preparations which have a tremendous role in cosmetics and pharmaceutical preparations. Inspite of its benefits, these preparations meet limitations in the delivery of hydrophobic drugs. So to complicit this limitation, emulgel preparations arrived which are able to deliver the hydrophobic therapeutic moiety. As the name indicates, these emulgel formulations are a combination of both emulsion and gels, and it also shows the unique properties of gels. In this article, a review of emulgel is presented by discussing shortly about topical drug delivery,advantages, rationale, drug delivery, marketed formulations, latest elevations and emulgel formulation parameters. Topical delivery means the application of formulation directly to the skin for the localized action. These formulations delivery across the skin is an important parameter in achieving these effects.Various penetration enhancers can improve such effects. So the study about its formulation parameters is an unavoidable step to bring together the properties of an emulgel. The emulgel preparations have several properties like maintaining stability of emulsion, improved patient compliance, drug release prolongation etc..The prepared emulgel is assessed by determining various parameters such as pH, viscosity, spreadability, drug release, drug content etc.

Keywords:-Emulgel, Topical drug delivery, Gelling agents.

INTRODUCTION

Topical delivery can be defined as the application of drug containing formulation to the skin directly with the intent of the pharmacological or other effect of the drug to the skin surface or within the skin. It is one of the simplest and easiest way of drug delivery where other route of drug delivery is not prominent or treatment needs only a localized

drug delivery. Usually topical drug delivery formulations available mainly as solid, semisolid or liquid forms. The drug present in the topical formulation is absorbed through the skin with minimal systemic drug absorption and bypassing the first pass metabolism. The therapeutic efficacy of topical formulation mainly depends on two factors such as the drug absorption through the skin and the release rate of drug from formulation. These two factors also can be elaborate with the subfactors such as favourable lipid/water partition coefficient, wetting effect of formulation on skin and the physiochemical properties of the carrier and the drug used [1].

Skin is the peripheral covering of the body and is the largest organ of the integumentary system. Skin composed of seven layers of ectodermal tissue and guards which underlying muscles, bones, ligaments and internal organs. Skin have greater influence in immunity role for protecting the body against pathogens and excessive water loss [2]. There are different formulations available as dermatological products which can applied to the skin with varying range in consistency from liquid to powder, but among them the prominent favoured ones are semisolid preparations. Gels are one of the semisolid preparation which can seize small drug particles and due to its cross linked network provides a controlled release pattern of drug delivery. Because of its mucoadhesive property it can extend the duration of medication over the skin to a long time. Mainly gels are of two types on the basis of solvents used, first one is organic solvent based and other is water based. Gels have a lot of advantages but one of the prominent disadvantage is the limitation in the delivery of hydrophobic drugs.

Emulgels are another semisolid preparations which have a combination of both emulsion and gel. It have almost all the properties of gels and one of the peak advantage is that, it can overcome the main limitation of gel ie, the delivery of hydrophobic drugs. To deliver the various drugs through the skin by emulgel, both water in oil and oil in water type emulsions are used as solvent.Mainly the three layers of skin are epidermis, dermis and the subcutaneous tissue. The three routes by which the molecule penetration happens through the skin are by stratum corneum layer, sweat ducts or through sebaceous follicle. Epidermis is the outer most layer of skin which is visible to the eyes. It is primarily made up of keratinocytes and again consists of a number of layers such as the innermost basal layer, pricle layer, granular layer and stratum corneum. Dermis is the middle layer composed of glycosaminoglycans and structural protein collagen. It mainly have two layers such as the papillary layer and the reticular layer. The deepest layer ie, subcutaneous tissue primarily composed of lipocytes.

Rationale

There are a lot of medicated products which is applied to the skin for the pharmacological aid and also thereby restores the normal functioning of skin. Some of the semisolid preparations among them are ointments, cream, lotions etc. All these formulations have its own advantages and purposes. But some of the common disadvantages usually found are stability problems, uneasiness to apply due to its sticky in nature etc..To overcome these disadvantages, gel formulations step-up in cosmetics and pharmaceutical field. A major limitation arised in case of gels are the delivery of hydrophobic drugs. This limitation was cover up successfully by the emulgel formulation. Emulgels easily delivered the hydrophobic therapeutic moiety.

Advantages

Incorporation of hydrophobic moiety

Hydrophobic drugs are not soluble in water phase and due to its lack of solubility in many of the solvents, an improper drug release may occur if these moieties are added directly to the formulations. So these agents are incorporated to the oil phase of emulsion and an o/w emulsion was attained. Then for convenience, the semisolid emulgel formulation is prepared to achieve better stability and release properties of drugs.

Better stability

Usually topical preparations faces amajor drawback in the stability attainment. Some of the cases generally seen are that creams shows breaking or inversion, powders shows hygroscopic nature, oil bases becomes rancid etc.. In most of these semisolid formulatons, emulgel can achieve a better stability.

Better loading capacity

One of the main problem arised in the case of novel drug delivery systems are their lesser entrapment efficiency due to its nanosize. But gels have greater loading capacity when compared to other delivery systems.

Low preparation cost

The cost for the production of emulgels are low because it doesn't need any specialized instrument for production and the materials used are cheaper and available easily.

Controlled release

The release pattern of drug can be attained in a controlled manner by the emulgel formulation and thereby enhance the therapeutic efficacy.

Delivery of Drug Across the Skin

Drug delivery across the skin is an important area for consideration, when discussed about the topical preparations. For developing a topical preparation two important factors which have to be noticed are the physiology of skin and the characteristics of drug. A brief description about the layers of skin were discussed in the introduction section. A surface area approximately 2 $m^2$is covered by the skin of an average adult body. An average of 40-70 hair follicles and 200-300 sweat ducts on every square centimeter of the skin were present on an average human skin surface. The skins pH of the skin surface depends on the sweat and fatty acid secreted from sebum. The rate limiting step of controlled drug delivery system is the release of drug from dosage form to the absorption site. The main factors affecting topical absorption of drug are categorized as two such as: physiological factors and physiochemical factors.

Physiological factors

1) Skin thickness
2) Lipid content
3) Density of hair follicles
4) Density of sweat glands
5) Skin pH
6) Blood flow
7) Hydration of skin
8) Inflammation of skin

Physiochemical factors

Partition coefficient
Molecular weight
Degree of ionization (only ionized drugs gets absorbed well)

Effect of vehicles.
The topical drug delivery system can be classified into four categories such as solid preparation, semisolid preparation, liquid preparation and miscellaneous preparation.
Examples for each type of preparations are given below:-
Solid preparations- topical powder, plaster
Semisolid preparations- ointments, creams, paste, gel
Liquid preparations- lotion, liniment, emulsion
Miscellaneous preparations- transdermal drug delivery system, liquid cleaner, topical aerosol.[3]

Emulgel Formulation- Ingredients

Vehicle

Some of the properties shown by the vehicles are:-

- The deposition of the drug on the skin will attain efficiently and distributed evenly.
- The release pattern of the drug is controlled to a certain extend and hence it can migrate freely to the site of action.
- Drug delivered to thetarget site.
- The therapeutic drug level is sustained for a sufficient duration in the targeted tissue and thereby produce a pharmacologic effect.
- The absorption rate and extend will different according to the nature of vehicle and also influenced by drug characteristics.

Aqueous material

Emulsions aqueous phase are generally obtained by aqueous materials.
Examples are water, alcoholetc..

Oils

The oil phase of the emulsion is obtained by oils. The emulsification process, the droplet size of the emulsion, drug solubility are very much depends the physiochemical properties of oil. So oil phase have a great role in the formulation of emulsion. Usually the oil for the formulation of an emulsion was selected according to the solubility characteristics of the choosed active constituent. Thus can attain a maximal loading of drug.
Eg:-castor oil, rose hip oil, myrrh oil

Emulsifier

The emulsification process and stability of the formulation is controlled by the emulsifiers. Emulsions are thermodynamically unstable systems. The addition of a suitable emulsifying agent will enhance the stability of the formulation.
Eg:- Span 80, Tween 80

Gelling agents

Gelling agents are the agents which determines the consistency of gel related formulations by forming a gelly like structure. It is also known as thickening agents.
Eg:-Carbopol 934, Carbopol 940, Xanthan gum

Penetration enhancers

These are the agents used to enhance the penetration of active moiety and thereby improves the therapeutic efficacy by overcoming the impermeability of skin. Penetration enhancing agents should have to follow some of the properties. It should be pharmacologically inert, non irritating, non toxic, colourless, odourless, tasteless, compatible with other ingredients, inexpensive etc.. Care should have to takenthat, it should not produce any worse effect on skin or therapeutic activity. Some of the penetration enhancers generally used in the emulgel preparations are oleic acid, clove oil, menthol etc..[4], [Table 1, Table 2]

Peparation Methods of Emulgel [15]

1st step:- Formulation of gel using gelling agent

Required quantity of Carbopol 940 was weighed and add to warm distilled water with continuous stirring. Then allow to cool for 1-2hours. With continuous stirring other excepients like Propylene glycol and Glycerol can add to it. Then the drug can introduce into it and have to disperse uniformly. Then the gel can sonicate for 15 minutes and keep for overnight.

2nd step:- Formulation of emulsion

According to the types such as oil in water and water in oil, emulsion can formulate.

3rd step:- Incorporation of emulsion to gel base

This is the last step of emulgel formulation in which the emulsion is incorporated in gel base.

Parameters for the Evaluation of Emulgel [16]

Physical appearance

Colour, homogeneity and consistency are inspected visually.

pH

The pH value of the obtained emulgel can evaluate using a pH meter.

Viscosity

The viscosity of the obtained emulgel can evaluate using viscometer. The determination of viscosity helps for studying the rheological properties of formulation. The average of triplicate checking are taken as the result usually.

Spreadability

The easiest method for determining the spreadability of semisolid preparations are parallel plate method. As the name of the method indicates, two glass plates arerequired and before doing the test, one of the glass plate have to pre-marked with a circle of 1cm diameter. Then a weight of 500g emulgel is placed over it and press with other glass plate. The spreading rate can be determined by noting the increase in diameter.

Extrudability study

It is also known as the tube test which is performed for the determination of the force required to expel the formulation from the tube. A clean lacquered aluminium collapsible metal tube is required for filling the formulation. Then later these tubes were pressed with the finger to expel the material. Extrudability depends upon the quantity in the percentage of emulgel. Emulgel which is extruded from the tube on application of weight in grams required to expel atleast 0.5cm ribbon of gel in 10 second. The average of triplicate checking helps for the determination of result.

Extrudability = Applied weight to extrude formulation from tube (gm)/ Area (cm^2)

Swelling index

For determining the swelling characteristics, swelling index is checked. To perform the test, a porous aluminium foil is taken and 1gm of gel is placed over it. Then a beaker containing 10ml of 0.1N sodium hydroxide is taken and the gel have to place separately in beaker. Then at different time intervals samples are taken out from beakers and after drying the samples, reweighed it.

Swelling index in % = (wt-w0)/w0 × 100

w0 – initial weight of emulgel

wt – weight of swollen emulgel

t – time

Drug content study

Drug content in emulgel is determined by spectrophotometer. Suitable solvent have to selected for dissolving gel formulation (1 gram). To obtain clear solution, filter it. Then the absorbance of the solution is noted by UV/visible spectrophotometer. From the calibration curve for drug, drug content can be measured.

In vitro release study

Franz diffusion cell can be used to conduct the invitro release study. Prepared emulgel have to apply evenly over the surface of an egg membrane. Diffusion cell mainly have a donor and receptor compartment. The receptor compartment is stirred using a magnetic stirrer. At suitable time intervals the samples are collected and analyzed by UV/visible spectrophotometer. As a function of time, the cumulative amount of drug released through the egg membrane is noted.

Patch test

It is for determining the skin irritation. A total set of 8 rats have to select for the test. Then its skin have to shave properly and the emulgel is applied on to the skin. A 24 hours observation can be conducted for noticing the change in skin morphology. The test is passed if there is no irritation. The study have to repeat if any irritation occurs in more than 2 rats.

Microbiological assay

For the semisolid formulations, microbilogical assay is one of the relevant test. Ditch plate technique can be adopted for this purpose. It helps for the bacteriostatic and fungistatic evaluation of a compound. Already prepared Sabouraud's agar dried plates are used for test. 3g of emulgel are placed in the ditch cut in the plate. Freshly prepared culture loops are streaked through the agar at a right angle from the ditch to the edge of the plate. It is incubated at 25ºc for about 18-24 hours. The fungal growth is noticed an the percentage inhibition rate is determined as follows.

Percentage inhibition = L2/L1×100

L1 – total length of the streaked culture

L2 – length of inhibition

Stability studies

Emulgel formulation issealed in a collapsible tube and sealed it. Triplicate method is followed. Then stability studies are attained at 5ºC, 25ºC 60%RH, 30ºC 65%RH and 40ºC/75%RH for about three months. The sample analysis will done at preselected time intervals for pH, physical appearance, rheological properties and drug content.

Kinetic studies

For the determination of the drug release mechanism, the release data can fitted to following equations.

i. **Zero-order equation**
   Q=k0t
   Q – Amount of drug released
   t – time
   k0 – zero-order release rate
ii. **First-order equation**
   In(100-Q) = In100 – klt
   Q – percent of drug release
   t – time
   k1 – first order release
iii. **Highuchi's equation**
   Q = k2√t
   Q – Percent of drug release
   t – time
   k2 – diffusion rate constan

Table 3 and 4

CONCLUSION

This reviews gives an overview of the emulgel topical formulation which are very safe and will be a promising dosage form for topical treatment than conventional dosage forms.Emulgel could help powerfully, to advance the targeting of the drug.

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Table No 1.Formulation Ingredients of Emulgel

Sl.No	Ingredients	Examples
1	Aqueous material	Water, Alcohol
2	Oil	Castor oil,Rose hip oil, myrrh oil
3	Emulsifier	Span 80, Tween 80
4	Gelling agents	Carbopol 934, Carbopol 940, Xanthan gum
5	Penetration enhancers	Oleic acid, Clove oil, Menthol

Table No: 2 Various Gel Formulations- Gelling Agents use as Polymers

Sl.No.	Drug	Type	Polymer	Purpose	Reference
1	Calcipotriol	Emulgel	Carbopol 934, HPMC	Effect of gelling agents on release	5
2	Nimesulide	Gel	HPMC, Carbopol 934, Natural polymer	Effect of gelling agents on release	6
3	Ketoconazole	Emulgel	Carbopol 934, Carbopol 940	Comparative study of polymer and drug release	7
4	Fluconazole	Liposomal gel	Carbopol 934	Increase permeation and deposition	8
5	Miconazole	Emulgel	Carbopol 934, Carbopol 940	Controlled delivery	9
6	Mefanimic acid	Emulgel	Carbopol 934, HPMC	Release study and pharmacologic action	10
7	Aceclofenac	Gel	Carbopol, HPMC, Sodium CMC	Carbopol gel shows superior release	11
8	Ibuprofen	Gel	Chitosan	Studies on topical system effect	12
9	Piroxicam	Emulgel	Pluronic R F127	Study of percutaneous permeation	13
10	Benzydamine	Gel	Sodium CMC, Hydroxy ethyl cellulose	Effect of gelling agents and formulation study	14

Table No: 3 Latest Elevations in the Preparation of Emulgel for Different Drugs

Drug	Aim	Route	Category	Use	Reference
Lacidipine	Proniosomes for transdermal delivery of Lacidipine	Topical	Calcium channel blockers	Antihypertensive	17
Flurbiprofen	Determination of the formulation parameters on functional and rheological properties of emulgel	Buccal	NSAID	Anti-inflammatory, Analgesic	18
Calcipotriol	For successful permeation of calcipotriol delivery as emulgel into the skin	Topical	Antipsoriatic	Psoriasis treatment	19
Amlodipine besylate	Transdermal delivery of amlodipine besylateemulgels and its in-vitro permeability studies	Topical	Calcium channel blockers	Transdermal delivery	20
Chlorphenesin	Optimization of Chlorphenesinemulgel	Topical	Antifungal	Candidiasis, Mycosis fungal infection	21
Diclofenac sodium	Determination of the antimicrobial efficacy	Topical	NSAID	Analgesic, Antiinflammatory	22
Terpinen-4-ol	Permeation profiles, release and rheological behaviour of the Terpinen-4-ol emulgel	Topical	Antimicrobials	Antimicrobial activity	23
Amphotericin B	Assesment of invivoleishmanicidal activity of Amphotericin B emulgel	Topical	Antibiotic	Leishmaniasis therapy	24
Clobetasol	Formulation of a new topical delivery system for the prolonged release of clobetasol propionate	Topical	Corticosteroid	Psoriasis treatment	25

Table No: 4. Different Marketed Emulgel Formulations

1	Diclobaremulgel	Diclofenac diethyl amine	Barakatpharma	Rheumatic diseases
2	Levoragemulgel	Hibiscus, Liquorice and natural extracts	THD ltd	Anal fissures
3	Bello photostable sunscreen emulgel	Bemotrizinol, Bisoctrizole	Bello pharmacy	Sunscreen
4	Voveranemulgel	Diclofenac diethyl ammonium salt	NOVARTIS	Anti-inflammatory
5	Kojitinemulgel	Arbutin, Kojic acid, Liquorice extract	KLM Laboratories pvt. Ltd	Skin lightening gel with sunscreen properties
6	Derma feet cream emulgel	Urea 40%	Herbitas laboratories	Hyperkeratosis
7	Tubelite gold emulgel cream	Glutathione	Aakaar laboratories	Skin lightening
8	Diclomaxemulgel	Diclofenac sodium	Torrent pharma	Anti inflammatory
9	Denacineemulgel	Clindamycin phosphate	Beitjala pharmaceutical company	Antiacne
10	Isofenemulgel	Ibuprofen	Beitjala pharmaceutical company	Anti-inflammatory
11	Cataflamemulgel	Diclofenac potassium	NOVARTIS	Anti-inflammatory
12	Diclonaemulgel	Diclofenac diethyl amine	Kuwait Saudi pharmaceutical industries	Anti-inflammatory
13	Dosanacemulgel	Diclophenac diethyl ammonium	Siam bheasach pharmaceutical company	Anti-inflammatory
14	Diclonemulgel	Diclofenac diethyl amine	Medpharma	Anti-inflammatory

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Vitamin D Deficiency among Patients with HCV Genotype 3A

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Received: 02 Feb 2020 Revised:04 Mar 2020 Accepted: 06 Apr 2020

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ABSTRACT

Vitamin D has several classical functions that are important for bone maintenance. Among patients with hepatitis C, vitamin D deficiency can lead toliver fibrosis, necro-inflammation, bone disease, and treatment failure. The aim of the current study was to determine thefrequency of vitamin D deficiency among patients with Hepatitis C virus (HCV) genotype-3a. This cross-sectional study was conducted from April 2017 to July 2018 at the outdoor department, Saidu teaching hospital, Swat. A total of 160 patients having HCV genotype 3a were selected. The data was recorded using a pre-designed questionnaire and patient's baseline characteristics were taken. Serum 25-hydroxyvitamin D [25(OH)D] level was also measured and severity of vitamin D deficiency was categorized as severe deficiency, deficiency, insufficiency and sufficiency. Data was analyzed using SPSS version 22. Of the 160 patients, 142(88.75%) had some degree of vitamin D deficiency, 62(38.8%) patients had insufficiency, 67(41.9%) had deficiency, and 13(8.1%) had severe vitamin D deficiency. Vitamin D levels were equally insufficient among HCV patients of both genders and insignificant association exist between the two variables (p-value > 0.05). In conclusion, Vitamin D deficiency is highly prevalent among HCV patients, emphasizing the need of Vitamin D supplementation.

Keywords : Chronic Hepatitis C, Genotype 3a, Vitamin D deficiency.

INTRODUCTION

HCV infection is one of the leading cause of chronic liver disease [1], globally 130 to 210 million people are affected with HCV while the prevalence rate is 6%in Pakistan [2]. Egypt has the highest worldwide prevalence of HCV, ranging from 6 to 40% (average 14%) [3]. More than seven HCV genotypes, and a large number of subtypes have been described lately [4]. The most common HCV genotype in Pakistan is 3a [5]. In 2004, a panel of 30 top

gastroenterologists reported that around 75-90% of HCV patients in Pakistan had genotype 3a [6]. Diagnosis of chronic HCV infection is based on the presence of both anti-HCV antibodies, detected by enzyme immunoassays, and HCV RNA, detected by molecular assays [7]. HCV genotype and subtype can be determined via direct sequence analysis, reverse hybridization, and genotype-specific real-time PCR [7]. Liver biopsy is still regarded as the reference method to assess the grade of inflammation and the stage of fibrosis [8,9]. HCV patient are usually observed with vitamin D deficiency, for which the exact mechanism is still not known. Low vitamin D serum level is related to severe fibrosis and low responsiveness to interferon (IFN)-based therapy in genotype 1 chronic hepatitis C [10]. Two recent randomized clinical trials examined the effect of vitamin D3 in combination with conventional IFN and ribavirin (RBV) therapy. Which showed that vitamin D supplementation significantly enhances the sustained virological response (SVR) rates among treatment-naive patients infected with HCV genotype 1 (86% for the supplemented group *vs* 42% of the controls) and for genotypes 2–3 (95% for the supplemented group *vs* 77% of the controls) [11, 12]. As in Pakistan the most common HCV genotype is 3a, so the rationale of this study was to determine the frequency of vitamin D deficiency among HCV genotype 3a patients. So, that Vitamin D supplementation can be added to the conventional therapy in order to obtain regulate SVR rate among HCV infected patients.

MATERIAL AND METHODS

A Cross-sectional study was conducted over a sample of 160 patients with HCV genotype 3a presented to the outdoor department, Saidu teaching hospital, Swat. The study continued from April-2017 to July 2018. All patients between 18 to 65 years of age with chronic HCV infection having genotype 3a were recruited. While patients receiving vitamin D for any other medical condition or those suffering from active rheumatologic or orthopedic conditions were excluded from the study sample. Written informed consent were taken from each patient before inclusion. The study conduction was in accordance to the declaration of Helsinki and all ethical principles were followed. The data was collected using a pre-designed questionnaire inquiring the patient demographic details and the vitamin D status for each HCV patient enrolled was also monitored and recorded. The presence of HCV was established on the basis of HCV RNA through HCV RNA PCR test. For Vitamin D assay, the serum sample of each HCV patient was collected and 25-hydroxyvitamin D [25(OH)D] level was measured and categorized as severe deficiency (< 7 ng/ml), deficiency (7–19 ng/ml), insufficiency (20–32 ng/ml) and sufficiency (32 ng/ml). The collected data was analyzed using SPSS version 22. All qualitative variables were given as frequency and percentages while mean and standard deviation was used for all quantitative variables. Chi-square test was used for significance testing where p-value<0.05 was considered significant.

RESULTS

A total of 160 patients diagnosed with HCV were enrolled in the study with the mean age of 42.96 ± 9.78 years. Of the total, 55.6% were males and 44.4% were females. The mean vitamin D level was 20.84 ± 7.09 (ng/ml) and around 88.75% of these patients were diagnosed with mild to severe Vitamin D deficiency while only 11.3% were having normal status as shown in table 1. The mean vitamin D level was insufficient among both genders i.e. in between the range of 20–32 ng/ml but the results indicated insignificant association (p-value > 0.05) (Table 2).

DISCUSSION

Hepatitis C has become the major healthcare problem globally [13]. Despite of the fact that the treatment modalities are discovered and are widely available, the probability for reducing the burden of chronic hepatitis C remains restricted [14]. It is evident from previous literature that Vitamin D deficiency is closely associated with HCV infection, as it controls several genes associated with immunity, cell proliferation, differentiation, apoptosis and angiogenesis [13]. Therefore, its deficiency subsequently leads to decreased immunity against such viral infections

[15]. Moreover, Vitamin D deficiency is also involved in treatment failure among HCV patients. As reported by a study, decreased serum Vitamin D levels among HCV patients being treated with pegylated IFN and RBV therapy displayed failure to achieve SVR [16]. In a study, Glecaprevir-pibrentasvir combination drug was given for eight weeks to 157 HCV genotype 3 patients resulting in the 95% SVR rate [17]. The response rate was similar in a twelve-week course of daclatasvir plus sofosbuvir i.e. 95% SVR among 76 HCV patients [18]. The prevalence rate among the studied population was high i.e. around 88.75% HCV patients were diagnosed with Vitamin D deficiencies of different severity levels. The results were consistent with another local study conducted in Lahore, reporting overall vitamin D deficiency among 78.3% HCV [13]. However, another study in support reported around 90% HCV patients with low vitamin D level where 31% had deficiency and 59% were observed with insufficient Vitamin D levels [14].

Vitamin D deficiency has been associated with an increased risk of cancer, cardiovascular, autoimmune and infectious diseases [19]. Which enhances the research interest to explore the role of vitamin D status among various infectious diseases. Some studies have shown that high levels of serum vitamin D level are an independent predictor of SVR following anti-viral therapy, and higher SVR is achieved with vitamin D supplementation in CHC individuals [16]. However, Lange and his colleagues discovered that a low SVR rate was common only among patients with chronic hepatitis C (CHC) genotype 2/3 (treated with PEG-IFN and RBV for 24 weeks) while no difference was observed among CHC genotype 1 patients [16]. Jazwinski et al., also displayed contrasting results i.e. no association was observed between vitamin D levels and SVR among genotype 1 CHC naive patients, treated with PEG-IFN and RBV [20].

Patients with normal vitamin D levels (11.3%) had comparatively low-level circulating HCV viral loads, consistent with this another study reported similar results [13]. Strengthening the hypothesis that the upregulation of vitamin D among deficient patients can be effective in correcting the HCV status thereby improving the immune response by achieving SVR. Ageing also affects the circulating level of Vitamin D i.e. high frequency of vitamin D deficiency was found in patients >40 years of age [13]. One of the major reasons for high prevalence of Vitamin D deficiency among the studied population was age (42.96 ± 9.78 years). Therefore, the level of Vitamin D should be tracked at all stages of the hepatitis C progression [10]. Vitamin D supplementation must be taken into account during the disease progression as well as throughout the treatment course. Although study results clearly indicated high prevalence of vitamin D deficiency among HCV patients but there were several limitations that need to be addressed. First and the foremost was the absence of control group. A descriptive comparative analysis is required to determine the frequency of the deficiency among the disease group and the counterparts. Moreover, we did not consider the impacts of other associated variables that might have altered the study outcomes including comorbidities, body mass index (BMI) and the factor altering Vitamin D levels among these patients.

CONCLUSION

It is con concluded from the study results that majority of the patients with hepatitis C genotype 3a are vitamin D deficient. Considering the adversities associated with inadequate vitamin D level, it is recommended to measure the 25(OH)D levels periodically among the HCV patients and adequate supplementation for disease management is essential. As vitamin D helps in achieving SVR and also halts the process of fibrosis among HCV patients.

CONFLICTS OF INTEREST

The authors have no conflicts of interest.

ACKNOWLEDGEMENT

The authors would like to acknowledge the Medical Affairs Department of Getz Pharma for providing their technical support during the preparation of this manuscript.

FUNDING

None.

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Table 1. Demographic characteristics of the study population

Characteristics		(n=160)
Age (Years)		42.96 ± 9.78
Gender	Male	89(55.6)
	Female	71(44.4)
Mean Vitamin D level (ng/ml)		20.84 ± 7.09
Vitamin D Status	Severe deficiency (< 7 ng/ml)	13 (8.1)
	Deficiency (7–19 ng/ml)	67 (41.9)
	Insufficiency (20–32 ng/ml)	62 (38.8)
	Sufficiency (32 ng/ml)	18 (11.3)

****values are given as mean ± SD or n(%)***

Table 2: Association of gender with mean vitamin D deficiency

Gender	**Mean Vitamin D level (ng/ml)**	**p-value**
Male	21.46 ± 6.782	0.22
Female	20.07 ± 7.425	

****p-value<0.05 is considered significant.***

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

REVIEW ARTICLE

Overview of Isolation and Identification of Different Bacterial and Fungal Species from Municipal Solid Waste

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Received: 01 Feb 2020 Revised:03 Mar 2020 Accepted: 06 Apr 2020

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ABSTRACT

The current review work was directed to find out the isolation and identification of bacteria from municipal solid waste (MSW) and degradation as organic manure or compost. From the recent study, it can be proved that useful bacteria which are friendly for bioconversion of solid organic waste might be isolated from the surrounding environment. Microorganisms play vital roles in the maintenance of many natural and man-made occurrences in the environment. They give out positive purposes which make our life easier. One such region that microorganisms are adopted is in waste management. The proper disposal of the capacious waste that humans produce in their daily activities is a big challenge that environmental agencies and government are repeatedly searching for better ways of addressing. Thus, this paper emphases the different types and applications of microorganisms in the management of municipal solid and liquid wastes also. It also reviews the several roles of microorganisms in the environment, such as in sewage and soil treatment in waste management, saline lakes, etc. It also talked about waste production and management methods, and some specific benefits of microorganisms (bacteria, fungi, algae, virus and protozoa) in waste management. It concludes by highlighting some current advances in microbiological waste management.

Keywords: Degradation, Environment, Identification, Isolation, Microorganisms, MSW.

INTRODUCTION

Municipal solid waste (MSW) is the waste produced in a community by household, institutional and/or commercial activities with the exception of industrial and agricultural wastes. Hence, MSW includes residential, commercial and

institutional wastes which are claimed to be non-hazardous. They also consist food wastes, plastics, textiles, glass, metals, paper, cardboard, wood, street sweepings, landscape and tree trimmings as well as general wastes from beaches, parks, temple, market places and other recreational areas. These wastes nonetheless often get infected with some hazardous materials like the batteries and electronics item. Local and regional factors, contribute to the dissimilarities in MSW composition, such as climate and level of commercial activity. In non-developed countries the content of organic matter in MSW is found to be higher due to the rapid use of fresh and unprocessed vegetables. Hence, they have the potential to be converted into organic fertilizer for farm use because these are bio-degradable in nature. On account of these about 53% of waste composition are the biodegradable fraction (paper, garbages, garden and food wastes). Therefore, the biodegradation of these wastes forms an important factor of an integrated solid waste management strategy, which will reduce both the volume and harmful effects of the MSW needs final disposal in a landfill. It is argued that city farming, through Urban Agriculture (UA) programs, consumes the generated urban solid waste and reduces the volume of waste to be collected and transported to distant dumps (Mohapatra, 2006; Amalraj, et al., 2006).

Municipal Solid Waste Management

In developing countries Municipal Solid Waste Management (MSWM) like Kenya faces lots of challenges due to weak economies, poor administrative capacities and inabilities to enforce environmental legislation. In most developing countries, the MSWM is of serious matter especially due to rapid urbanization. If not properly tackle, insufficient management of wastes can cause harm to human health and the environment (Suess and WHO, 1985; Khajuria et al., 2008). Daily municipal waste formation per capita ranges from 2.75 to 4.0 Kg in high income countries and in countries with low incomes is 0.5 to 0.8 Kg (Cointreau, 1982; World fact sheet, 2001; Zurburgg, 2002). Nairobi generates solid waste on daily basis about 4,000 tonnes (Allison 2010) while in Kisumu, about 400 tonnes in everyday (Waston, 2009; Munala and Moirongo, 2011). Dandora dumping site has been documented to negatively affect the health of thousands of Nairobi residents which covering an area of about 26 hectares. Besides composting, other MSWM practices include combustion/ incineration, landfills, source reduction, and recycling. Composting is more desirable because it is cheap, prevents emissions of greenhouse gases, saves energy, reduces pollutants, and conserves resource .With this it can also supply valuable raw materials for agriculture and reduce the need for new landfills and combustors (Colon and Fawcett, 2006).

Bhubaneswar is the capital of the state of Odisha in India. An emerging Information Technology (IT) and education hub, in recent years Bhubaneswar is one of the fastest developing cities of India. 11.02 per cent of the population lived in 99 unauthorized slums and 47 authorized slums according to 2001 census report. However, the number of slums in Bhubaneswar increased to 377, with most of them being unauthorized in 2009. The growth of slums is due to migration from rural areas and neighbouring states, which are a major challenge to the city's growth .The Orissa Municipal Act, 1950 and the Orissa Municipal Rules, 1953 govern the constitution and functioning of Bhubaneswar Municipal Corporation (BMC).. Urbanization and industrialization influence the quantity of garbage produced in city. In India, Mumbai, with a population of 13.8 million, is the largest (8,000 t d-1) MSW generator. Delhi generates 6,000 t d-1 of MSW for a population of 10 million. The MSW generation rate in Bhubaneswar is about 360 g per capita per day (GPCD) and the total generation is about 3, 00 t d-1 (Chattopadhyay et al., 2007).

Physical and Chemical Characteristics of Solid Waste

Waste generation is the first materials of waste management processes. It is a precondition to anywaste management plan to have sufficient knowledge of the generators of waste, its physical and chemical attributes. The waste features vary not only from city to city but even within the same city, as it depends on components such as the nature of local activities, food habits, cultural traditions, socio-economic factors, season and climatic conditions. The physical and chemical features aid in determining the desired frequency of collection of wastes, necessary precautions to be taken during transportation, and methods of processing and disposal (Assmuth and Strandberg, 1993).

Landfill waste is diverse in nature and biological degradation takes place within the landfill in microenvironments. About 40 percent of the total mass paper and paper-related products are the biggest constituents in MSW (Barlaz et al., 1990). These paper products contain holocellulose, which is the sum of cellulose and hemicellulose, and lignin, which together is called lignocellulose is major part of biomass. Degradation of lignocellulose is necessary for operation of the global carbon cycle. About 91percent of methane potential in cellulose and hemicellulose of MSW (Barlaz et al. 1989).

Use of microorganisms in waste management

The microorganisms which include bacteria, fungi, algae, protozoa, and other higher animals settle in the aerobic biological treatment systems. In a particular industrial waste disposal system the growth of all types of microorganisms will depend upon the chemical features of the industrial waste, the environmental restrictions of the particular waste system and the biochemical characteristics of the microorganisms. All of the microorganisms contribute to its over-all characteristics, both good and bad that grow in a specific industrial waste disposal system. It is important to identify the contributions to the over-all stabilizationmade by each type of organism of the organic wastes if the waste treatment system is to be perfectly designed and performed for highest potency (Beede and Bloom, 1995; Metin et al., 2005).

Characterization of Bacteria

Bacteria can be described and classified in three major ways, namely; microscopic examination, molecular phylogenetics and cultural characteristics. The study of microbial diversity constitutes a major opportunity for advances in biology, microbiology and biotechnology. In the biosphere there is a enormous volume for genetic diversity of bacteria. One of the major issues in the environment that hamper studying the bacterial diversity is the inability to obtain many of bacteria in culture. Over the years, diverse natural microorganisms have provided important biological materials in medical, industrial and agricultural fields which are useful to humans. Recent development in ecological molecular microbiologymanifest that the level of microbial diversity in nature is far greater than previously thought (Atalia et al., 2015).

The basic biological units in aerobic waste treatment systems are the bacteria. It makes possible for them to metabolize mostly due to various biochemical character of bacteria, if not all, organic compounds found in industrial wastes. In all aerobic waste treatment systems bacteria are found which are obligate aerobes and facultative. Growth of any distinct species is reliant on its adverse capability to get a portion of the obtainable organic material in the system. Predomination of bacteria will typically split itself into two major groups: the bacteria utilizing the organic compounds in the waste, and the bacteria utilizing the lysed products of the first group of bacteria. In waste the bacteria utilizing the organic compounds and will regulate the characteristics of the treatment system. The secondary predomination extension will depend upon the length of starvation. Depletion of the organic substrate is followed-by death and lysis of the predominate bacteria. Bacteria release the cellular component that permits other bacteria to grow up. Since secondary predomination will occur, as a safety factor all biological treatment systems are normally overdesigned. The most important characteristics of bacteria is their ability to flocculate. All of the aerobic biological waste treatment methods rely on the flocculation of the microorganisms and their separation from the liquid phase for absolute stabilization (McKinney, 1957).

It was first thought that flocculation was caused by a single bacterial species, *Zoogloea nigeria*, but recent studies have shown that there are many different bacteria which have the ability to flocculate. Autoagglutination is to permit the bacteria to multiply and to be rapidly motile. Autoagglutination, or flocculation, starts only after the bacteria lack the energy of motility. The process of composting takes place through microflora diversity by three phases, that are mesophilic, thermophilic, and maturation. These microflora include esmesophilic and thermophilic bacteria, fungi and actinomycetes to turn and stabilise the organic waste to humus. Throughout various phases, the physiochemical

condition like oxygen, temperature, nutrient availability and moisture content determine the development of microbial populations during composting. The microbial produces different enzymes which disintegrate the complex organics substances to a stable and simple form and eventually produce as product as humus and biogas (Zeng et al., 2001).

Microbes which are extremophiles, adapted to grow in conditions such as extreme pH, temperature, pressure, salinity, ionizing and UV radiations etc. However, many extremophiles have been isolated from other extreme conditions. For example, alkaliphiles have been isolated from places like acidic and neutral environments in which they would not be supposed to grow. Alkaliphilic bacteria are widely distributed in naturally occurring environments like alkaline or non-alkaline. Due to the unusual combination of climatic, geological and topological conditions stable alkaline conditions are formed. The most stable high pH environments on Earth represented by Soda lakes and commonly have pH values above 11.5. Alkaliphilic cyanobacteria which provide fixed carbon that is utilized by a vast range of alkaliphilic aerobic and anaerobic chemo organotrophs , Bacilli, Clostridia methanogens and notably Halomonads (Grant et al., 1990). The microbial community of Soda Lake carries alkaliphilic specimen of all the major trophic groups of bacteria and archaea. Cyanobacteria, notably *Arthrospira platensis* and Cyanospirarippkae are responsible for primary photosynthetic production in dilute lakes and likewise an insignificant contribution to primary productivity is made by anoxygenic phototrophic bacteria of the genus *Ectothiorhodospira* (Jones et al., 1998). *Cyanobacteria* and *Anoxygenic phototrophs* from the genus *Halorhodospira* and *Rhodobaca* were reportedthat may be responsible for primary effectiveness in hyper saline lakes. Commercial processes such as beverages, food, soda ash, textile, , cement manufacturing and paper and hide processes liberate alkaline conditions due to the chemistry of the components used. However, such environments have a relatively limited variety of alkaliphilic inhabitants, generally Bacillus or related species (Milford et al., 2000).

An alkaliphilic bacterium, *Bacillus marmarensis* sp. nov., and protease producer was isolated from mushroom compost from Marmara region, Turkey. (Denizci et al., 2010). Purple sulfur bacteria of the family ectothiorhodospiraceae were isolated from brackish, moderately saline steppe and hypersaline lakes. Haloalkaliphilic Bacilli contains of distinct genera such as *Alkalibacillus*, *Gracilibacillus* and *Halobacillus* were isolated from various different saline environments (Romano et al., 2005; Echigo et al., 2010). *Haloalkaliphilic* strains like *Alkalibacillus silvisoli* (Usami et al., 2007), *Bacillus oshimensis* (Yumoto et al., 2005), *Halalkalibacillus halophilus* and *Geomicrobium halophilum* gen. nov., sp. nov. were isolated from forest and garden (non-saline) soils of Japan (Echigo et al., 2010).

Characterization of Fungus

Fungi play a vital role in the stabilization of organic wastes. The fungi can metabolize almost every type of organic substances found in industrial wastes like the bacteria,. The fungi have the potentiality to predominate over the bacteria but they do not include under unusual environmental conditions. The filamentous fungi found in industrial wastes makes them unpleasant since they do not form a tight dense loc and settle easily. The filamentous fungi predominate over the bacteria at low pH, at low oxygen tensions and at low nitrogen. Metabolism does not proceed to carbon dioxide and water under reduced oxygen levels, but stops with the formation of aldehydes, organic alcohols, and acids. If the system needs adequate buffer, the organic acids weaken the pH to the more favourable range for fungi. Thus, it can be visualize that low oxygen tension and pH can be interrelated. Most of fungi grow at pH 4 to 5 while some bacteria are allowed to grow well sufficient to compete. Fungi need less nitrogen than bacteria per unit mass of protoplasm. The fungi are able to integrate in nitrogen deficient wastes, more active masses of protoplasm than the bacteria from the wastes and predominate. Bacteria average 10 to 12% nitrogen while fungi range from 5 to 6% nitrogen approximately. Fungi will be present and will aid in the stabilization of the organic matter under normal environmental conditions. But the fungi are of secondary importance and will not predominate. Hence, microorganisms are vital to humans and the environment, as they participate in the nitrogen and carbon cycles, as well as fulfilling other vital roles like recycling other organisms' dead remains and waste products through

decomposition. Microorganisms also have an important place in higher multicellular organisms as symbionts (Mohawed et al., 1986).

CONCLUSION

Municipal waste is a blend of various substrates in this way it is a perfect advancement media for the development of various microorganisms. Microorganisms in this environment are metabolically dynamic which prompts the creation of different chemicals and bioactive compounds contrasted with other ecological condition. Therefore, it is important to understand the waste derived microorganisms in ecological terms and also as a resource for biotechnology. This review paper revealed that municipal waste dump site is a potential source for wide spectrum of microorganisms and industrial enzyme producing bacteria. Moreover the utilization of microorganisms with strong degrading capability for processing MSW would be of great economic and aesthetic value to the environment since most metropolis are littered with MSW without any proper processing for further usage.

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Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Vietnamese High School Students' Perception of Self-Esteem

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Received: 18 Feb 2020 Revised: 20 Mar 2020 Accepted: 22 Apr 2020

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ABSTRACT

The primary purpose of this research was to explore Vietnamese high school students' perception of self-esteem. The sample was composed of 339 students (130 boys and 209 girls from grades 10, 11, and 12) of 16–18 ages attending five high schools in Ho Chi Minh City, Vietnam. They completed the self-Esteem scale of Toulouse in Viet Nam, which included Physical self, Academic-Future self, Emotional self, Social Self, and Family self. The Vietnamese people group's research then gathered data. Descriptive and inferential statistics were used to analyze the data. Cochrane methodology was applied to determine the sample size, and the MANOVA test was used to assess differences between each group. The results found that regarding the self-esteem towards research scores, the self-esteem of males was found to have higher than females. Moreover, the self-esteem grade 11 that found to be higher than those of grade 10 and grade 12 on the self-esteem towards research scores. The high school students who had gone under the research showed, and they would be interested in self-esteem family self and physical self-more than Academic-Future self, Emotional self, and Social self.

Keywords: Self-Esteem, High school, Student.

INTRODUCTION

Self-esteem is described as the positive or negative reflection which the person has for himself and the belief that he can cope with the fundamental challenges of life (Galanou, Galanakis, Alexopoulos, & Darviri, 2014). This is an excellent method for collecting data for use in this research ofOrth and Robins (2014) found that: (a) Self-esteem increases from teen to middle adulthood, (b) Self-esteem is stable over time, (c) Prospectively, high self-esteem predicts achievement and well-being in life areas such as relationships, employment, and health(Orth & Robins, 2014). This was based on previous experience within the research team that showed that the self-esteem for students

had a relatively small negative relation with anxiety(Stupnisky, Perry, Renaud, & Hladkyj, 2013). Compared to a similar area of research self-esteem perceived control, the findings are a more powerful predictor of the GPA for first-year university students(Stupnisky et al., 2007). An essential component of the research is data collection and analysis of self-esteem partially mediated social support's influence on life satisfaction and positive influence, while fully resolving social support's impact on adverse effects. However, in many instances, such metrics can take different life satisfaction values or influences between higher and lower social support groups when global self-esteem is low (Kong, Zhao, & You, 2013). Several research groups have been working on the design Mendelson and White (1985)indicate on three age groups (8.5–11.4, 11.5–14.4, and 14.5–17.4 years) these were used to analyse the development of overweight and average-weight self-esteem. There were 47 females and 50 males; 48 were overweight in that. The findings of this preliminary research showed similar self-esteem at a younger age, excess weight, and healthy weight. Obese males adversely affected self-esteem in the middle-age but not in overweight females. Self-esteem in overweight females was impaired at the oldest age but not in overweight males. Overweight at all ages had a lower body-esteem than average weight did(Mendelson & White, 1985).

Galanou et al. (2014) found that women can have higher self-esteem than men(Galanou et al., 2014). However, Orth, Trzesniewski, and Robins (2010) indicate found that women had lower self-esteem than did men in young adulthood. Moreover, previous research has found that about the self-esteem students German high school (M = 19.6 years, SD = 0.9; 55% female) the show's development of self-esteem yet shows interdependencies with the accomplishment of age-specific challenges in the transition to young adulthood(Wagner, Lüdtke, Jonkmann, & Trautwein, 2013). Research in this area is of great interest, and more optimism and self-esteem were less stressed than those who were more pessimistic or lower self-esteem(Hewitt, 2009). The results suggest that self-esteem has a significant prospective impact on real-world life experiences and that high and low self-esteem are not mere epiphenomena of success and failure in prestigious life domains(Orth, Robins, & Widaman, 2012). However, the application of the Self-Esteem Scale of Toulouse (ETES) in Vietnam for measuring self-esteem(Trinh, Tran, & Ngo, 2017)in Vietnamese high school students is still limited. We have been conducting studies to fill this gap further. Our research is conducted to empirically explore high school students' self-esteem in Ho Chi Minh City, Vietnam.

METHOD

Research Design

A 5×2 factorial design was used. The independent variables were two students' characteristics: grate (grade 10, grade 11, grade 12), and gender (male and female). Five dependent variables were measured: Physical self (PS), Academic-Future self (AF), Emotional self (ES) Social self (SS), and Family self (FS). The following null hypotheses were tested: H_{01} (main effect): There is no significant difference between male and female groups of students when they are compared simultaneously on the Physical self (PS), Academic-Future self (AF), Emotional self (ES) Social self (SS), and Family self (FS). H_{02} (main effect): There is no significant difference between grade10, grade 11 and grade 12 groups of students when they compared simultaneously on the Physical self (PS), Academic-Future self (AF), Emotional self (ES), Social self (SS), and Family self (FS). H_{03}(interaction effect): There is no significant interaction between grades and gender groups of children when they are compared simultaneously on the Physical self (PS), Academic-Future self (AF), Emotional self (ES) Social self (SS), and Family self (FS).

Research Sample

The convenience sampling method was used to recruit students who volunteered to help with the research and administer the survey. The survey instrument was distributed to 350 Vietnamese participants of five high schools located in Ho Chi Minh City, Vietnam, of which 339 surveys were returned, for a 96.5% return rate, which exceeds the 30% response rate most researchers require for analysis(Dillman, 2000). The sample of this research was drawn

from 339 respondents who completed the survey instrument. There were more girls (61.7%) than boys (38.3%) among the 339 Vietnamese participants who were surveyed. Of these, 116 (34.2 %) were grade 10, 117 (34.5%) were grade 11, and 106 (31.3%) grade 12. Table 1 shows the distribution of participants in grade by gender.

Research Instruments and Procedures

Participants were asked to complete the following questionnaire: the Vietnamese versions of the Self-Esteem Scale of Toulouse (ETES) in Viet Nam for students based on the original(Trinh et al., 2017). The ETES consists of five subscales: Physical self (PS), Academic-Future self (AF), Emotional self (ES) Social self (SS), and Family self (FS). All participants were instructed to read the questionnaire questions carefully and choose the responses that best described themselves. The ETES consists of 33 items measured on a 5-point Likert scale in which the 1 indicates a response of 'strongly disagree', while the value of 5 corresponds to 'strongly agree'. The internal consistency reliability (Cronbach's alpha) estimate for this sample was high at 0.92(Bowling, 2014). Alpha coefficients for each subscale were as follows: Physical self (PS):0.9, Academic-Future self (AF): 0.87, Emotional self (ES):0.9, Social self (SS):0.88, and Family self (FS):0.92 (Linh, T.T., Huong, T.T. and Trang, 2017). The internal consistency reliability estimate for this sample was .92. Then calculated the scores for scale, as well as the sum of all questions on the students' Self-Esteem Scale of Toulouse in Ho Chi Minh, Viet Nam.

Data Analysis

Descriptive and inferential statistics (Cohen 1988) were performed using the Statistical Package for the Social Sciences (SPSS) version 20.0. Descriptive statistics were used to analyze the data collected. A two-way MANOVA was presented with two independent variables (grade and gender) and subscales of The Self-Esteem Scale of Toulouse (ETES) as dependent variables. The average item means average standard deviation, F values, and effect from MANOVA were calculated for each of the scales of the ETES. These analyses were used to investigate differences in the Physical self, Academic-Future self, Emotional self, Social self, and the Family self of Vietnamese high school according to grade and gender.

RESULTS

Descriptive Analysis

According to the norms from the ETES, the participants scored in the average range on the Self-Esteem Scale of Toulouse. The mean score for the sample on the ETES (total score) was PS 97.55 (SD =11.70). The mean score for the AF subscale was 14.93 (SD =3.22). The mean score on the ES subscale was 15.44 (SD = 3.67). The mean score on the SS subscale was 10.40 (SD =2.00). The mean score on the FS subscale was 34.84 (SD = 5.02). Table 2 presents descriptive statistics of dependent variables including PS, AF, ES, SS, and FS results by grate and gender groups.

Inferential Analysis

The null hypotheses were tested using a two-way multivariate analysis of variance (MANOVA). To use MANOVA, the multiple dependent variables should be related to each other at a low to a moderate level(Pallant, 2016). More specifically, a high correlation (.50 to 1) among dependent variables shows multicollinearity and small to medium correlation (±.10 to ±.49) among dependent variables show singularity. Table 3 revealed that all values were more than -.16, which provides controlling singularity assumption. Besides, Pallant (2016)stated that correlations around .80 or .90 cause violation of multicollinearity assumption. Since all values were under .80, multicollinearity, the assumption was also checked.

The researchers tested all the assumptions, and the results were positive except the Box's test of equality of variance. MANOVA is robust to violations of homogeneity of variance/covariance matrices if the sizes of groups are nearly equal or if the size of the largest group is less than about 1.5 times the size of the smallest group(Leech, Barrett, & Morgan, 2005). Although the largest group in this research (n = 79) was about 2.10 times larger than the smallest group (n = 38), the multivariate homogeneity of covariance matrices tested with Box's M test revealed that the M value of 67.098 was not significant (p = .805). Therefore, the assumption of homogeneity of covariance matrices was satisfied.

The MANOVA revealed a significant multivariate effect for the grade group, Wilks' lambda = .99, F(10, 658) = .319, p < .01, partial η2 = .976, and a non-significant multivariate effect for gender, Wilks' lambda = .966, F(5, 329) = 2.34, p > .05, partial η2 = .034. A non-significant multivariate effect for interaction was also found, Wilks' lambda = .974, F (10, 658) = .88, p > .05, partial η2 = .013. Therefore, the results suggested that the first hypothesis (Ho_1) was rejected, but the second hypothesis (Ho_2) and third hypothesis (Ho_3) were not rejected. Based on the significant effects found from the MANOVA, a separate two-way univariate analysis of variance (ANOVA) for each of the dependent variables was conducted without undue inflation of the experiment wise Type I error(Grimm & Yarnold, 1995). The Levene's test revealed that the assumption of homogeneity of variances was met for SS[F(5, 333) = .36, p > .05], FS [F(5, 333) = .30, p > .05], AF [F(3, 333) = .55, p > .05], ES [F(3, 333) = .58, p > .05] and for PS [F(5, 333) = .77, p > .05].

The ANOVA results as shown in Table 4 revealed the interaction effect was non-significant (p >.05). A significant grade effects on AF [*F(1, 339)* = 2.02, *MSE =10.40, p* <.84, Partial η2= .01], SS [*F(1, 339)* = 1.06, *MSE =4.03, p* <.30, Partial η2= .03], FS [*F(1, 339)* = 0.02, *MSE =25.5, p* <.96, Partial η2= .00], ES [*F(1, 339)* = 7.20, *MSE =25.5, p* <.157, Partial η2= .006], PS [*F(1, 339)* = 5.0, *MSE =26.92, p* <.26, Partial η2= .015] among the grade 10, grade 11 and grade 12 (grade 10: M_{AF}= 15.13, M_{SS}=10.22, M_{FS}=34.67, M_{ES}= 15.24, M_{PS} = 21.90, M $_{total}$=19.42grade 11:, M_{AF}= 14.86, M_{SS}=10.44, M_{FS}=35.14, M_{ES}= 15.44, M_{PS} = 22.38, M $_{total}$=19.65; grate 12:, M_{AF}= 15.00, M_{SS}=10.47, M_{FS}=34.70, M_{ES}= 15.34,M_{PS} = 22.10, M $_{total}$=19.52). Significant gender effects on AF [*F(1, 339)* = 7.21, *MSE* = .73, *p* < .008, Partial η2= .03], ES [*F(1, 339)* = 7.20, *MSE* = 13.30, *p* < .008, Partial η2= .021], SS [*F(1, 339)* =1.06, *MSE =4.03, p* < .03, Partial η2= .003], FS [*F(1, 339)* = .002, *MSE* = 25.5, *p* < .96, Partial η2= .00], ES [*F(1, 339)* = 7.20, *MSE* = 13.30, *p* < .008, Partial η2= .021], ES [*F(1, 339)* = 7.20, *MSE* = 13.30, *p* < .008, Partial η2= .021], PS [*F(1, 339)* = 5.0, *MSE* =26.91, *p* < .26, Partial η2= .015] among males and females (Male: M_{AF}= 15.25, M_{SS}=10.26, M_{FS}=34.85, M_{ES}= 14.80, M_{PS} = 22.81, M $_{total}$=19.59; Female:, M_{AF}= 14.74, M_{SS}=10.45, M_{FS}=34.82, M_{ES}= 15.88, M_{PS} = 21.50, M $_{total}$=19.48).

DISCUSSION

The main goal of the present research was to explore high school students' perception of self-Esteem in Ho Chi Minh City, Vietnam. The main findings indicate that there was the relationship between five ETES subscales: Physical self (PS), Academic-Future self (AF), Emotional self (ES), Social self (SS), and Family self (FS). It is a crucial conclusion for further upcoming studies advanced regarding self-esteem among Vietnamese high school students that need to be conducted as soon as possible with high efficiency. This research reported that high school students' perception of self-esteem was high. We also found that there was no significant difference between male and female groups of highschool students when they are compared simultaneously on the ETES subscales. This result different from what has been found in the previousresearch that in females on self-esteem was higher than males (Orth et al., 2010). Orth et al. (2010) found that females had lower self-esteem than thesemales in young adulthood. Our research also indicated that grade 11 higher is self-esteem was grade 10,12 higher. Although more research is needed to investigate whether high school students' have a different self-Esteem might be more pronounced in Vietnam, the results suggest that perceptions may prove essential to understanding the self-esteem in Vietnamese high school students.

This research has several limitations. The primarydefect arises from the sampling process used. The sample was drawn from only one city of Ho Chi Minh City, Vietnam. The random selection of participants alleviates this concern

to a significant degree but does not entirely remedy that shortcoming. The second limitation is related to the sample and the self-reported measurements. It might bias the findings as well, and was cross-sectional research, which does not allow. Future studies should address these limitations. All results obtained after this research are necessary for the cognition of high school students in ho chi Minh city, Vietnam, about self-esteem. The research is done with an expectation of acting as stimulation in extending similar investigations on bridging the gap between research and its needed practices in the high school in Vietnam.

CONCLUSION

The current research explored the self-esteem of high school students in Ho Chi Minh, Vietnam. It shows that the self-esteem of high school students is high. It is the first research to investigate the understanding of self-esteem by high school students in Ho Chi Minh City, Vietnam, to the best of authors' knowledge. All results obtained after this research are necessary for them to enhance the self-esteem of high school students in Ho Chi Minh City, Viet Nam. In addition to the minimal research on this in Vietnam, Vietnamese school educators need to get a better understanding of their tasks.

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Table 1: Number of participants in the grade by gender

Grate		Gender group		
		Male	Female	Total
	Grate 10	44	72	116
	Grate 11	38	79	117
	Grate 12	48	58	106

Table 2: Number of participants in grade by gender groups

Gender	Grader Group				
		Grade 10	Grade 11	Grade 12	Combined
Male		44	38	48	130
PS					
M		22.80	23.50	22.12	22.75
SD		5.893	4.354	5.334	5.262
AF					
M		15.68	14.97	15.10	15.26
SD		3.581	3.166	2.868	3.200
ES					
M		14.95	15.03	14.40	14.77
SD		3.766	3.149	3.407	3.447
SS					
M		10.16	10.37	10.25	10.25
SD		2.011	2.307	1.896	2.047
FS					
M		35.00	34.92	34.63	34.84
SD		4.700	5.380	5.397	5.129
Female		72	79	58	209
PS					
M		20.99	21.27	22.26	21.44
SD		4.772	5.448	5.128	5.136
AF					
M		14.57	14.75	14.90	14.73
SD		3.280	3.123	3.323	3.221
ES					
M		15.53	15.85	16.28	15.86
SD		3.849	3.796	3.558	3.744
SS					
M		10.28	10.51	10.69	10.48
SD		1.908	2.044	1.958	1.971
FS					
M		34.35	35.37	34.76	34.85
SD		4.804	4.907	5.279	4.974

Table 3: Correlations matrix

	PS	AF	ES	SS	FS
PS	1	.46**	-.12*	.14*	.26**
AF		1	-.16**	.22**	.21**
ES			1	.27**	.35**
SS				1	.33**
FS					1

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Table 4: Combined univariate ANOVA table

Source	Dependent Variable	Type III Sum of Squares	Df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	SS	10.487[a]	5	2.097	.520	.761	.008
	FS	43.406[b]	5	8.681	.340	.888	.005
	AF	38.472[c]	5	7.694	.740	.594	.011
	ES	123.441[d]	5	24.688	1.860	.101	.027
	PS	233.611[e]	5	46.722	1.736	.126	.025
Intercept	SS	34093.387	1	34093.387	8457.732	.000	.962
	FS	384366.141	1	384366.141	15066.426	.000	.978
	AF	71218.569	1	71218.569	6848.400	.000	.954
	ES	74510.545	1	74510.545	5613.489	.000	.944
	PS	155462.720	1	155462.720	5775.586	.000	.945
Gender	SS	4.265	1	4.265	1.058	.304	.003
	FS	.047	1	.047	.002	.966	.000
	AF	21.051	1	21.051	2.024	.156	.006
	ES	94.364	1	94.364	7.109	.008	.021
	PS	134.495	1	134.495	4.997	.026	.015
Grade	SS	4.020	2	2.010	.499	.608	.003
	FS	14.800	2	7.400	.290	.748	.002
	AF	3.727	2	1.864	.179	.836	.001
	ES	2.034	2	1.017	.077	.926	.000
	PS	13.092	2	6.546	.243	.784	.001
Gender* Grader	SS	1.710	2	.855	.212	.809	.001
	FS	17.107	2	8.553	.335	.715	.002
	AF	14.349	2	7.174	.690	.502	.004
	ES	25.531	2	12.765	.962	.383	.006
	PS	83.456	2	41.728	1.550	.214	.009
Error	SS	1342.334	333	4.031			
	FS	8495.308	333	25.511			
	AF	3462.967	333	10.399			
	ES	4420.070	333	13.273			
	PS	8963.434	333	26.917			
Total	SS	37965.000	339				

	FS	420112.000	339				
	AF	79088.000	339				
	ES	85354.000	339				
	PS	172482.000	339				
Corrected Total	SS	1352.820	338				
	FS	8538.714	338				
	AF	3501.440	338				
	ES	4543.510	338				
	PS	9197.044	338				

a. R Squared = .008 (Adjusted R Squared = -.007)
b. R Squared = .005 (Adjusted R Squared = -.010)
c. R Squared = .011 (Adjusted R Squared = -.004)
d. R Squared = .027 (Adjusted R Squared = .013)
e. R Squared = .025 (Adjusted R Squared = .011)

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Study on Morphology and Taxonomic Classification of Ectoparasite

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Received: 06 Feb 2020 Revised: 08 Mar 2020 Accepted: 10 Apr 2020

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ABSTRACT

Ectoparasites of mammalian hosts are the causative agents of various diseases. Many ectoparasites are known to be vectors of pathogens, which the parasites typically transmit to hosts while feeding or defaecating.Ectoparasites are organisms which inhabit the skin or outgrowth of the skin of another organism (the host) for various periods, and may be detrimental to the latter. A wide variety of crustaceans feature prominently as fish ectoparasites and some species adversely affect domestic fish stocks.There are almost 18 genera, about 900 species. Mainly 2 families are seen i.e., *Ixodidae* (hard tick)-700 species, and *Argasidae* (soft ticks)-200 species.Another rare family is Nutalliellidae-1 species.They have 4 stages of life cycle: egg, larva, nymph andadult. *Ixodidae* family has a hard dorsal scutum or plate with mouth parts anteriorly attached and visible dorsally. They are 3-5mm in size, 6 legs; each having 4 segments, 2 antennae and 3 black spots on abdominal region. For the study of morphology and taxonomic classification of ectoparasites, the samples of ectoparasites are collected from different animals and the morphological characters are studied. For the taxonomical classification of the ectoparasites the study of their body parts, habits, and habitat are studied. The collection of the sample includes the specimens of ectoparasites from cows, goats, human, buffaloes. The ectoparasites collected are of different species and both males and females. After the collection of samples they are preserved in formalin. For the morphological study the specimen collected are kept under the microscope. Each body parts are separately marked and recorded.This study will help in identifying the new ectoparasitic species. It will identify the specimen and helps to place under the taxonomic key of classification.The process of identifying the ectoparasites will help in further future studies and related diseases caused by the ectoparasites. It will help in future to identify new species of ectoparasites.

Keywords: Ectoparasites, Morphology, Taxonomic, Classifications, Tick, *Ixodidae*, *Argasidae.*

INTRODUCTION

Ectoparasites are organisms which inhabit the skin or outgrowths of the skin of another organism (the host) for long periods and may be detrimental to the latter. Ticks were considered parasites of domestic animals as early as 400 B.C

(Dobbelarece D, and Heussler V, 1999). Various ectoparasites cause significant infestations in many kinds of domestic animals including livestock, pets, laboratory animals, poultry, fish and bees (Flynn,1973 and Hopla, 1982).Manyof these ectoparasites (e.g. most lice) are host specific, while others (e.g. many ticks) parasitize a wider range of hosts. Several ectoparasites currently associated with domestic animals have been acquired by the introduction of either host or parasite into new regions, as animals have become domesticated throughout the world (Hopla, 1982).For example, cattle, goats, and other important domestic livestock species have been introduced into much of Africa, where they may now fall victim to the ravage s of native tsetse flies (*Glossina* spp.) and ticks, as well as pathogens transmitted by these parasites. Relatively rapid intercontinental transportation of these animals has compounded the problem. The vast majority of ectoparasites are invertebrates. Most invertebrate ectoparasites are arthropods; insects and arachnids typically parasitize terrestrial domestic animals, while crustaceans are associated with fish. (Hopla*et al*.,1994).

Arthropods constitute the largest animal phylum, and yet a relatively small number of species are directly or indirectly related to public health (Fritsche, 2003). Still, the relative importance of medical and public health entomology seems to be increasing (Goddard,2012) with a worldwide resurgence of certain arthropods like bed bugs (Doggett *et al*., 2012) and expanding ranges of others (e.g., mosquitoes and ticks). There are many ways in which arthropods can be of public health or medical importance. Most notably, they can be biological vectors of disease-causing organisms, including those organisms that cause malaria, filariasis, yellow fever, dengue fever, plague, babesiosis, typhus, lyme disease, chagas disease, and many others. Other arthropods are passive carriers (i.e., mechanical vectors) of disease-causing organisms, for example, flies transporting pathogenic enteric bacteria from feces to foodstuffs (Ebling, 1978). Several species are ectoparasites like Scabies mites, Chiggers, Lice, Ticks, and Fleas) or sub-dermal or visceral parasites such as myiasiscausing flies, Tunga fleas, and pentastomids (GA, 2012,) and may cause localized pain, itching, dermatitis, or vesicular eruptions. Other arthropods may also be nuisance pests and cause similar dermatologic manifestations by incidental contact of millipedes, stinging caterpillars, and blister beetles (Hellier andWarin, 1967), biting and stinging are horse and deer flies, biting midges, bed bugs, avian mites, and ants, respiratory problems from inhalation by cockroaches and dust mites and their feces).

In particular, envenomation by bee, ant, and wasp stings can be potentially deadly for patients who have a hypersensitivity to their venom (Vetter and Visscher, 1998), while the bites and stings of spiders, scorpions, centipedes, and others can also be dangerous and even deadly. The brown dog tick *Rhipicephalus sanguineus* is the most widespread tick in the world, even considering that many ticks currently identified as *Rh. sanguineus* might actually represent other closely related species of *Rhipicephalus turanicus*. This tick is a parasite of dogs that can occasionally parasitize other hosts, including humans. Moreover, *Rh. sanguineus* is a vector of many disease agents, some of *Coxiella burnetii, Ehrlichia canis, Rickettsia conorii*, and *Rickettsia rickettsii* are being of zoonotic concern (Dantas, 2008). Ticks belong to an ancient lineage, with specimens found in Burmese amber dating back to about 100 million years ago (Mans *et al*.,2011). They are a very successful group of arthropods and have adapted to feeding on practically every terrestrial mammal, bird and reptile, and have attacked humans and infested the animals that they have domesticated for thousands of years. During the past 150 years they have become an important concern of veterinary and medical research, not only because of the direct effects they have on their hosts, such as injury at their points of attachment, blood loss and paralysis caused by toxins in their saliva, but also because they are efficient vectors of a wide variety of micro-organisms.Many ectoparasites are known to be vectors of pathogens, which the parasites typically transmit to hosts while feeding or defaecating. Ectoparasites are organisms which inhabit the skin or outgrowth of the skin of another organism (the host) for various periods, and may be detrimental to the latter. A wide variety of crustaceans feature prominently as fish ectoparasites and some species adversely affect domestic fish stocks. There are almost 18 genera, about 900 species. Mainly 2 families are seen i.e. *Ixodidae* (hard tick)-700 species, and *Argasidae* (soft ticks)-200 species.Another rare family is Nutalliellidae-1 species and they have 4 stages of life cycle: egg, larva, nymph, adult.

Ixodidae family have a hard dorsal scutum or plate with mouth parts anteriorly attached and visible dorsally. They are 3-5mm in size, 6 legs, each having 4 segments, 2 antennae and 3 black spots on abdominal region. The focus of this review is to help provide useful information for the handling, processing, identification, and reporting of ectoparasitic arthropods routinely submitted to clinical or reference diagnostic laboratories for identification, with an emphasis on the arthropods encountered in the United States. These include hard ticks, soft ticks, scabies mites, chiggers, demodex mites, zoonotic biting mites, lice, bed bugs, and fleas. Arthropods commonly encountered by tourists in countries outside the United States, such as *Tunga* sp. and myiasis-causing flies, are also discussed. Most of the arthropods submitted to clinical and diagnostic laboratories are those that reside for a length of time on or near the human host for most or part of their life cycle (e.g., ticks, lice, myiasiscausing flies, fleas, scabies mites, bed bugs, and biting mites). Adult forms of blood-feeding flies (e.g., mosquitoes and biting midges, etc.) may not be typically submitted for such identification. Ticks are the most important group of vectors of pathogens of phylum Arthropoda. (Hoogstrall,1985; Marcondes,2009)

Globally a total of close to 900 tick species have been described, of which slightly more than 700 species belong to the *Ixodidae* or hard ticks and the remainder to the *Argasidae* or soft ticks. Ticks are particularly abundant in the Afrotropical region with its rich animal fauna and climatic zones ranging from arid to tropical. Approximately 200 ixodid tick species (hard or shield ticks) and 40 argasid tick species (soft ticks or tampans) are present in the Afrotropical region, but only a small number are of veterinary and medical importance. Many of the ticks and tick-borne diseases occur usually in specific geographical areas but with globalisation and climate change their range may expand and may even spread intercontinentally. Although it is common to consider domestic animals as being the preferred host of ticks, most species occur on wildlife, and several would not be able to complete their life cycles without the presence of small wild mammals or birds as hosts for their immature stages. Most importantly, at the livestock/wildlife interface transfer of tick-borne pathogens frequently occurs and poses a risk to livestock farming and development. (Walker and Bouattour, 2003.)

There are two parasitic species of lice on the human host. They are further divided into two sub-species based on location of host and biological differences (Kim *et.al.,* 1986). Approximately 450 species of sucking lice are identified. Lice are wingless insects which are classified either as a single order of Phthiraptera or as two order (Anoplura-sucking lice and Mallophaga-chewing or biing lice). Only about 20 of these species are pests of domestic animals, they can occur in huge numbers which can cause host irritation or dermatitis. Two sucking louse genera are: *Haematopinus* and *Linognathus*. Their body size is about 1-11mm and they are host specific. The body structure of lice is divided into three parts;thorax- 3 regions called pro thorax, meso-thorax, meta thorax; abdomen- 9 segments; head- 3 segments. (Kim and Pratt, 1986).

The head lice are not agent disease causing factors and are very common tend to seen in teenaged girl children (Veracx and Raont, 2012). Some of the lice are blood sucking, these lice are generally more adept at transmitting pathogens to domestic animals than are chewing lice, although chewing lice on cattle can also transmit *T. verrucosum*. The chewing lice (Mallophaga) may be classified into three groups; Ischnocer, Amblycera and Rhynchophthirina. Chewing lice are more diverse than the sucking lice with approximately 2,600 species described world-wide. The male and female are differentiated by some characteristic differentiation, in male the male genitalia is clearly visible than female. The female has setae and vulva with a round structure. Male and female varies from species to species and female is bigger and broad than male.

MATERIALS AND METHODS

Collection of Samples

For the study of ectoparasites the specimen collection is the major part to study the morphology of ectoparasites. The specimens for the study are collected from different animals like cows, buffalos, goats, and human ticks. The

specimens ware collected from Centurion University of Technology and Management, Jatani, Bhubaneswar farm and from Bhadrak district. The ectoparasites are the outgrowth of animals, they are carried out from the animals by using hands with gloves. They are collected and kept in a jar with a lid over it so that they can't escape from the jar. Ectoparasites from goats are also collected and kept in jar with a lid. Samples of ectoparasites of human beings are also collected from the head of a girl child.

Preservation

All the specimens are were collected and preserved in a jar having alcohol or formalin. Ticks of humans, ticks of cows, buffaloes, and the ticks of goats are kept in a jar which should have lid over it. For the storing of the specimens the samples are kept in wet preservation. For the preservation of lice the permanent slides are prepared. Permanent slides are prepared of glass or plastics, slides are approximately 1x3 inches and between 1mm- 1.2mm thick.

- Selection of specimens
- Decolorisation- 20% aqueous KOH solution.
- Neutralization- 10% aqueous acetic acid for 30-40 minutes.
- Staining- highly concentrated aqueous acid fuchsin for 8-16 hours.
- Dehydration- 40%, 70% and absolute (or 96%) ethanol for 30-40 minutes in each concentration.
- Clearing- pure clove oil for 24 hours at least.
- Dissection- in clove oil.
- Labellingof slides.
- Mounting- neutral Canada balsam in xylene.
- Drying- in oven 50-55 degree for 3 weeks at least.

Identification

The specimens collected before are taken in a petri plate with alcohol, the specimens are separated by looking to their outer structure under Laicastereosome S91. The identification of lice is done by the microscopes where the permanent slides of lice are prepared. Photographs of dorsal, ventral side, mouth part of the specimens, anal parts, leg segments of the specimen is taken and some outer important structures are captured. After differentiating the specimens are compared with the identification key present before. The identification is done according to their anterior, posterior, leg segments, anal part, mouth structure they are identified into different taxonomical orders.

RESULTS

The specimens that collected from the cows, goats, and humans are identifiedas; *Ixodidae ixodes, Ixodus ricinus, Boophilus annulotus, Rhipicephalus sanguinecy, Humanus pediculus, Hyalomma anatolicum, Hyalomma dromendarri***.**

Ixodidae ixodes

Characters: It is commonly known as black-legged ticks, six legged. Mouth parts are long, with relation to basis capituli, dorsal shield is inornate. There no festoons or eyes, inverted U shaped anal groove. Their length is about 3 to 4mm, mouthparts are anterior. Scutum is present in the female and a conscutum in male. The samples are collected from cows.

Ixodes ricinus

Characters: It is a hard bodied tick, relatively small ticks where females are slightly larger than female. They have a

sclerotized dorsal plate known as scutumthat protects them from damage. It lacks eyes but consisting a sensory organ called Hallers organ. It is a sucking type tick that sucks blood from host to moult to the next stage and produce eggs. The samples are collected from cows.

Boophilus annulatus

Characters: These are short, compact palps with lateral ridges and a hexagonally shaped basis capitulum. It is having shades of brown and inornatescutum. Adult ticks has eyes and oral grooves which is present in front or anterior of anus. The samples are collected from cows.

Rhibicephalus sanguineus

Characters: The scutum and conscutum are yellowish to reddish brown in color. The eyes are slightly convex. The males are having marginal grooves and are sharply visible. The body wall of male has a salmon pink color and at the time of engorged it extends beyond the conscutum. In females the cervical fields are slightly depressed and scalpel-shaped. It is collected from cows.

Haemaphysalis

Adult *Haemaphysalis* black in color with short and broad mouthparts. The legs are paler and may be ringed by paler bands. Eyes are absent and it is a three host tick.The host is goat where the tick completes its life-cycle, the postero-median groove is deep and narrow, also known as hard tick. The samples are collected from goats.

***Pediculushum anuscapitis*(head lice)**

Characters: It is generally confined to the scalp, and commonly known as head louse mainly seen in human. They are soft bodied and wingless. Body is dorsoventrally flattened, eyes and antennae are present. Mouth is sucking type and also piercing mouthparts are present. Three pair of legs that helps in grasping hairs. The samples are collected from human head.

DISCUSSION

Determination of the species of tick by its size because all ticks are extremely tiny in their immature stages like larva and nymph get progressively larger as they mature through their life cycle of adult males and femalesand larger still as they become engorged with blood. Some of the soft tick species exhibits extremely rigid host specificity nature and some *Argasidae* ticks are may be resistant to starvation without taking blood meal for many of the years. Identification of tick species is an important step in epidemiological studies, in order to establish tick species distribution maps and to characterise tick fauna. (Dantas-Torres F, Chomel BB and Otranto D, 2012). Ticks that have been successfully identified as ixodesscapularis the blacklegged tick (deer tick) can be tested for *Borrelia burgdorferi*, the causative agent of Lyme disease. The tick is tested using a DNA based technique known as Polymerase Chain Reaction (PCR). The tick can be tested whether it is alive or dead (Drummond and Roger, 2004). Ixodid ticks are characterized by the presence of a dorsal shield (scutum) and by having their mouthparts visible from above (Keirans JE and Litwak TR, 1989).Ixodid ticks typically takes blood meals for their developmental processes. In male Ixodid ticks, the dorsal shield covers most of the dorsum, whereas in the females, it is usually restricted to the anterior third. Key characteristics in identifying ixodid ticks to the genus level include the length of the mouthparts (palps, in relation to the basis capituli), presence or absence of eyes, presence or absence of festoons, color or markings on the dorsal shield, and shape and orientation of the anal groove. Ixodid ticks typically take one blood meal in each of the developmental stages. It is typically the female tick that takes a blood meal and is therefore the

greatest threat for disease transmission (Mathison and Pritt, 2014). The following key will help facilitate identification of medically important Ixodid ticks to the genus level. References for regional keys for species-level identification are given below for each genus under their individual section (Keirans andLitwak, 1989).

- Inverted, U-shaped anal groove extending anteriorly around the anus, eyes absent; festoons absent in Ixodes. Anal groove never extending anteriorly around the anus, eyes present or absent, festoons present or absent.
- Eyes absent—Haemaphysalis.
- Basis capituli hexagonal, posteriorly directed inwards in Rhipicephalus. Basis capituli rectangular.
- Palps is about as long as basis capituli; second palpal segment about as long as it is wide Dermacentor. Palps much longer than basis capituli; second palpal segment longer than it is wide.
- Dorsal shield ornate, coxa I with large paired spurs of unequal length, festoons regular in *Amblyomma* sp. Dorsalshield inornate, coxa I with large paired spurs of equal size, festoons irregular in *Hyalomma* sp.

The *Ixodidae* family mainly seen in cattles like cows, rabbit etc., they lay eggs and hatched into 6 legged larvae. The larvae gets attached to the host body and feed on the first host body. The larvae molt into nymph and leave the first host and attached to the second host. Then after leaving the second host the nymphs of ixodidaemolt into adults in summer season. At the arrival of spring the adult ticks attached to the third host and mating is started (Walker and Bouattour, 2003). Argasid ticks lack a dorsal shield in their body and have their mouthparts hidden above when viewed dorsally (Cooley and Cohls, 1944). The nymphs and adults of *Argasidae* do not reside on the, but feed for a short time period of timebefore returning to the sheltered location. Unlike ixodid ticks, they are intermittent feeders and do not remain on the host for a prolonged length of time. Their habits are very similar to those of bed bugs, given that they feed for very brief periods of time, spending most of their time in secluded areas (cracks and crevices in homes, rodent burrows, and under rugs and carpeting, etc.) Because of these secretive habits, they are not routinely submitted to diagnostic laboratories for identification. Adults are oval in shape and dorsoventrally flattened. They typically have a very rough, granulate texture to their dorsal tegument. Medically, the most important species are in the genus *Ornithodoros*. Several argasid ticks in the genera *Argas* and *Carios* that normally feed on bats and birds may come into homes and feed on humans in the absence of their normal host.

The life cycle of *Argasidae* ticks starts after the eggs hatched into 6 legged larvae and attached to their first host and starts feeding on them. The larvae startsmolting into first nymphal stage after leaving the first host and feed on a second host. The nymphs' then move out from the host and molt in shelter area and feed on a third host and the cycle is repeated up to the seven nymphal stages. After the 2-7 nymphal stages they leave the last host and molt into adults in the shelter area. Adults feed on the host and the process of mating and egg laying started (Cooley and Kohls, 1944). The Pediculus humanus capit is works similarly to the body louse but is generally confined to the scalp only (Goddard, 2012). The life cycle of Pediculosis(head lice and body lice) lays eggs by the adult lice, the eggs molt into first nymph, second nymph, third nymphs and finally to adults. They show growth and development in these three nymphal stages. The adult female grows up to 3-4mm, with six legs ending in raptorial claws, eyes present, one pair of antennae, wings areabsent and the male is smaller than the female and cause irritation and itching in human head (Goddard,2012).

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Fig.1. Sample collected from cow

Fig.2. Sample collected from cow

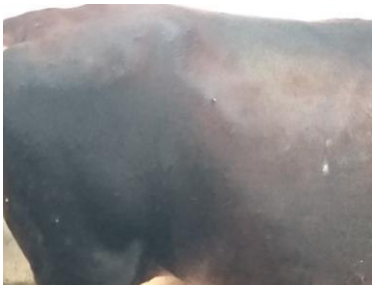

Fig.3. Ticks in the cow

Fig.4. Collected sample preservation in laboratory

Fig.5. *Ixodidae ixodes* dorsal view

Fig.6. *Ixodidae ixodes* Ventral view

Fig.7. *Ixodes ricinus* Dorsal view

Fig.8. *Ixodes ricinus* Ventral view

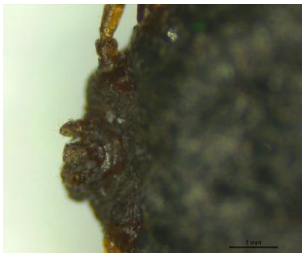

Fig.9. *Ixodes ricinus* Mouth part

Fig.10. *Boophilus annulatus* Dorsal view

Fig.11. *Boophilus annulatus* Ventral mouth part

Fig.12. *Boophilus annulatus* Ventral view

Fig.13. ***Rhibicephalus sanguineus*** **Dorsal view (male)**

Fig.14. ***Rhibicephalus sanguineus*** **Ventral view (male)**

Fig.15. ***Rhibicephalus sanguineus*** **Mouth part (male)**

Fig.16. ***Rhibicephalus sanguineus*** **Anal part (male)**

Fig.17. ***Haemocephalis*** **Dorsal and ventral view**

Fig.18. ***Haemaphysalis*** **Mouth part**

Fig.19. ***Pediculus humanus capitis*** **Dorsal view**

Fig.20. ***Pediculus humanus capitis*** **Ventral view**

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Exploring the Potential of using Spinach Powder for the Visualization of Latent Fingerprints

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Received: 18 Feb 2020 Revised: 20 Mar 2020 Accepted: 22 Apr 2020

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ABSTRACT

There have been several attempts in past to use chemicals, pigments, dyes to visualise latent fingerprints which are the prime evidence in the crime scene. The disadvantages of using such material motivated the scientist in search of non-toxic and an easily available material to replace the toxic compounds. The present work has been designed to study the possibility of using Spinach powder to visualize latent finger prints. In order to enhance retention of pigments, blanching and dehydration process was adapted to prepare the spinach powder. This is evaluated based on TLC separation where the color and size of spot was compared with the control sample. The prepared powder was tested on various surfaces and compared with already reported natural material, turmeric and a chemical dye, Eosin yellow. The powder dusting method was followed to visualize the fingerprints.The retention of pigment has been enhanced by blanching and dehydration treatment of Spinach. The Spinach powder gave impressive results on various surfaces like, wood, floor, rubber, glass, plastic, CD, and foil.The spinach powder prepared by this method is not only safe, but cost effective as well and can be used to decipher latent fingerprints on various surfaces in an eco-friendly manner.

Keywords: Forensic, Latent fingerprint, powder dusting, *Spinacia oleracea.*

INTRODUCTION

Fingerprints are defined as the tiny ridges, whorls and valley patterns on the tip of each finger. The uniqueness of the fingerprint is considered a valuable tool of physical evidence in identification especially in forensic science. Fingerprints can be found on any solid surface, including human body. There are three categories of fingerprint based on their visibility on different surfaces. Visible and plastic prints can be visualized and photographed directly without using any additional technology. Latent prints found on a variety of surfaces, mainly hard surfaces are not readily visible and hence, detection of such type of fingerprints often requires many techniques like the use of

fingerprint powders, chemical reagents or alternate light sources [1]. Some of the inorganic salts used for the visualization of latent fingerprint includes Mercury, Cadmium and Lead are reported to cause serious health effects in fingerprint experts [2]. The organic substances like fluorescein and rhodamine B are also used for the visualization [3]. The metallic powders such as silver powder containing aluminium flake and quartz powder and gold powder containing bronze flake and powder quarts also elicit toxic effect to users [4]. The dyes such as Eosin blue, anilline blue, cyano blue dye, azure dye are also used in the visualization which reported to have several side effects on continuous exposure [5]. The possible health hazards after getting expose to various chemical compounds used by finger print specialists working with Federal Bureau of Investigations was also investigated [6]. The review on traditional developing methods like powder dusting method, cyanoacrylate fuming, chemical method and small particle reagent method was reported [7]. The use of fluorescent nanomaterials like quantum dots and rare earth upconversion fluorescent nanomaterials to develop latent fingerprints was also emphasised by the same author.

Spinach (*Spinacia oleracea*) belongs to Chenopodiceae (Amaranthaceae) family and is considered to be native to central and south western Asia. This popular vegetable is cultivated throughout the world as cool season annual leafy vegetable. Chlorophyll is the important constituent of leaf responsible for photosynthesis and green coloration. The color of the leaves changes from green to olive green or brown on thermal processing mainly due to conversion of chlorophyll to pheophytin and pyropheophytin applying the non-enzymatic pathway [8,9]. Formation of chlorophyllin and arresting further conversion is a major challenge but could be achieved through the controlled processing for obtaining a highly acceptable dehydrated leaf powder enriched with carotenoids, vitamin C and vitamin E content. Hence this powder is a good source of carotenoids, porphyrin colors, flavonoids and betalains colours, apart from nutritional value, it may be used as the replacement of artificial color as demand of natural pigment is increasing in present era [10]. An attempt was made to explore the potential of using non-toxic, easily available, eco-friendly material i.e., spinach for the identification of latent print.

MATERIALS AND METHODS

Spinach Powder Preparation

A bunch of *Spinacia oleracea* (palak) leaves were taken and subjected to the process of blanching and dehydration. Steam blanching as pre-treatment was provided to spinach leaves for a period of two minutes in a developed precision steam blancher consisted of two interconnected chambers, one for steam generation and another as blanching chamber for effective and reproducible blanching process. The obtained leaf sample were dehydrated at 80°C for 1hr and then at 60°C for next 2 hr. After this process, the leaves were manually crushed before using mixer grinder to get spinach leaf powder [11]. This powder was stored in a sealed container for further use.

Thin layer Chromatography (TLC)

We analysed the spinach powder by TLC to find out the effect of dehydration and blanching process on the pigments of spinach leaves. To make control sample 1g of spinach leaves along with 1g of anhydrous Magnesium sulphate and 2g of sand were grinded in mortal and pestle to get a paste. This was transferred to a test tube using 2-4 ml of acetone, corked and shaken vigorously for 1 min and allowed to stand for 10 min. The upper green solvent was transferred into another test tube using pipette, covered and stored. To prepare the experimental solution, the spinach powder prepared by blanching and dehydration was taken in a test tube and 4 ml of acetone was added, covered and shaken vigorously for 1 min and allowed to stand for 10 min. The upper green solution was transferred to another test tube, covered and stored. TLC plate was taken and marked on coated side approximately 1 cm above from base. Dots of control and experiment solutions were placed on the line using capillaries with a proper distance between them. The coated plate was carefully placed inside the TLC chamber filled with developing solvent (mixture of 100 ml petroleum ether, 11 ml isopropanol and 5 drops of distilled water) up to 0.5 cm. Care was taken to keep the

solvent level below the sample dots. This setup was kept at room temperature and allowed to develop till the solvent reached near the top [12]. The results were noted and compared for the best results.

Development of Latent print

The test latent prints were collected with sebum mainly from face and forehead. The method used for the development of latent prints is powder dusting. It is a physical method of enhancement of latent fingerprints and works on the mechanical adherence of the fingerprint powder particles to the oily components of the skin ridge deposits. Application of powder to the print by brushing is a simple and easy technique. But the disadvantage of this method is destruction of ridge when the brush is used on the fingerprints [13]. In order to preserve the ridge characteristics, special care was taken while using the brush. The powder is sprinkled over a surface using fibre brush and then excess of powder is removed by tapping in order to get a clear print. In order to check the comparative evaluation, the spinach powder was applied on both porous and non-porous surfaces. The types of surfaces used in the present study range from normal paper, aluminum foil, wooden surface (sun mica-glossy), to plastic, tile, painted steel, and top as well as writing surface of CD. All the results were noted and photographed.

RESULTS

The possible order of separated pigments (in order of decreasing Rf value) may be Carotene (yellow), Pheophytin a (grey), Pheophytin b (grey), Chlorophyll a (blue-green), Chlorophyll b (green) and Xanthophylls (3spots of yellow).The enhancement or retention of the pigments in our sample preparation by blanching and dehydration treatment is very evident in the TLC plates (Fig. 1B). The color and size of the spots in the experimental solution were more prominent as compared to control. All the three spots of xanthophylls in the experimental solution is bigger than the control sample.The spot visible between chlorophyll a and b is not there in control sample. Similarly the sopts of pheophytin a, b and carotene are merely visible in control sample. This proves the retention of pigments in the experimental solution which is prepared by blanching and dehydration.

The result of the latent fingerprints developed using spinach leaves powder on different surfaces are shown in Figs. 2 – 13. The different surfaces were selected to ensure the efficacy of spinach powder on the commonly encountered surfaces in the crime scene. The results were evaluated based on the comparison with already reported natural powder (turmeric) and a chemical dye (eosin yellow dye) used for the development of latent finger print. The latent fingerprints developed on wood, speaker, door handle, floor, both the sides of CD rubber surface and steel gave better results with spinach powder and eosin yellow dye in terms of ridge details(Figs. 2-8, 13). The ridges were of good quality and very impressive for all the three powders on various surfaces like plastic, Glass foil and note book (Figs. 9-12). In most of the surfaces the quality of the fingerprint developed by spinach powder are in equivalence with the eosin dye. The visibility and clearness of the ridge details achieved with spinach powder may be due to the size and shape of the particle. The lack of sensitivity using turmeric powder may be due to the coarse nature of the powder and its deposition between the ridges.

DISCUSSION

The comparative evaluation on different surfaces with spinach powder reveals its potential of its use in the deceipherment of the latent fingerprints. The reason of adherence of spinach powder to latent fingerprints can be the pigments of spinach leaves which got enhanced by blanching and dehydration sample preparation. Kumari et al., (2011) [14] reported similar results and got impressive latent finger prints using synthetic food and festival holicolor. They tested the selected colors on various surfaces like paper, aluminium foil and both the surfaces of CD. Garg et al. (2011) worked on the visualization of latent finger marks using turmeric powder obtained from rhizomatous herb on nine different substrates and found to give clear results on majority of substrates. The selected surfaces include

paper, wooden surface, aluminium foil and CD. The comparison of turmeric powder with standard black powder has also been included in their study. Badiye et al., (2015) [15] visualized latent finger marks on twenty-four matrices using Robin blue powder (used as post wash whitening agent) and observed better results on majority of substrates specifically on multicolored surfaces. Similar impressive results using fuller's earth was aslo reported and the comparison was made with standard black, gray, Magnum and white powder. They tested this powder on various surfaces like wooden, plastic, steel and glass [16]. It is concluded that spinach powder is a simple, easily available and non-toxic, eco-friendly material which can be successfully used to develop fingerprints on porous and non-porous surfaces in crime investigations. This is the preliminary investigation which suggests that spinach powder can be successfully used as a substitute to visualise fingerprints on majority of surfaces except skin and cloth. Further studies on the effect of different conditions like humidity, temperature, season on the development of latent finger print is required for better understanding. Work on decipherment of aged fingerprints and the stability of these developed finger prints are in progress.

ACKNOWLEDGEMENT

We would like to express our deepest appreciation to Miss Renu Yadav (MDU), who helped us finalising the project aim, assemble the parts and gave suggestion regarding the project.

Abbreviations

TLC: Thin Layer Chromatography
CD: Compact Disc
Rf: Retardation Factor

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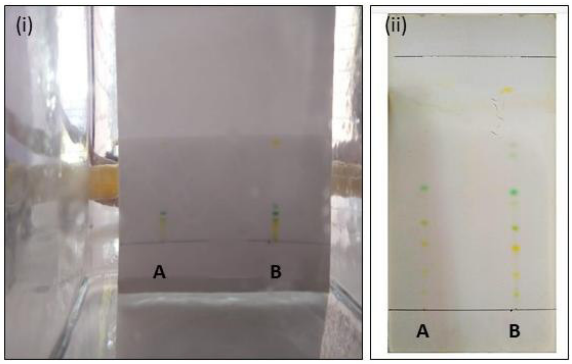

Figure 1. Results of TLC showing more pigment spots in the spinach powder prepared by blanching and dehydration. (i) TLC plate in the solvent chamber (ii) TLC plate after separation, A. Control B. Sample prepared by blanching and dehydration.

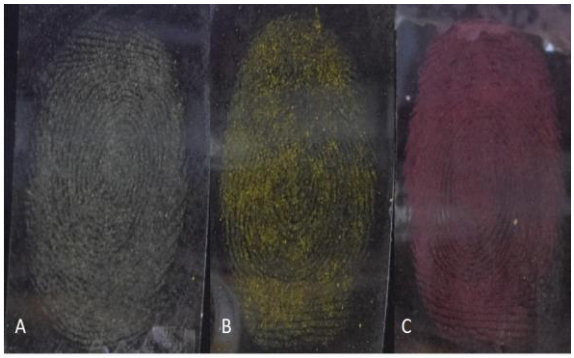

Figure 2. Latent finger prints developed on wood A. Spinach powder B. Turmeric C. Eosin yellow dye

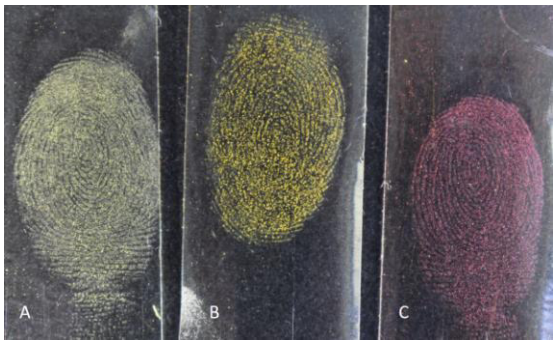

Figure 3. Latent finger prints developed on speaker A. Spinach powder B. Turmeric C. Eosin yellow dye.

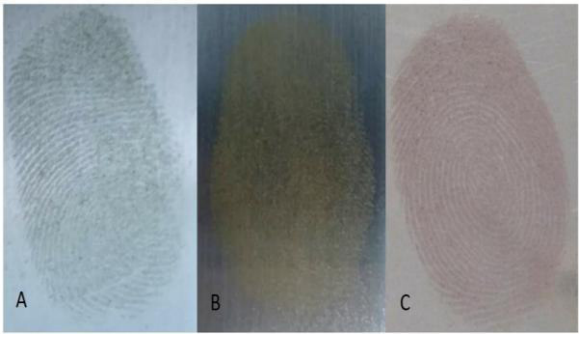

Figure 4. Latent finger prints developed on door handle. A. Spinach powder B. Turmeric C. Eosin yellow dye.

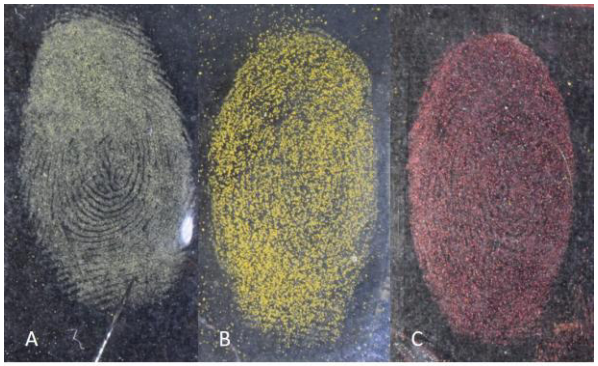

Figure 5. Latent finger prints developed on Floor surface. A. Spinach powder B. Turmeric C. Eosin yellow dye.

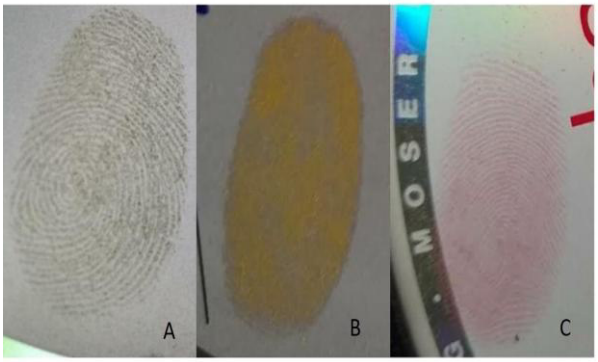

Figure 6. Latent finger prints developed on CD A. Spinach powder B. Turmeric C. Eosin yellow dye.

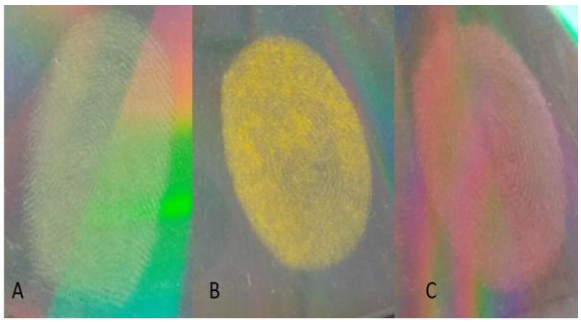

Figure 7. Latent finger prints developed on reflecting CD surface. A. Spinach powder B. Turmeric C. Eosin yellow dye.

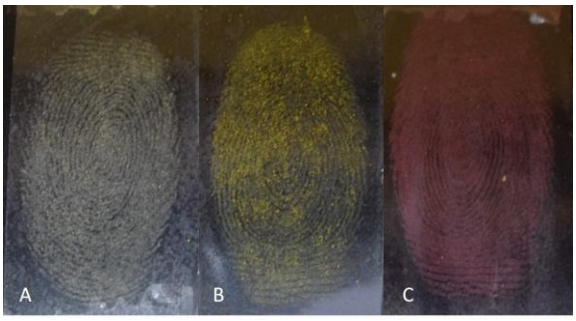

Figure 8. Latent finger prints developed on rubber surface. A. Spinach powder B. Turmeric C. Eosin yellow dye.

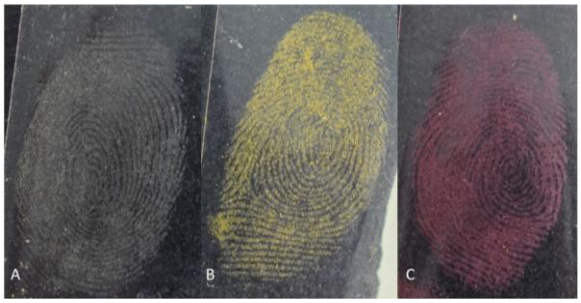

Figure 9. Latent finger prints developed on plasticglass. A. Spinach powder B. Turmeric C. Eosin yellow dye.

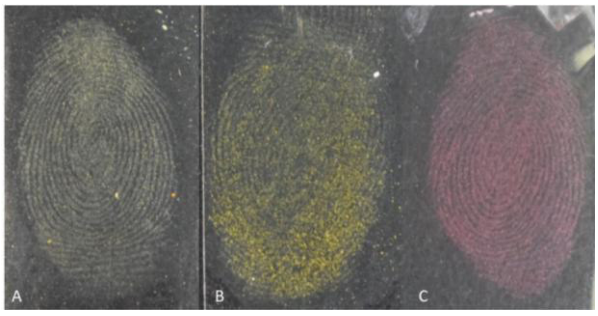

Figure 10. Latent finger prints developed on Glass A. Spinach powder B. Turmeric C. Eosin yellow dye.

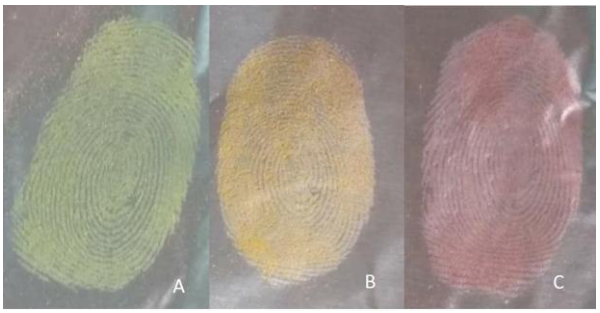

Figure 11. Latent finger prints developed on aluminum foil. A. Spinach powder B. Turmeric C. Eosin yellow dye.

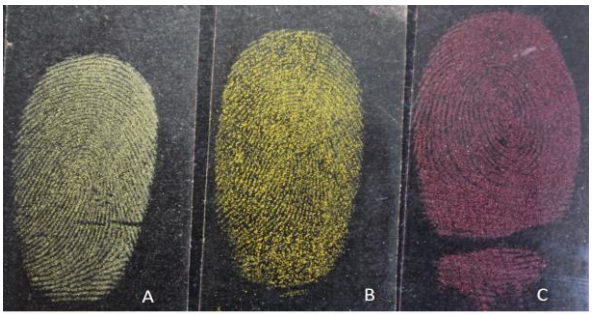

Figure 12. Latent finger prints developed on Notebook A. Spinach powder B. Turmeric C. Eosin yellow dye.

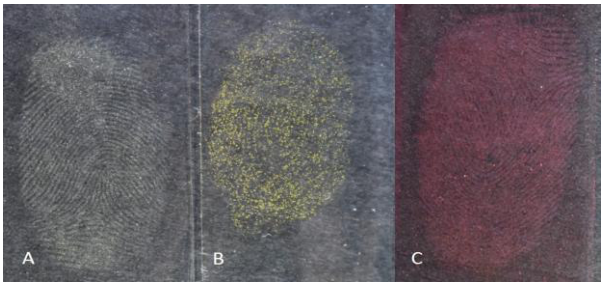

Figure 13. Latent finger prints developed on painted steel (Almira) A. Spinach powder B. Turmeric C. Eosin yellow dye

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Earthworm Growth Activity with Various Concentration of Fly Ash inVermicomposting for Agricultural Application

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Received: 06 Feb 2020 Revised: 08 Mar 2020 Accepted: 10 Apr 2020

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ABSTRACT

This research work focuses on experimental study on soil fertility from a serious environmental causing industrial waste Fly Ash (FA) for sustainable agricultural growth by Vermicomposting (VC). The application of vermicomposting soil is considered a good management practice that stimulate soil microbial growth and activity, enhance the soil fertility for any agricultural production with subsequent mineralization of plant nutrients. The experimental study has been initiated with Fly Ash (FA) collected from Ib Thermal industries of Jharsuguda, Odisha, India. Preferably the air dried samples of soil and organic matter (OM) in the form of cow dung were collected from the mentioned experiment area and mixed with Fly ash for proportional set up of different ratios (4:1,3:2,2:3,1:4).The experimental set ups were introduced by red earthworm *Eisenia foetida* in the treatment of various proportion of FA up to 65 days. From the observations it is found that high concentration of lead ,nickel, copper iron, zinc and sulphur in fly ash results maximum output of vermicasts and population of juveniles produced to 4:1 and then to 3:2 as compared to other proportion of vermicompost. Vermicomposting is a low cost technology using certain species of earthworm like *Eosinia foetida,* of earthworms able to consume organic material residuals and possibly heavy metals, fragment into much finer particles vary rapidly. Based on this analysis the further experiment & observations to be carried out with addition of earthworms to various proportion set ups of FA and chemical analysis of vermicompost to yield bioavailability for agricultural benefits.

Keywords: Fly ash(FA), Heavy metals, Organic matter(OM), Vermicomposting(VC)

INTRODUCTION

A rapid expansion in population growth, urbanization, agricultural production and industrialization are demanded for meeting their power energy supplied by number of the Thermal power plants.In India, the coal fired power plants produce electricity out of which about 79% of the entire electricity met for various purposes (Singh & Siddiqui, 2003). Fly ash is an earnest source of air pollution since it remains air borne for a long period of time and causes health hazards (T.E.R.I., 1998). India is an astronomically immense consumer of non-coking coal having a high ash content of about 30-40% (Senapati, 2011). FA contains silica, aluminium and oxides of iron, calcium, magnesium, arsenic, chromium, lead, zinc and other toxic metals. It has been regarded as an earnest ecumenical environmental solid waste all over the world. FA has great potential as a resource material in construction, agriculture and other cognate areas. The presence of considerable amount of K, Ca, Mg, S and P in FA has been proposed for utilization on agricultural sectors (Singh et al., 1997). Extensive research is being carried out in order to utilize FA in sundry sectors as it does not come under the category of hazardous wastes (Latifi et al., 2015).

In India a "fly-ash mission'' in 1994 was commissioned under the Department of Science and Technology for proper management and disposal of fly-ash. Sustainable remediation practices can only way to resolve this quandary (Tilman et al., 2002). Ergo, it is consequential to surmount these quandaries not only by opportune and safe disposal, but withal through conversion of these materials to value-integrated products (Bhattacharya & Chattopadhyay, 2002). FA, avail in amending crop magnification having both the soil amending and nutrient enriching properties to yield even in low fertility acid lateritic soils. FA contains both macro and micro-nutrients (S, Ca, Mn, P) with deficiency of nitrogen and phosphorus which can sustain plant magnification (Negi & Meenaxi, 1991).Presently there are various bio- processing technology are used and among them vermi-conversion has been recommended as preferable option to stabilization various kinds of solid wastes.Some species of earthworms are kenned to be potential accumulators of cumbersomely hefty metals and ergo they have been prosperously demonstrated in mitigating the toxicity of industrial and municipal waste by vermicomposting technology (Saxena et al., 1998).Vermicomposting is a more expeditious method for abbreviating organic waste than traditional composting. This approach utilises the action of earthworms as well as bacteria to break down organic waste. The resultant material (vermicompost) can be a highly efficacious fertiliser, or soil conditioner, when applied correctly.Composting is defined as biological decomposition of solid complex organic material into humus material .Organic factors are degraded by some decomposers like earthworms and microbes.

Finally produced compost eco-friendly for plants,during this; process pathogenic microbes and undesirable weeds are destroyed.Vermicomposting has gained popularity in both industrial and in domestic field thus it is compared to conventional composting. Vermicomposting is a non-thermophilic biodegradation of organic material engendered finer particles by fragmentation during passing through a grinding gizzard while maintain nutrient (Shrestha et al., 2011).*Eisenia fetida*, the tiger or brandling worm most familiarly used earthworm species for vermicomposting (Haimi & Huhta, 1900). Other suitable species include *Lumbricus rubellus, Eudrilus eugeniae* and *Perionyx excavatus*, an Asian species (Edwards et al., 1995) and *Eisenia andrei*(Haimi & Huhta, 1900). Vermicomposting need ideal earthworm species should be prolific breeder, salubrious eater, resistant, etc. They are consequently a utilizable implement for ecological assessment of soil and land (Rombke et al., 2005).The end product of vermicomposting isproduced as vermicompost a natural fertilizer. Vermicomposts prepared from different organic wastes, possessed considerably higher calibers of major nutrients - N, P, K, Ca and Mg compared to that of the other wastes; these findings are in conformity with those of earlier authors (Edwards, 2004; Garg et al., 2006; Kitturmarth et al., 2007; Pattnaik & Reddy, 2009).Vermicasts are withal believed to contain enzymes and hormones that stimulate plant magnification and deter pathogens (Abbasi and Ramasamy, 1999; Szczeck, 1999). The main objectives of the present study were to find out the appropriate proportion of flyash–soil-cowdung for sustainable and efficient vermicomposting to obtain vermicompost reduce with heavy metal concentration.

MATERIALS AND METHODS

Collection of samples

The present work has initiated with procuring of Fly Ash (FA) from IB Thermal industries of Jharsuguda, Odisha, India. The required soil and organic matter (OM) in the form of cow dung are collectedfrom Centurion University of Technology & Management, Bhubaneswar, Odisha, India and air dried exposed to sunlight and chopped into smaller particles. These chopped soil and cow dung were allowed for sieving with a sieve of diameter 2.36mm. The collectedfine materials of both were used for the next procedure of proportional set up.

Experiment on laboratory condition

In preliminary step different combinations of fly ash (FA) with soil (S) and organic matter (OM) in the form of cowdung was prepared taking each samples individually in various proportion of FA+S+OM (1:2:2)for 20% FA, FA+S+OM (2:1.5:1.5) for 40% FA, FA+S+OM (3:1:1) for 60% FA, FA+S+OM (4:.5:.5) for 80%FA,FA(100%) and S+OM (1:1) of 1kg for each.All the mixtures are taken into plastic containers (50cm L-length, 27cm B-breadth and 12cm H-height) and kept in shaded laboratory zone. Two whole set labelled as SET-I and SET-II were prepared having each with 6 containers named Stock(S+OM), Proportion-1(20%-FA), Proportion-2(40%-FA), Proportion-3(60%-FA), Proportion-4(80%-FA),Experimental-(100%-FA) were carrying the above mentioned ratios .

Mixture of FA were carried out with sprinkling of water in regular intervals per day for maintaining moisture content was obtained as feed mixture normally aid to grow earthworm. The endemic earthworm are maintained in the vermiculture unit of the Centurion University and Technology and Management(CUTM)campus, Bhubaneswar, India, were collected and added in vermicomposting sets. There were 6-8 number ofearthwormswere introduced into each of the pots approximately having length of 8-12 cmbought from the above each mentioned areaand kept for 65days. Black granular compost in upper surface of the containers was indicating of formation of vermicompost. The vermicompost and earthworms after rinsing used for various physico-chemical analysis.Within this period regular monitoring of water sprinkling, adding of cow dung slurry and amalgamation of mixture were carried out for each vermireactorwithin time interval. However, cowdung essentially required for nutrition and survive of earthworm. Two sets of the feed mixture were conducted for first run.After 65 days, the proportion sets were observed with castings generation of juveniles and formation of vermicompost.

Chemical Analysis

From the chemical analysis report it was collected that the FA is contained oxides of Al, Si, P, S, K, Ca, Ti, V, Cr, Mn, Fe, Ni,Cu,Zn,Ga,As,Rb,Sr,Y,Zr,Nb,Sn,Ba,Eu,Yb,Ir,Pb,Th and Re. Similarly the Soil is obtained with oxides of all as previous except Ba and cow dung contained Cr,Ni, Zn,Pb,Cu,P and other trace elements.

RESULT AND DISCUSSION

The physical parameters like temperature, moisture content and water holding capacity are easily controllable and indicatedthe progress of the composting and vermicomposting process.The output of vermireactors from first attempt of the mixturesoil+cow-dung and fly-ash in various proportions of SET-I and SET-II were described in TABLE-1 and TABLE-2 with their physico-chemical characteristics of the feed mixture. The P^H and Electro conductivity (EC) of the soil and fly-ash proportion was studied according to their manure activity with the help of the main bio-reactors (earthworms) for the effect of vermicomposting into fertile soil.

In the present studies the physico-chemical activity of P^H and Electrconductivity wasstudied according to the differences of vermireactor sets from recorded initial,final reading measurements in the laboratory.

Observation-1

From observation of SET-I it was studied that the electroconductivity gradually decreased from the difference value of Stock onwards Proportional-1 and Proportional-2 while it increased onwards Proportional-3, Proportional-4 and in experiment sets. It is measured in v/w(vermicompost/water)((Alidadiet.al., 2005; Munnoli and Bhosle,2009, 2011)

Observation-2

Similarly, in pH value of SET-I the difference from initial and final readings was increased in Stock, Proportion-3 and in Experimental as compare to other proportions.

Observation-3

The observation of SET-II was indicated that the electrical conductivity gradually decreased from the difference value of control onwards proportional-1 and proportional-2 while it increased onwards proportional-3, proportional-4 and in experimental-FA sets same as to SET-I having closely approximate values.

Observation-4

In reading of pH value of SET-II the difference from initial and final readings was increased in Stock, proportion-3 and in experimental-FA as compare to other proportions also has equal significant output as compared with SET-I.

Observation-5

Table-3 was described about the survival of EWin both SET-Iand SET-II containing each of theirStock, proportional sets and experimental-FA with various proportion of FA.In SET-I and SET-II it was observed that 8 and 6 number of adult EW respectively wereintroduced in each proportions and were survived by monitoring in regular interval of 7 days. Increase in population ofjuveniles were observed and counted after 20 days in Stock, Proportion-1 and Proportion-2 while decrease in Proportional-3, Proportional-4 with retardation of reproduction capacity according to environment condition.The survival of earthworm was found difficult in Experiment at beginning with recording of 2 dead in SET-I and 1 dead in SET-II but further it showed population growth though it was in slower rate may due to adopted with composting environment.

Observation-6

The estimation of growth were measured by taking the length of adult EWs after each interval of 15 days of the total period of establishment in vermireactors. The length was increased in Stock,Proportion-1and Proportion-2 in both SET-1 and SET-II while in Proportion-3, Proportion-4 and in Experiment was observed less increase in length. It was indicated that the growth of EWswere reciprocal to their respective containers.

From the present study and tabulated data was found that the electro conductivity readings were indicated the presence of soil salinity and organic matter level for existence of micro and macro organism which enhance the soil fertility for sustainable crop production. The pH value was increased in respective proportion towards alkalinity above than 7 and less than 8 which indicated the survival of earthworm which changed during vermicomposting from slight acidic medium by adding of slurry as nutrition. In Experimental the FA was contained high acidic initially which converted to slight alkaline due to the activity of EWs with addition of nitrogenous substance as casts. The population and survival of EWs was observed in almost all containers still it was best in Proportion-2 and least in Experimental. The population of EW is slower and survival rate was estimated only adding of EWs after precomposting. 2 EWs and 1EWs out of 8 in SET-I and 6 in SET-II respectively was found dead of initial introduction in Experimental later all other survived may due to adaptation. The process of castingwas another parameter which indicated the population of all containers of both sets. The amount of cast was found more in Proportion-2 as

compared to others. In Stock and Proportion-1 the survival and population of EW was increased with refer to it's growth from initial observation to final but less than Proportion -2. In Proportion-3 and Proportion-4 of both sets was shown approximately same result of population also slower than proportion-2 but more than Experimental. The survival rate of EW was preferably found in the sets contained less amount of FA whereas the population of EW was more in less contained FA while moderate in more than less amount and growth was retarded in high % of FA.

CONCLUSION

The estimated results of undertaken sample proportions by taking organic matter (OM) and fly-ash (FA) were variesin all containers of each set whereas the approximate data obtained in comparison to both SET-I and SET-II during vermicomposting. The survival of earthworm and their population were measured through the growth parameter in respect to various concentration of fly ash were the successful indicator for vermicompostingThe investigation was taken place about a 60 days where the high level of population obtained in 40% of FA.This present study is clearly suggest that vermicompost must be obtained from the heavy toxic FA by adding suitable proportion of cow dung and soil for precomposting before introduce earthworm.

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TABLE. 1 Physical Parameter Analysis of SET-I

Sl.No	Observation	Sample in proportion	Electroconductivity			pH		
			Initial	Final	Difference	Initial	Final	Difference
1	Stock-100%	OM(100%)	1.929	1.284	0.645	6.98	7.24	0.26
2	Proportion-1	FA+OM(1:4)	1.768	1.303	0.465	7.62	7.68	0.06
3	Proportion-2	FA+OM(2:3)	1.485	1.043	0.442	7.69	7.30	0.39
4	Proportion-3	FA+OM(3:2)	0.888	0.729	0.159	7.50	7.58	0.08
5	Proportion-4	FA+OM(4:1)	1.243	0.638	0.605	7.55	7.46	0.09
6	Expt.(FA)	FA (100%)	1.527	0.672	0.855	8.67	7.24	1.43

TABLE-2 Physical Parameter Analysis of SET-II

Sl.No	Observation	Sample in proportion	Electroconductivity			pH		
			Initial	Final	Difference	Initial	Final	Difference
1	Stock-100%	OM(100%)	1.917	1.263	0.654	6.94	7.22	0.22
2	Proportion-1	FA+OM(1:4)	1.772	1.297	0.475	7.60	7.66	0.06
3	Proportion-2	FA+OM(2:3)	1.457	1.104	0.353	7.66	7.28	0.38
4	Proportion-3	FA+OM(3:2)	0.897	0.725	0.172	7.51	7.55	0.04
5	Proportion-4	FA+OM(4:1)	1.243	0.666	0.577	7.54	7.50	0.04
6	Expt.(FA)	FA(100%)	1.521	0.668	0.853	8.71	7.32	1.39

TABLE-3 (Survival of Earthworm in vermireactor)

Sl.No.	Sample in Proportion	Survival no. of EW in SET-I (Counting of EW population from their introduction to final stage)		Survival no. of EW in SET-I (Counting of EW population from their introduction to final stage)	
		Initial Reading	Final Reading	Initial Reading	Final Reading
1	Stock-100%	8	39	6	36
2	Proportion-1	8	34	6	29
3	Proportion-2	8	47	6	39
4	Proportion-3	8	27	6	23
5	Proportion-4	8	18	6	13
6	Expt.(FA)	8	13& 2 dead	6	11& 1 dead

EW-Earthworm

TABLE-4. Estimation of Growth of Earthworm

Sl.No.	Sample in Proportion	Estimation of growth by measuring of EW length in SET-I		Estimation of growth by measuring of EW length in SET-II	
		Mean of Initial length Before vermicomposting	Mean of Final length After vermicomposting	Initial Length Before vermicomposting	Final Length After vermicomposting
1	Stock-100%	8.3cm	14.7cm	7.8	12.9cm
2	Proportion-1	6.4cm	12.3cm	7.3	13.1cm
3	Proportion-2	8.1cm	11.6cm	6.7	10.6cm
4	Proportion-3	7.5cm	8.8cm	6.6	7.8cm
5	Proportion-4	8.2cm	8.6	7.1	7.4
6	Expt.(FA)	7.7cm	8.1	7.6	7.9

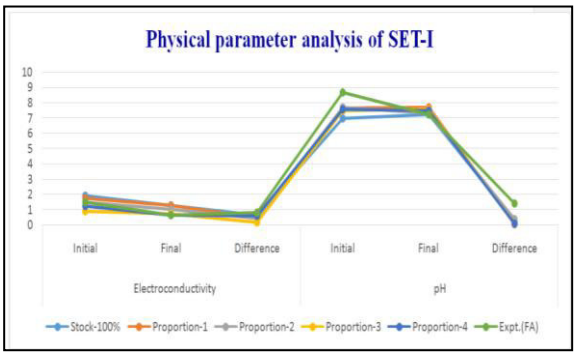

Fig.1. Graphical presentation of Electroconductivity and pHvalue of TABLE-1

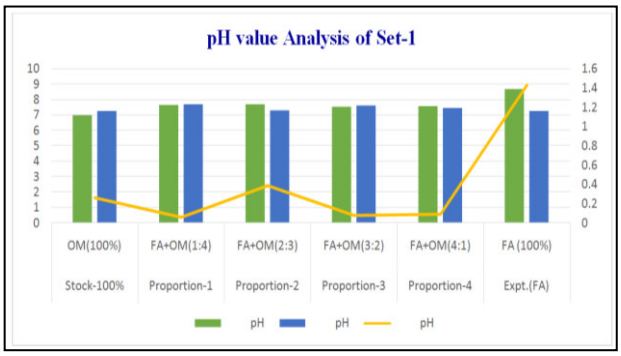

Fig. 2. Graphical presentation of pH value of TABLE-1

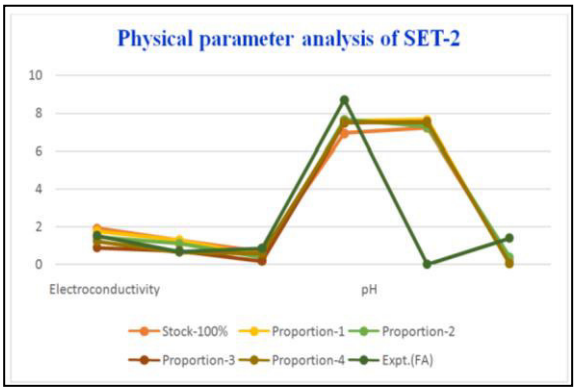

Fig.3. Graphical presentation of Electroconductivity and pH value of TABLE-2

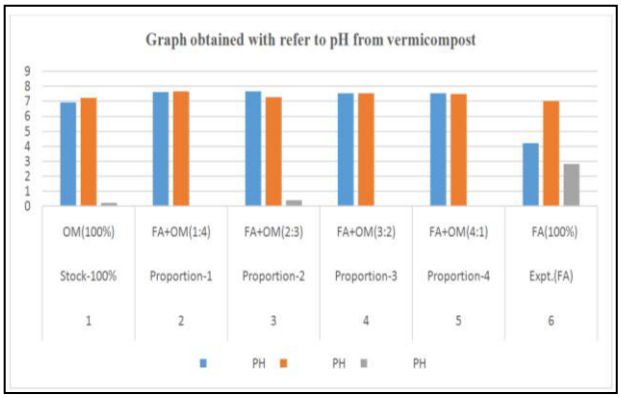

Fig. 4. Graphical presentation of pH value of TABLE-2

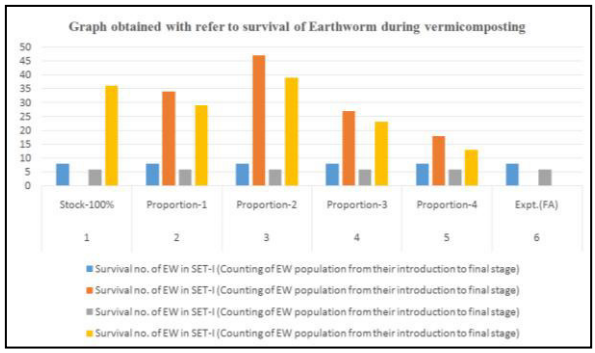

Fig.5.Graphical presentation of survival of EW of TABLE-3

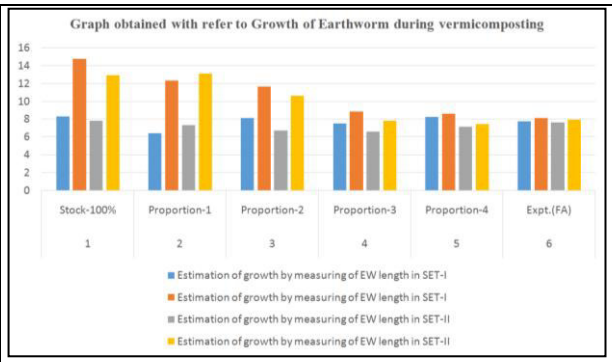

Fig. 6.Graphical presentation of Growth of EW of TABLE-4

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Study on Estimation of Cholesterol Content in Different Species of Prawn

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Received: 05 Feb 2020 Revised: 07 Mar 2020 Accepted: 10 Apr 2020

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ABSTRACT

A quantitative study was made on the total cholesterol content of flesh of five commercially important prawns *Macrobrachium rosenbergii, Macrobrachium malcomsonii, Fenneropenaeus indicus, Penaeus monodon, Penaeus vannamei* collected from the market of Balugaon obtained from Chilika lagoon. People living in the coast of Chilika consume fish, shellfish, as the main source of nutrition. So, it is essential to study the nutritional value of prawns. Prawns are a good source of nutrition but they are sometimes criticised for their high cholesterol content. The cholesterol content in prawns varies from species to species. Mean of cholesterol content of five commercially important species of prawn are found to be very noteworthy .The cholesterol content is found to be highest in the muscle tissue of *Macrobrachium malcomsonii* i.e, 2.8874±0.234mg/g whereas *Fenneropenaeus indicus* have the least amount of cholesterol i.e, 1.2892±0.547mg/g among all five species.

Keywords: Cholesterol, Prawn species, Chilika Lake

INTRODUCTION

Prawns are marine as well as freshwater shellfish. They belongs to the class crustacea, order decapoda and suborder natantia. They are cosmopolitan in distribution. Along with fresh water and marine water they also found in brackish water and estuaries as well. They possess sufficient quantity of organic and inorganic constituents.The vital constituents are protein,carbohydrate, cholesterol.Including this, prawns also have a good proportion of vitamins like vit-A,vit- C,vit-D and minerals such as calcium, phosphorous, magnesium, manganese, and chlorine (Ferdose, and Hossain, 2011).Inspite of having all essential nutritional values prawns are sometimes criticised due to their high

cholesterol content. Cholesterol is a non-polar lipid substance belongs to sterol group.It is an alcohol with a cyclic nucleus, found either free or esterified with fatty acids (Kannan,et al.,2014) Cholesterol is a soft waxy fat like substance found in all animals except plants.It is insoluble in blood and thus it is distributed in the blood stream by molecules known as lipoproteins. The molecules lipoproteins are named on the basis of their density. The (HDL) high density lipoprotein is better than (LDL) low density lipoproteins. High density lipoproteins are good and helps in carrying cholesterol to the liver where as low density lipoproteins is bad fat and have major contribution for atherosclerosis and consequently leads to (CHD) coronary heart diseases. Thus, HDL provides protection against coronary heart diseases (Ezomoh, and Madukosiri, 2016).

Cholesterol is an essential precursor of bile acids, steroid hormones, moulting hormones, vitamin D3 and prostaglandins,which are involved in the moulting process in shrimp(Kannan, etal., 2014)Cholesterol is essential to build the structure of cell membranes.It performs various biological functions in humanbeings. It help in the synthesis of steroid hormones like adrenocortical hormones, sex hormones, placental hormones etc. Apart from humanbeings cholesterol is remarkably essential in crustaceans. It plays an important role in the decapod crustaceans development. Supplementation of cholesterol in the diets improves various biological functions of prawn *Penaeus japonicus*, *Penaeus monodon*, lobsters *Homarus americanus,* mud crab *Scylla serrata* and Pacific white shrimp *Litopenaeus vannamei* (Mahalingam et al.,2009). Since cholesterol have the history of causing cardiovascular diseases it is essential to determine the cholesterol content in most liked prawn species. So, the aim of the study was to determine the concentration of cholesterol of five common species of prawns that are usually preferred and consumed by people of coastal areas as well as urban areas. Thus current investigation is carried out on species like *Macrobrachium rosenbergii, Macrobrachium malcomsonii, Penaeus monodon, Fenneropenaeus indicus,* and *Penaeus vannamei* for cholesterol estimation.

MATERIALS AND METHODS

Procedure

Five species of prawns were collected from the fish market of Balugaon.They were obtained from Chilika .They were brought to laboratory and were identified by using taxonomic keys. The collected prawns were identified as

- *Macrobrachium rosenbergii*
- *Macrobrachium malcomsonii*
- *Penaeus monodon*
- *Penaeus vannamei*
- *Fenneropenaeus indicus*

After identification the length, breadth and weight were measured by using thread and weighing machine respectively. The carapace and exoskeleton of prawns were removed and prawns were weighed again. The flesh of prawns were allowed to blend through mortar pestle then blended flesh were kept in oven for 24 hour at 100 ^{0}C. The dried samples were powdered, for the preparation of standard solution. 100g of raw flesh of prawn were taken for determining the cholesterol content. Stock solution was prepared by dissolving 100mg of dry ash in100ml of chloroform. From the stock solution standard solution was prepared. 2ml of stock solution dissolved in 18 ml of chloroform i.e the stock solution was diluted in 1:10 proportion with chloroformto bring into a working standard.Both the stock solution and standard solution were kept in refrigerator until use. From the working standard 0.2, 0.4, 0.6, 0.8 and 1 ml of solution were taken and made to 5ml by adding chloroform.

The method of Stadtman (1957) Libermann–Burchard reaction was followed for the determination of cholesterol concentration. 10gram of raw flesh was taken and homogenized with 80 %ethanol and acetone (1:1) using mortar

and pestle for deprotenization. Slurry was prepared from the raw tissue. Prepared slurry was centrifuged at 1300rpm for 20 minutes. The supernatant was discarded and the pellet was kept in water bath for 10 minutes at 50^0C for complete recovery of the cholesterol.The sample was again centrifuged and the supernatant was discarded. The pellet was again kept in water bath where the samples were evaporated to dryness. To the residue 2ml of glacial acetic acid and sulphuric acid was added and stirred well. The mixture is then kept in incubator at 18^0C for 15 minutes.The blue green colour was measured at 625 nm in spectrophotometer. The optical density was plotted against the concentration of cholesterol.

RESULTS

The study resulted that the cholesterol content is highest in *M. malcomsonii* and lowest in F. indicus among all the different species. In the present study cholesterol content of five adult prawn samples of five different prawns were subjected for computation of mean value. Mean of cholesterol concentration ranged from 1.892±0.4888 (*Fenneropenaeus indicus*) to 2.8874±0.3308mg/g *(Macrobrachium malcomsonii).*The concentration of cholesterol occurred in these five species noteworthy .*The* quantity of cholesterol that were found in the prawns indicates that they were having a good nutritional value. The maximum cholesterol content was observed in the filet of *Macrobrachium malcomsonii* where as in the filet of *Fenneropenaeus indicus* minimum cholesterol was observed.

The graph reveals that out of the five species taken for cholesterol analysis *P.monodon* acquired the maximum length as compared to others .Simultaneously the graph depicts that the member of *P.vannamei* is having the minimum length with content of cholesterol slightly higher than that of *M. malcomsonii*. Kannan, et al.(2014) reported that as the size of the shrimp increases the concentration of cholesterol also increases .This phenomenon is true among the members of a same species but when we consider the members of two different species it might not be true. Thus from the mentioned graph we can concluded that the concentration of cholesterol sometimes depend upon the body length and weight of the animal at the same time it also vary from species to species.

DISCUSSION

Various studies were carried out on the different species of prawn from different parts of the world. The present study concerned with the level of cholesterol in five commercially important species of prawn that are found in Chilika lagoon. Cholesterol is reported to be one of the essential nutrients for growth and survival of crustaceans (Kanazawa 1971). Polyunsaturated fatty acids are highly essential for the growth and development of crustaceans like *M.rosenbergii (*Abramo and Sheen 1993*), Penaeusindicus (Read 1981) Penaeus duodarum (*Sick and Andrew 1973).
Comparison of cholesterol content between the five species of prawn shows that high quantity of cholesterol were reported in the *M.malcomsonii* and lower level of cholesterol was noticed in the species named *F.indicus.* The proximate composition of prawn varied significantly from species to species and even within the same species from one individual to other (Stans by 1962). Cholesterol is essential for the normal functioning of the body as well as for conducting various metabolic activities. Cholesterol is an essential precursor for the synthesis of bile acids, steroid hormones, moulting hormones, vitamin D3 and prostaglandins (Akiyama 1992). In the present study an attempt has been made to evaluate the approximate level of cholesterol in five commercially important species of prawn**.** The concentration of cholesterol not only depends on the length and weight rather it also depends on the diet intake, habitat (i.e, the place of origin) as well as the sex of the animal.

CONCLUSION

According to the results of the present study the five species of prawn has proven to have a good cholesterol contents. They are no way inferior to other species of prawn. A significant difference in the amount of cholesterol content was observed in each species. Cholesterol levels varied from species to species. The analysis confirmed that

high cholesterol was observed in the species of *Macrobrachium malcomsonii*. (2.8864± 0.234mg/g) followed by member of *Penaeus vannamei* having cholesterol quantity 2.69±0.242mg/g. *M.rosenbergii* contains moderate level of cholesterol i.e, 2.413±0.113mg/g followed by members of *P.monodon* which possesses 2.145±0.436mg/g cholesterol. The species of *F.indicus* has proven to bear least amount of cholesterol among the five species i.e, 1.289±0.547mg/g. Just because prawn contains a good quantity of cholesterol we cannot avoid consuming prawns rather we should opt for the species of prawn having least quantity of cholesterol.

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Table.1. Cholesterol concentration in filet of five species of prawn in mg/g

Species	Mean length (in cm)	±SD	Cholesterol content (in mg/g)	±SD
P.monodon	14.82	±0.952	2.145	±0.436
F. indicus	12.02	±0.952	1.289	±0.547
M.rosenbergii	12.38	±1.338	2.413	±0.113
M.malcomsonii	9.08	±1.427	2.887	±0.234
P. vannamei	5.04	±0.32	2.69	±0.242

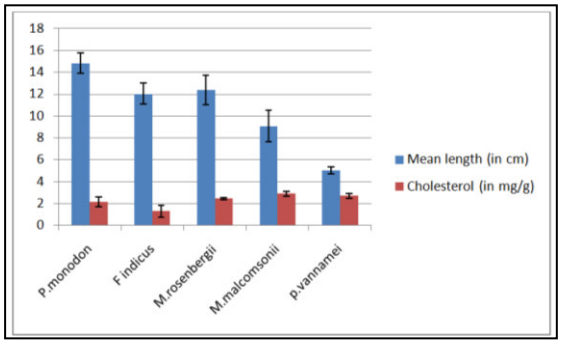

Fig.1.Graph depicting the correlation between length and cholesterol content in five different species of prawn

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Green Synthesis of Reduced Graphene Oxide from Waste Dry Cells using *Azadirachta indica* leaf extract

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Received: 07 Feb 2020 Revised: 09 Mar 2020 Accepted: 11 Apr 2020

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ABSTRACT

Graphite electrodes so obtained from waste zinc-carbon batteries were washed several times with water to remove impurities and made into powder and then treated with mixture of HCl and HNO_3(3:1) followed by drying at 60^0C to get pure graphite powder that was used as a starting material to synthesize graphene oxide(GO) by improved Hammer's method.The graphene oxide(GO) again treated with *Azadirachta indica* leaf extract and converted to reduced graphene oxide(RGO).The rGO so obtained was characterized by UV-VIS Spectrophotometer, Fourier Transform Infrared Spectrophotometer, X-ray Diffractometer and Scanning Electron Microscope. The characterization data gave clear evidence on effective formation of reduced graphene oxide.

Key words: waste zinc-carbon batteries, graphene oxide (GO), *Azadirachta indica* leaf extract, reduced graphene oxide(RGO).

INTRODUCTION

Waste zinc- carbon battery cells are primary cells that once used is considered as a solid waste which is a source of numerous environmental pollution by discharging toxic materials into soil as well as water. All most all batteries use graphite electrodes which has no use after the cell ceases to function. Several researches have been carried out to synthesis rGO from GO using graphite and reducing agents such as $NaBH_4$ [1] and hydrazine [2] in wet chemical methods but due to hazardous effects of these reducing agents and the use of these rGO doesn't found suitable in many bio and environmental applications. However green synthesis of RGO using ascorbic acid [3], amino acid [4], tea solution[5], wild carrot root [6], phytoextracts [7], melatonin [8], grape extract [9,10], fenugreek seeds[11] and Nigella sativa seed extract [12] leads the research to a new horizon where the product can be used in various fields without any environmental limitations. Synthesis rGO from Plant Leaf Extracts [13], yedoensis leaf extract [14], Eucalyptus leaf extract [15], Annona squamosa leaf extract [16], green tea extract [17], kariff lime peel extract [18] has also been reported .

In the present research *Azadirachta indica* leaf extract is used as a reducing agent which is easily available, cheap , environmental friendly, non toxic as well as non corrosive in nature.Also it acts as a strong and powerful reducing agent without causing any harm to the environment and gives the same product like other reducing agents.The graphene oxide so prepared by improved hammers' method was undergo reduction by *Azadirachta indica* leaf extract and subjected to further characterization to get conformity of it's formation.

EXPERIMENTAL

Materials

The graphite powder used as a raw material here was derived from waste battery cells. HCl, HNO_3, H_2SO_4, $NaNO_3$, $KMnO_4$, H_2O_2 were purchased from merck and used without further purification .

Recovery of graphite powder from waste batteries

Waste batteries were collected from various sources to separate the electrodes. After separation they were rubbed with sand papers slowly and washed several times with water to remove impurities like carbon, MnO_2 and metal particles. After drying the electrodes were grinded to powder by crushing .Again the powders so obtained were treated with HCl and HNO_3 (3:1) mixture in a beaker and heated for 3hrs. Then the solution was centrifuged and washed several times with distilled water to bring it to normal pH. The Graphite Powder G(R) so obtained was dried in oven at 60^0C for 24 hrs [19].

Synthesis of GO from Graphite Powder G(R)

To 75mL concentrated H_2SO_4, 3g graphite powder and 3g $NaNO_3$ were added and Stirred in ice bath until dissolve of all the contents about 4hr.Then 9g KMO_4 was added slowly with vigorous stirring for 30 minutes at a temperature below 20^0C. Then again stirred for 6hrs at room temperature. Immediately after that 100mL of distiiled water was added drop wise and refluxed for 3hrs at 98^0C.Then it was cooled and 5mL of H_2O_2 was added to remove excess of $KMnO_4$ present and confirmed by colour change of the solution to yellow. The final solution was centrifuged and washed several times with distilled water and then was poured into petridice and put in oven 90^0C for 24 hrs .After drying it was made to powder by mortar and pestle and kept for further processing. The yield was 3g

Preparation of *Azadirachta indica* (Neem)leaf extract

Azadirachta indica (Neem)leaves were collected from it's tree and washed several times with distilled water and dried. Then cut into small pieces and poured into a beaker containing 100mL of distilled water and heated to boiling for 30 minutes. The extract now collected by filtering with normal filter paper.

Synthesis of rGO from GO

2 g GO powder was added to 100 mL Neem extract and stirred for 6hr at 35^0C with magnetic stirrer .Then cooled to room temp and filtered by whatmann filter paper and dried over it at room temperature and made to powder in mortar and pestle. The yield was 1 g.

Characterizations

UV-Vis absorption study of the dispersed samples in water were carriedout by perkin- Elmer Lamda 25 spectrophotometer. IR Spectra of the graphite powder G(R), graphene oxide (GO) and reduced graphene oxide

(rGO) were recorded on a perkin Elmer spectrometer(Spectrum RX1,Perkin Elmer, Singapore)using KBr pellet technique, in the range 4000-500 cm^{-1} with a resolution of 2cm^{-1} using 4 scans per sample. X–Ray diffraction (XRD) of the composites were carried out with PAN Analytical Xpert Pro Diffractometer with Co Kα radiation (30mA 40kV).The range of diffraction was 5 to 80^0 with a scanning speed of 1^0 per minute.The sample structure was also known by characterizing through analysis of Vibrational modes by means of Raman Spectroscopy with the help of LabRam HR800UV Spectrometer using a 633 laser light for excitation of the samples. The surface structure of the nanocomposites were viewed through Scanning Electron Microscope (JEOL 6510 LV, Japan).

RESULT AND DISCUSSIONS

UV-Visible spectra

The UV-Visible spectra of the water dispersed graphite powder G(R), GO and RGO were shown in figure-2. G(R) gives the absorption peak at 272nm where as GO shows two absorption peaks ,one around 237 nm due to π-π^* transition of aromatic C=C bonds and the other at 307 nm due to n-π^* transition of C=O bonds[20].Reduced graphene oxide(rGO) shows an absorption peak at 270 nm gives an indication of red shift from 237 nm (GO) to 270 nm(rGO) and the absorption peak 307nm is missed due to removal of oxygen from the functional groups.

Infrared Spectroscopy

The FTIR Spectra of the synthesized samples were shown in figure-3.The spectrum of G(R) showed a band at 3648cm^{-1} of OH due to presence of water molecules between graphene layes [21].The spectrum also shows an intense band at 1753cm^{-1} which is due to C=O stretching.The low intense bands at 1559 cm^{-1},1361 cm^{-1} and 1222 cm^{-1} are due to presence of C=C stretching, angular deformation of H_2O and C-O-C stretching. The GO spectrum shows same bands as that of G(R) with lower transmittance (%) indicating presence of oxygen groups in the structure due to formation of alcohols,carboxylic acids ,aldehydes, ketones ,ethers and epoxides [22] as it is already reported that GO is formed from oxidation of graphite by insertion of oxygenated functional groups into the structure. Finally the spectrum of reduced graphene oxide(rGO) shows a considerable decrease in intensity of bands compared to GO which is due to removal of oxygen groups by partial reduction [23]. So the OH stretching (3125cm-1), the angular deformation of water (1396 cm-1) , C-O-C stretching (1217cm^{-1}) creates in decrease in transmittance and the C=O group has totally disapplered from the spectrum confirming the partial reduction of the GO was successfully carried out using *Azadirachta indica* (Neem)leaf extract as a reducing agent.

X- Ray Diffraction (XRD)

The results of X-ray diffraction measurements of G(R),GO and rGO are present in figure-4 in a comparative way to identify the presence of crystalline phases of the materials. G(R) shows a high intense peak corresponding to (002) plane , $2\theta = 26.55^0$ that corresponds to the hexagonal arrangement and stacking of atomic layers in the structure of graphite [24].The interlayer distance d = 0.336 nm was calculated by using Bragg's equation [25].After oxidation a new diffraction peak at 11.54^0 corresponding to (002) plane and interlayer distance of 0.765nm confirmed the successful preparation of GO.The reason behind an increase in interlayer distance is due to incorporation of oxygen containing functional groups like epoxy, carboxyl,hydroxyl and carbonyl groups.Another groups at $2\theta = 43^0$ corresponds to turbostatic band of disorder of carbon materials.After phyto chemical reduction of GO by *Azadirachta indica* (Neem)leaf extract the diffraction peak at 11.54^0 disappeared and a broad peak appeared at 24.6^0 for reduced graphene oxide(rGO) with a corresponding interlayer distance of 0.361 nm confirming the elimination of oxygen containing functional groups from graphene oxide(GO) [23].The broad peak also suggests that the loss of crystallinity decreased as compared to G(R) and GO [26].so the result suggests that *Azadirachta indica* (Neem)leaf extract is an effective reducing agent for synthesis of rGO from GO.

Raman Spectra

The Raman spectra of GO and RGO was shown in figure-5.The D band represents the breathing of k point phonons A_{1g} related to structural defects and disorder where as the G band represents the first order scattering of the E_{2g} Vibrational mode in graphene sheets(sp^2 atoms) [27].GO shows the D band at 1359 $cm^{-1}$and a G band at 1596 cm^{-1}. After reduction of GO by *Azadirachta indica* (Neem)leaf extract RGO shows D band at 1347 cm^{-1} indicating the disorder in the sp^2 hybridized carbon system and the G band at 1602cm^{-1}.The I_D/I_G value of GO(0.96) is less than I_D/I_G value of RGO(0.98) due to reestablishment of graphene network(sp^2 carbon).

SEM Studies

The surface morphologies of G(R), GO and RGO was shown in the figure-6.The image of a typical graphite flake is shown in 6(a)with compact structure and regular edges. The images 6(b)and 6(c) of GO and RGO respectively show an exfoliation of the graphene sheets by insertion of functional groups into the structure of G(R).

CONCLUSION

Graphite powder has successfully recovered from waste zinc-carbon battery cells by acid treatment.The UV-Visble spectra reveals that there is absorption at 237 nm due to π-π^* transition of aromatic C=C bonds and the other at 307 nm due to n-π^* transition of C=O bonds for GO but there is a red shift from 237 to 270nm and missing of absorption at 370 confirming removal of oxygen from the functional groups for rGO. FT-IR data gave a strong evidence of absence of C=O functional group in RGO. XRD data confirmed the diffraction peak at 11.54^0 disappeared and a broad peak appeared at 24.6^0 for reduced graphene oxide(rGO) with a corresponding interlayer distance of 0.361 nm confirming the elimination of oxygen containing functional groups from graphene oxide(GO).Raman study proved that the I_D/I_G ratio of RGO is higher than GO due to restoration of the conjugated graphene network(sp^2 carbon) after removal of the functional groups.The SEM images showed an exfoliation of the graphene sheets by insertion of functional groups into the structure of G(R). So above all studies proved that RGO was successfully synthesized from GO by eco friendly phyto-chemical reduction method using *Azadirachta indica* (Neem) leaf extract cheaply and effectively.

ACKNOWLEDGEMENT

The authors are highly grateful to the authorities of Centurion University of Technology and Management, Odisha for their continuous support and encouragement to carry out this research.

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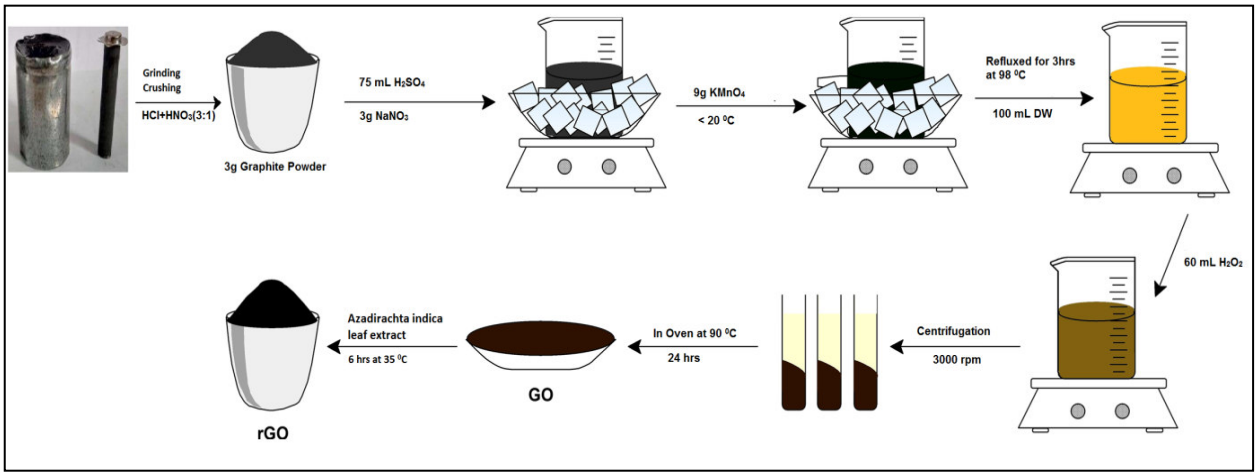

Figure. 1. Systematic procedure of synthesis of GO and rGO from graphite powder obtained from waste battery cells.

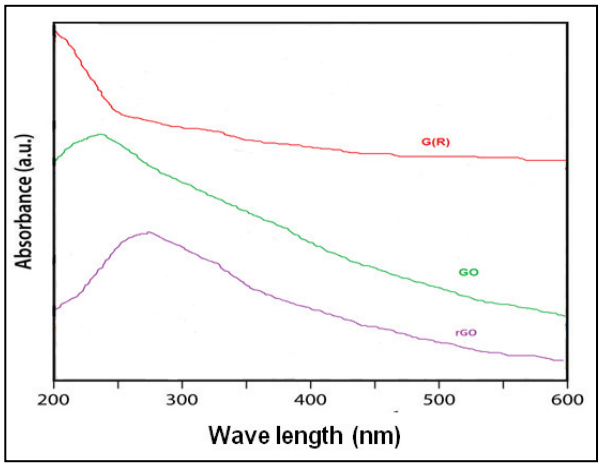

Figure.2. UV-Vis spectra of G(R),GO and rGO.

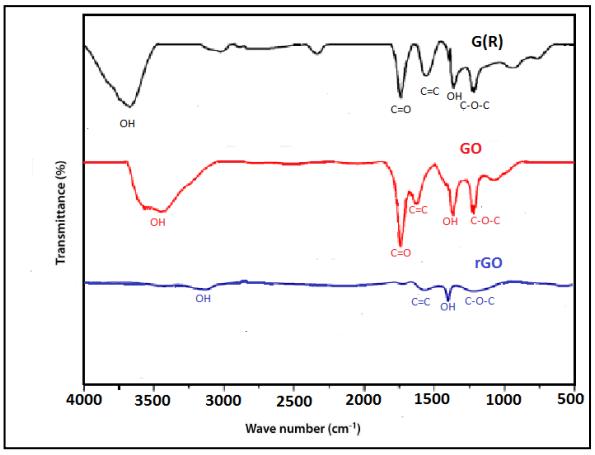

Figure.3: FTIR Spectra of G(R),GO and rGO

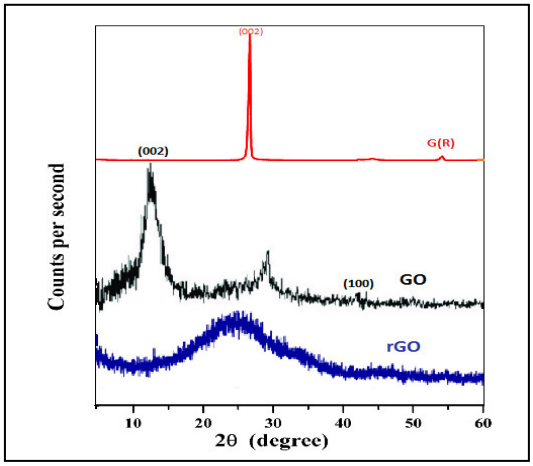

Figure. 4. XRD Pattern of G(R),GO and rGO

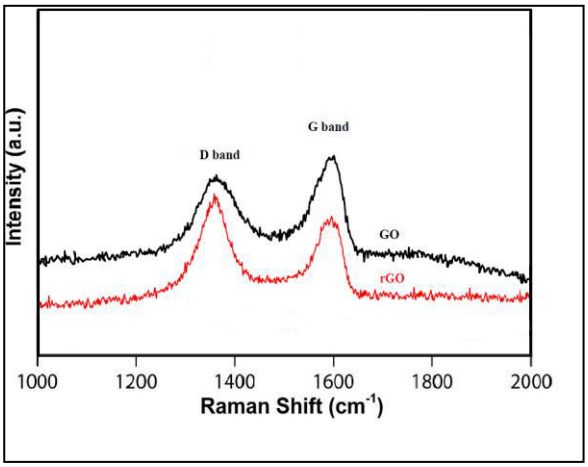

Figure. 5. Raman Spectra of GO and RGO

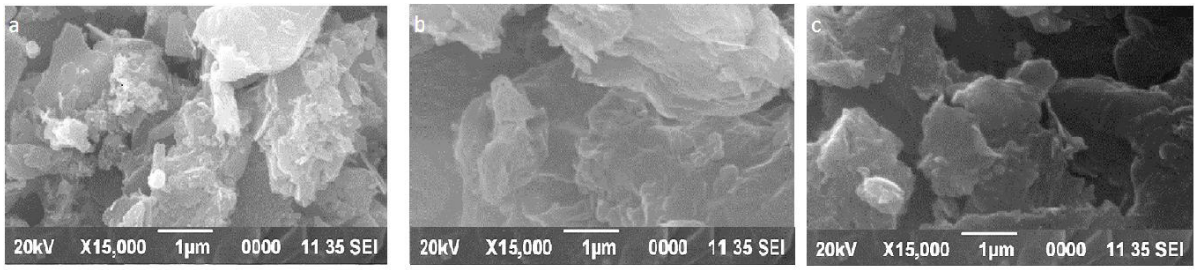

Figure.6. SEM Studies (a) G(R), (b) GO and (c) rGO

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Level of Satisfaction of Social Networks Site (SNS) usage among Engineering College Students: A Case Study

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Received: 26 Feb 2020 Revised: 27 Mar 2020 Accepted: 29 Apr 2020

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ABSTRACT

Information Communication Technology facilitates communication with a group of students, share their experience or discuss with similar interested areas is popularly known as social networks. This paper attempts to know the satisfaction level of the students of Engineering College, Dindigul District are differ while using the social networks sites and so on. Findings of the study reveals that majority of the respondents were using social network site in devices like Mobile. The Conclusion is that the number students are increasing every day in the social network link all over the World.

Key words: Social networking site, Usage of Devices, user Perception,Overall Satisfaction

INTRODUCTION

The word 'culture' is commonly used to denote many varied aspects of society, sometimes without much thought about the meaning of the term. Definitions developed by researchers give some insight into what 'culture' might mean for an entire society, or groups within that society. Social networks in simple term are online networks with a purpose of collaborative information sharing and exchange. A social network is a group of relationship and interaction with a group individual, which plays a fundamental role as a medium for the spread of information ideas and influence among its members. Social networking is another aspect of social media, in which individuals are in communities that share ideas, interests, or are looking to meet people with similar ideas and interests.

Major Social Networking Sites

Amongst the popular social networking sites, 'Facebook' became the most renowned website with the majority of Indian users surfing it, followed by 'Orkut', 'LinkedIn', and 'Twitter' and so on. Currently, the most popular social networking communities are 'Facebook', 'Twitter', 'Orkut' and 'Linkedin'.

Social Networking: A Platform for Open Access to Knowledge

Social networking sites are constantly promoting open access to knowledge. Open access is the term used to refer resources that are openly available to users with no requirements for authentication or payment. It is a model that presents free access to publications. In an open access platform, users are not charged for access to articles or other resources and are free to read, download, copy, distribute, print, search, or link to full texts of these resources, provided they do not violet respective copyright rules. The feature of social networking allows users to search, browse, filter, find, collaborate and have online open access to knowledge and contribute to web content.

Research Objectives

- To study the awareness level of usage of different social networking sites
- To study the level of trust over the information received from social networking sites.
- To study the type of social issues discussed on social networking sites.

MATERIALS AND METHODS

Data Collection

Primary data were collected through a structured questionnaire, which was distributed among the respondents of engineering college students, Dindigul District. The questionnaire contained open-ended questions and it also incorporated various parameters that were identified for analyzing those parameters.

Sample Size

The sample size consists of 185 respondents who had visit engineering college Library during the study period. Convenience sampling technique was used for a period of 2 months (November – December 2019).

Research Design

The question-wise analysis was carried out with the help of Microsoft Excel Workbook and SPSS version 20.0. The questionnaire was based on different variables, which were considered to be significant while using social media. Some analytical techniques like tables, percentages were used to analyze the collected data.

DATA ANALYSIS AND DISCUSSION

The collected data were classified and tabulated according to the objectives and hypothesis stated. For the proper arrangement of the data, a master table was prepared with the help of percentages and averages.

The above Table no. 1 shows gender wise analysis of social network site, Out of 185 respondents, 121 (65.41%) of the respondents are male and the remaining 64 (34.59%) are female. It is inferred that male respondents are greater than the female respondents in utilizing the social network site.The above table shows the age-wise social network site users in the sample area. 111 (60.00%) of the respondents were below 18 years, 54 (29.19%) of the respondents belong to the age group of 19 to 20 years and the remaining 20 (10.81%) of the respondents were of above 21 years. Majority of the respondents for the present survey were of below 18 years.An analysis of education wise social network site users, 143 (77.30%) of the respondents were graduate and the remaining 42 (22.70%) of the respondents were postgraduate.

Table no 2 explains that frequency wise respondents. of the total respondents, 135 (72.97%) are using social network site daily, 37 (20.00%) users are using weekly once and the remaining 13 (07.03%) users are using monthly once.Table no 3 mentions the social networking access place. 98 (52.97%) respondents are using social network site inside the college campus and 168 (89.19%) respondents are using outside the college area. Majority of the respondents used social network site in outside the college area.Table no 4 shows the devices for use of the social network site by the sample respondents. Among the 185 sample respondents, 171 (92.43 %) respondents have used Mobile.
37 (20.00 %) respondents have been using iPod. 155 (83.78%) respondents have used Laptop and 74(40.00%) respondents expressed their usage of Personal Computer. Majority of the respondents of the present survey used social network site in Mobile.

Table 5 presents the results of perception and the researchers found that 139 (75.14%) respondents were of the view of sharing information through the social network: 111 (60.00%) respondents opinioned for Increase the Friendship; 169 (91.35%) respondents were in favour of the time pass and 22 (11.89%) respondents prefer social network to develop skills. Majority of the respondents of the present survey perception on the benefit for Time pass.

From Table no.6 it is evident that the respondents' opinion regarding overall satisfaction, 163 respondents highly satisfied with the statement, and 16 respondents satisfied with the statement, while 6 respondents dissatisfied with the statement respectively. Regarding the gender wise analysis, 110 male respondents and 53 female respondents are highly satisfied. For the age-wise analysis highly satisfied respondents 96 respondents under the age group Below 18 years, 49 respondents under the age group 19 - 20 years and the remaining 18 respondents under were above 21 years. Regarding the Educational qualification wise analysis, 126 undergraduate respondents and 37 postgraduate respondents are highly satisfied.

The below table shows that Gender wise analysis of overall satisfaction among the engineering college students, Calculated value 3.63 is less than 5.90 (0.05) at df 2. The hypothesis is accepting.The table also shows that Age wise analysis of overall satisfaction among the engineering college students, the calculated value 01.75 less than 9.48 (0.05) at df 4. The hypothesis is accepting. The table shows that Educational wise analysis of overall satisfaction among the engineering college students, Calculated value 2.43 is less than 5.90 (0.05) at df 2. The hypothesis is accepting.

FINDINGS

- It is inferred that male respondents are greater than the female respondents in utilizing the social network site.
- Majority of the respondents for the present survey belong to the age group of below 18 years.
- Majority of the respondents for the present survey using social network site in outside the college area.
- Majority of the respondents of the present survey used social network site in Mobile.
- Majority of the respondents used social network for Timepass.

CONCLUSION

The use of these websites is growing rapidly, while other traditional online is on the decrease. Social network users are staggering, vastly increasing the exposure potential to the educational industry through advertising industry. Social networks offer people great convenience for networking. It allows people to keep in touch with friends, and with family members, meet new people, and even conduct business meeting online. We can find people with similar interests as we get to know them better, even if they are in outside of one's domicile country. Every day people are joining in the social network and increasing all over the World.

SUGGESTIONS

Product Innovation: Offer comprehensive services and adopt learnable things from others social media sites provide more things based on young people's needs, such as jobs, news, weather, or exams which relate to their life closely.

Infrastructure Management: social network properties by adopting real name registration policy. Consider making money from individual users; looking for new business partners and integrate e-commerce platform.

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Figure 1. Social Network Site

Table 1: Demographic factors of respondents

GENDER WISE RESPONDENTS		
GENDER	**RESPONDENTS**	**PERCENTAGE**
Male	121	65.41
Female	64	34.59
Total	**185**	**100.00**
AGE WISE RESPONDENTS		
AGE	**RESPONDENTS**	**PERCENTAGE**
Below 18 Years	111	60.00
19 Years- 20 Years	54	29.19
21 Years and above	20	10.81
Total	**185**	**100.00**
EDUCATIONAL QUALIFICATION WISE RESPONDENTS		
EDUCATIONAL	**RESPONDENTS**	**PERCENTAGE**
Graduate	143	77.30
Post Graduate	42	22.70
Total	**185**	**100.00**

Source: Primary data

Table 2: Frequency Wise Respondents

FREQUENCY	RESPONDENTS	PERCENTAGE
Daily	135	72.97
Weekly	37	20.00
Monthly	13	07.03
Total	**185**	**100.00**

Source: Primary data

Table 3: Place of Access

PLACE	RESPONDENTS	PERCENTAGE
Inside College	98	52.97
Outside College	165	89.19

Source: Primary data *Percentage out of 185

Table 4: Usage of Devices

Devices	Gender		Total
	Male	Female	
Mobile	114 (61.62%)	057(30.81%)	171(92.43%)
i Pod	024(12.97%)	013(07.03%)	037(20.00%)
Laptop	101(54.59%)	054(29.19%)	155(83.78%)
Personal Computer	041(22.16%)	033(17.84%)	074(40.00%)

Source: Primary data *Percentage out of 185

Table 5: Perception-wise respondents

Perception of the Benefit	Gender		Total
	Male	Female	
Sharing Information	098(52.97%)	041(22.16%)	139(75.14%)
Increase The Friendship	087(47.03%)	024(12.97%)	111(60.00%)
Time Pass	110(59.46%)	059(31.89%)	169(91.35%)
Develop Skills	017(09.19%)	005(02.70%)	022(11.89%)

Source: Primary data *Percentage out of 185

Table no. 7 Overall Satisfaction

FACTORS		Level of Satisfaction							
		Highly Satisfied		Satisfied		Dissatisfied		Total	
GENDER	Male	110	59.46%	9	4.86%	2	1.08%	121	65.41%
	Female	53	28.65%	7	3.78%	4	2.16%	64	34.59%
	Total	**163**	88.11%	**16**	8.65%	**6**	3.24%	**185**	100.00%
AGE	Below 18 Years	96	51.89%	10	5.41%	5	2.70%	111	60.00%
	19 Years- 20 Years	49	26.49%	4	2.16%	1	0.54%	54	29.19%
	21 Years and above	18	9.73%	2	1.08%	0	0.00%	20	10.81%
	Total	**163**	88.11%	**16**	8.65%	**6**	3.24%	**185**	100.00%
EDUCATION	Under Graduate	126	68.11%	11	5.95%	6	3.24%	143	77.30%
	Post Graduate	37	20.00%	5	2.70%	0	0.00%	42	22.70%
	Total	**163**	88.11%	**16**	8.65%	**6**	3.24%	**185**	100.00%

Source: Primary data

Table 10: Chi-Square Analysis of Overall Satisfaction

Factor	Calculated $\chi 2$ Value	Table Value (0.05)	D.F	Remarks
Gender	3.63	5.90	2	Accepted
Age	1.75	9.48	4	Accepted
Educational	2.43	5.90	2	Accepted

Source: Primary data

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Influence and Optimization of Machining Process Parameters on Surface Roughness in Die-sinking EDM for Mild Steel using Taguchi Method

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Received: 05 Feb 2020 Revised: 08 Mar 2020 Accepted: 11 Apr 2020

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ABSTRACT

The Electric Discharge Machining is an extensive non-traditional machining process using thermoelectric source of energy for economic machining of the exceptionally low machinability materials and intricate jobs. The optimized settings of different input parameter in this type of Machining has been determined in the present study. The key input parameters chosen are Pulse Current (I), Pulse on time (T_{on}), and Pulse off time (T_{off}) with output as Surface Roughness (R_a).Taguchi L9 orthogonal array was used for design development and experiments were carried out with Brass, Copper and Aluminium electrodes separately on Mild Steel. ANOVA approach was used to evaluate the effect of input parameters on output response with help of MINITAB 17 software. To obtain minimum roughness, the process parameters were optimized. The experimental outputs were eventually checked by confirmatory tests.

Key words: Die-sinking EDM, Electrode, Process Parameters, Taguchi Method

INTRODUCTION

Highest machining technique without need of manpower, expected qualitative result with minimum tolerance work and exotic materials leads for more demand of Electric Discharge Machining (EDM). It is a thermo-electric material removal process which removes material by erosion effect of series of sparks between tool and work piece immersed in a dielectric fluid. Material properties do not have any influence on machining process in EDM. The electrodes used with sinker EDM (typically graphite or copper) are often machined in a mirror or reverse image of the workpiece shape, although round, square and other shapes are also used. As the industry moves toward the fourth industrial revolution, EDM machining is expected to become more intelligent to enhance the productivity and efficiency of the system.

Aditya Kumar et al. [1] analysed various parameters of wire EDM using taguchi method to check MRR and roughness of surface. They develop a model for optimising by hybrid genetic algorithm and recommended grouping parameters. Arshad Noor Siddiquee et al. [2] optimized the parameter of deep drilling in CNC lathe by the use of solid carbide cutting tool. They used AISI 321 austenitic stainless steel material basing on Taguchi L18 orthogonal array process to minimise the roughness of surface. Srinivasa Rao et al. [3] analysed various methods such as grey, fuzzy and Taguchi process for welding of TiO_2-Al_20_3-CaO. From experimental result they found that rate of performance were improved by 15.72% and 11% at 32, 34 volt respectively. S. Assarzadeh et al. [4] used tungsten carbide-cobalt composite in EDM to optimise process parameter. From experimental results they observed that all response found changed in accordance with rate of discharged energy. Chen et al. [5] used Taguchi design technique for optimizing EDM process parameter in machining A6061-T6 aluminum alloy. From experiment they found optimum machining data and its impact on roughness of surface by use of ANOVA and analysis of means method. Nikalje et al. [6] analysed the impact of process parameter and found that optimum value of factors for tool wear rate and surface roughness were equal but differ from the optimal level of the factors for RWR and MRR. Kodlinge and Khire [7] investigated on material removal rate of Tungsten carbide in EDM using Kerosene as dielectric fluid. 23 factorial designs had taken into consideration and from ANOVA result it was observed that, three factors had more effect on material removal rate.

Das et al.[8] investigated optimising result of machine parameter on MRR in electric discharge machining using EN31 tool steel. Machine parameters were designed by Taguchi's L27 Orthogonal Array. They found that current had most effective role on material removal rate. Dhanabalan et al. [9] observed the effect of machine parameter on surface roughness, material removal rate and total wear rate.They used two types of Titanium grades using brass electrodes. They had also explained multi objective optimisation on orthogonal array with grey relational analysis in electric discharge machining.

S. Gopalakannan et al. [10] used metal matrix composite of aluminum 7075 reinforced with 10 % wt. of B4C to study impact of process parameter and their relation with surface roughness, material removal rate and electrode wear rate. They found that MRR rises with rise in pulse on time and further rise in time lowers the rate of material removal. Current and time had found remarkable effect on surface roughness and electrode wear rate. Pragya Shandilya et al [11] used response surface method to optimise parameter of process during operation of SiCp/6061 Al metal matrix composite in wire EDM. They verified that parameters had major role in minimising kerf. They also developed mathematical model to analyse roughness, kerf and microstructure. Alikbari [12] used taguchi method to optimise process parameter in rotary EDM with X210CrNi12 alloy material and three copper electrodes having no hole, one concentric hole and two symmetric eccentric hole. Number of hole in tool rises SR and MRR. S Chandramouli and K Eswaraiah [13] experimented 17-4 precipetation hardening stainless steel in EDM to optimise process parameters. They demanded that selection of input parameter will have vital role on MRR and surface roughness. Influence of input data on output were checked and analysed in their work using ANOVA with help of MINITAB 17 software. S Chandramouli and K Eswaraiah [14] optimised input parameters to get maximum MRR. They have used ANOVA with MINITAB 17 to analyse the impct of input parameter on MRR. They also demanded that choosing proper input parameter will have major role on MRR.

This paper emphasizes on EDM process which was carried out with three controlling input data Pulse current, Pulse on time, and Pulse off time, while machining of the Mild Steel with three different types (Copper, Brass & Aluminium) of electrodes. Then, different condition of machining were optimized for better result using Taguchi technique which approaches logically and sequentially to optimize the process parameters.

Details of Research

The work piece material used were Mild Steel Plates (Length-135 mm, Width- 20 mm & Thickness 10 mm). The mild steel contains Fe (96.840%), Mn (0.709%), P (0.029%), S (0.132%), Si (0.720%), Al (0.143%, K (0.272%), Ca (0.196%), C (0.924%). Three electrodes of same diameter i.e 15 mm were used.

Composition of Copper Electrode: P (0.267%), Cl (0.142%), Ca (0.183%), Fe (0.124%), Ni (0.128%), Cu (97.176%), Zn (1.619%), As (0.020%), Sn (0.046%), Er (0.166%) & Pb (0.129%)
Composition of Brass Electrode: P (0.211%), Cl (0.101%), Ca (0.117%), Cr (0.010%), Mn (0.038%), Fe (0.308%), Ni (0.264%), Cu (58.498%), Zn (36.468%), Sn (0.403%)
Composition of Aluminium Electrode: Al (90.162%), Si (0.343%), Cr (0.273%), Fe (0.228%), Cu (1.846%), Zn (6.537%).
ZNC-25 (JK Machines) die-sinking EDM machine was used for operations. Portable stylus-type profilometer, Talysurf (Taylor Hobson, Surtronic 3+, UK) was used for measuring roughness of surface.

EXPERIMENTATION METHODOLOGY

Three different input parameter with their levels are shown in Table 1, Table 2 represents plan of experimentation and responses (surface roughness). Lower-the-better quality characteristic is followed.

Table 1: Input parameters with their different levels

Parameters	Levels		
Pulse Current, I, amp	15	20	30
Pulse on time, T_{on}, µs	40	50	60
Pulse off time, T_{off}, µs	5	10	15

Table 2: Experiment Data

Sl.No	Pulse Current, I, amp	Pulse on time, T_{on}, µs	Pulse off time, T_{off}, µs	Roughness, Ra, µm
Copper Electrode				
1	15	40	5	**3.19**
2	15	50	10	**3.98**
3	15	60	15	**1.18**
4	20	40	10	**2.42**
5	20	50	15	**3.57**
6	20	60	5	**3.41**
7	30	40	15	**1.11**
8	30	50	5	**2.12**
9	30	60	10	**3.18**
Brass Electrode				
1	15	40	5	**2.01**
2	15	50	10	**1.95**
3	15	60	15	**0.86**
4	20	40	10	**2.36**
5	20	50	15	**2.93**
6	20	60	5	**2.24**
7	30	40	15	**2.46**
8	30	50	5	**2.71**
9	30	60	10	**2.77**
Aluminium Electrode				
1	15	40	5	**2.23**
2	15	50	10	**2.73**

3	15	60	15	**2.69**
4	20	40	10	**2.31**
5	20	50	15	**2.32**
6	20	60	5	**2.35**
7	30	40	15	**3.91**
8	30	50	5	**2.66**
9	30	60	10	**3.17**

RESULTS AND DISCUSSION

Results of Analysis of variance (ANOVA) conducted is shown in tables.

Table 3.Analysis of Variance for Means (Copper Electrode)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
I	2	1.534	1.535	0.767	0.50	0.023
T_{on}	2	1.491	1.491	0.745	0.49	0.673
T_{off}	2	0.529	0.529	0.264	0.82	0.548
Residual Error	2	3.068	3.067	1.533		
Total	8	8.621				

Table 4. Table representing means (Copper Electrode)

Level	I	Ton	Toff
1	2.907	2.783	2.240
2	3.193	3.133	3.223
3	1.953	2.137	2.590
Delta	1.240	0.997	0.983
Rank	1	2	3

Table 4 representing Means. The R^2-value (coefficient of determination) is obtained as 95.2%, along with S = 0.239, R-Sq (adj) = 89.8%.

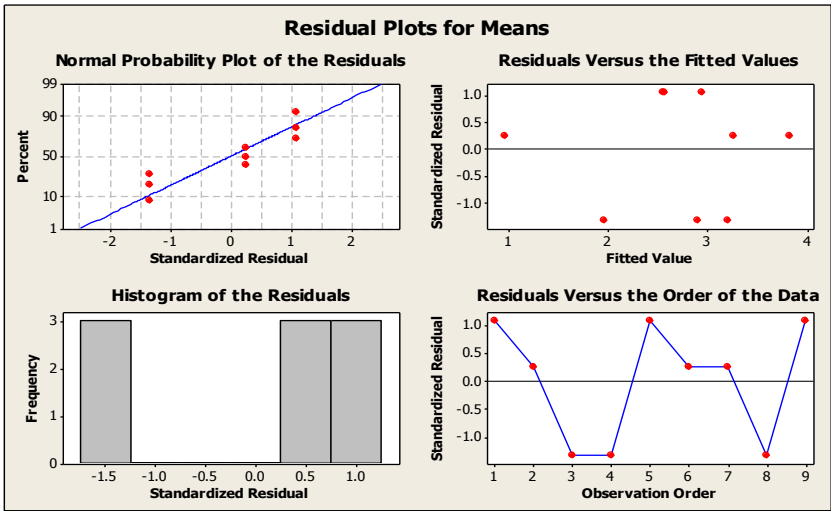

Fig .1.Residual plots (Copper Electrode)

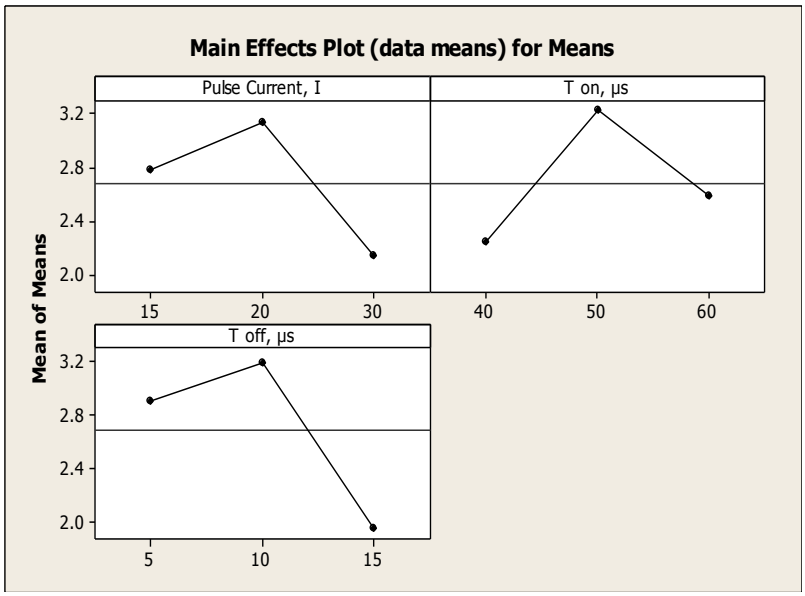

Fig. 2. Main Effect Plot (Copper Electrode)

Table 5. Analysis of Variance for Means (Brass Electrode)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
I	2	1.916	1.916	0.958	3.59	0.018
T_{on}	2	0.495	0.495	0.247	0.93	0.519
T_{off}	2	0.134	0.134	0.067	0.25	0.799
Residual Error	2	0.534	0.534	0.267		
Total	8	3.080				

Table 6. Representing Means (Brass Electrode)

Level	I	Ton	Toff
1	1.607	2.277	2.320
2	2.510	2.530	2.360
3	2.647	1.957	2.083
Delta	1.040	0.573	0.277
Rank	1	2	3

The R^2-value (coefficient of determination) is obtained as 97.2%, along with S = 0.517, R-Sq (adj) = 90.8%.

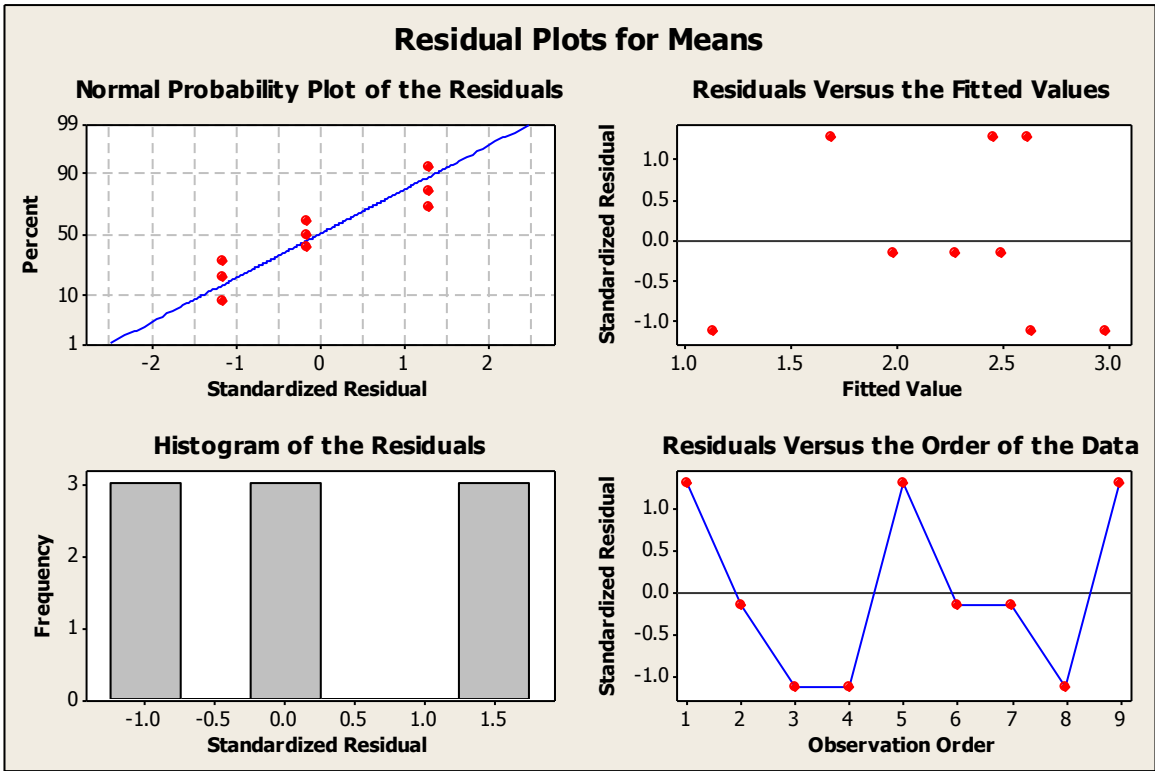

Fig .3.Residual plots (Brass Electrode)

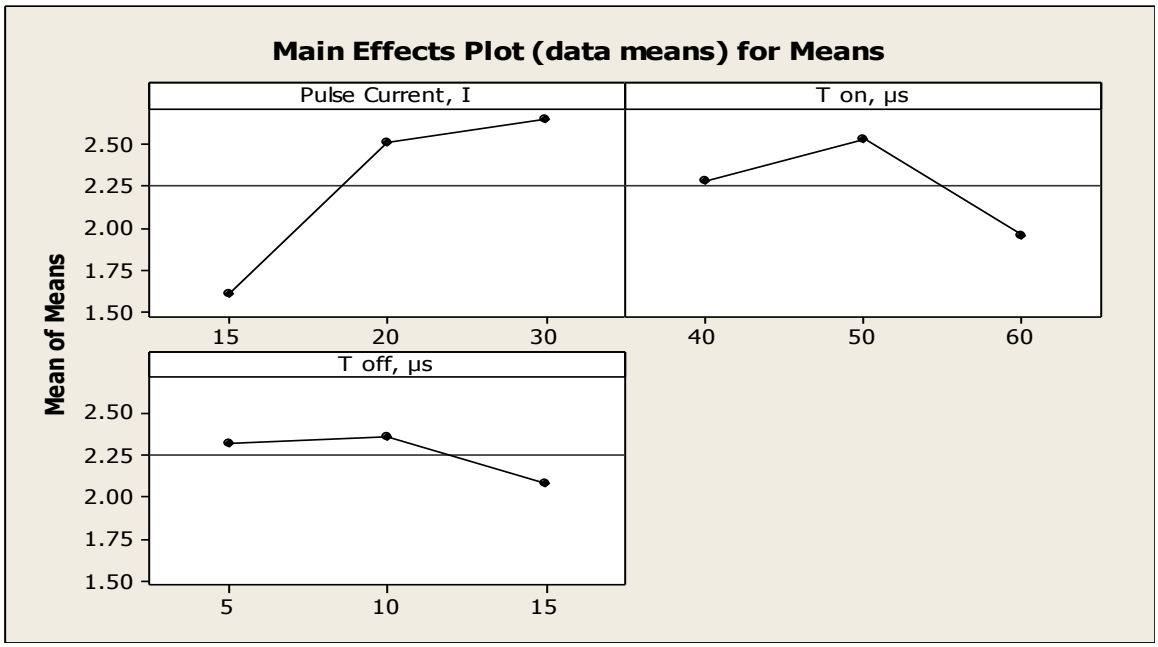

Fig .4.Main Effect Plot (Brass Electrode)

Table 7.Analysis of Variance for Means (Aluminium Electrode)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
I	2	1.381	1.381	0.690	3.67	0.038
T_{on}	2	0.095	0.095	0.047	0.25	0.798
T_{off}	2	0.474	0.474	0.237	1.26	0.442
Residual Error	2	0.376	0.376	0.188		
Total	8	2.326				

Table 8.Representing Means (Aluminium Electrode)

Level	I	Ton	Toff
1	2.550	2.413	2.817
2	2.327	2.737	2.570
3	3.247	2.973	2.737
Delta	0.920	0.560	0.247
Rank	1	2	3

The R^2-value (coefficient of determination) is obtained as 96.6%, along with S = 0.433, R-Sq (adj) = 87.2%.

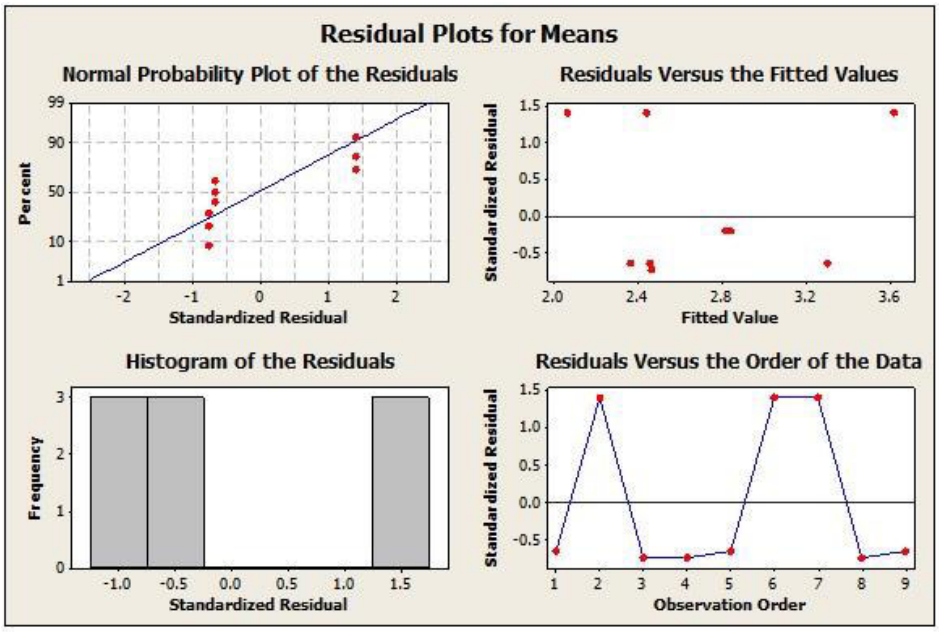

Fig .5.Residual plots (Aluminium Electrode)

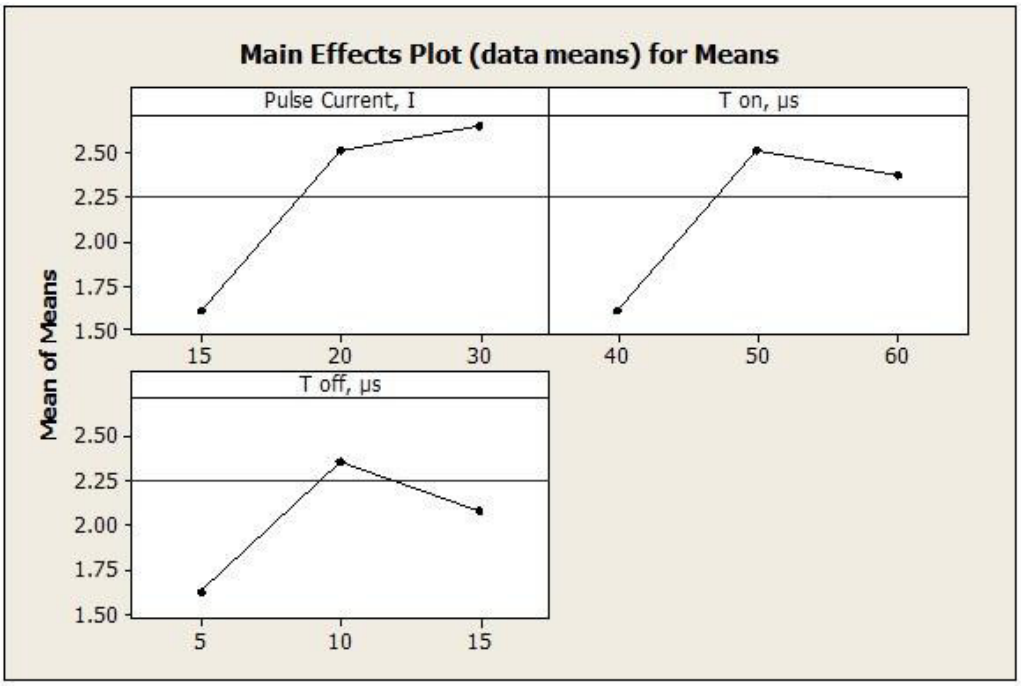

Fig .6.Main Effect Plot (Aluminium Electrode)

Table 9.Optimal Parameter Settings for Roughness Minimization

Electrode	Pulse Current, A	Pulse on Time, µs	Pulse off Time, µs
Copper	30	40	15
Brass	15	60	15
Aluminium	15	40	5

Table 10.Predicted Values & Confirmatory Results

Electrode	Pulse Current, A	Pulse on Time, µs	Pulse off Time, µs	Roughness, µm
Copper	30	40	15	1.03
Brass	15	60	15	0.97
Aluminium	20	50	5	1.89

A confirmatory test was performed. The variation in experimental surface roughness and estimated/predicted values is observed to be 5% by ANOVA. Therefore it is concluded that Taguchi's experimental evaluation was implemented accurately & the most favorable (optimum) combination of the input parameters was sufficiently good to minimize surface roughness.

CONCLUSION

- Discharging current was found to have major role on roughness followed by pulse on time. Pulse off time had minor role in comparison with other different parameter.

- A good correlation between the pulse current and the pulse on time is advised i.e to operate with high pulse current values and low pulse on time values.
- Increase in either pulse current or pulse on time increases spark energy; increase in spark energy increases the MRR; and increase in the MRR results in a coarser surface finish.
- Brass electrode produces less surface roughness as compared to Aluminium & Copper (Due to low thermal & electrical conductivity, and low MRR).

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Comparative Safety and Efficacy of Sitagliptin and Glimepiride in Combination with Metformin in Type 2 Diabetic Patients

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Received: 18 Feb 2020 Revised: 21 Mar 2020 Accepted: 23 Apr 2020

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ABSTRACT

Combination therapies are becoming vital for the appropriate glycemic control; Metformin is recommended as the first line treatment for type 2 diabetes mellitus (T2DM) while dipeptidyl peptidase-4 inhibitor Sitagliptin and Glimepiride are being utilized as add-on therapy to improve the efficacy profile. The purpose of this study was to compare the safety and efficacy of Sitagliptin and Glimepiride in combination with Metformin in T2DM patients.An observational, comparative, single center study was conducted on a sample of 180 T2DM patients presented at the Medicine & Endocrinology Department of Lady Reading Hospital (LRH), Peshawar. Patients were randomized to either Sitagliptin (50-100 mg) and Glimepiride (1-2 mg), both in combination with Metformin (500 -1000 mg)/day. Follow-up visits were planned twice after the baseline visit i.e. after 3 months and 6 months. Data regarding patients' clinical characteristics were collected and analyzed using SPSS Version 22. The mean HbA1c level (%) was significantly reduced in group 1 as compared to group 2 (-2.26 vs -2.39), the same pattern was observed for the mean PPG reduction as well ($p < 0.05$). While for FPG (mg/dl) the mean reduction rate was more prominent in group 2 (-50.07 vs -46.76). Minor adverse effects like weight gain and hypoglycemic incidences were common among group 2 patients administering Glimepiride, in contrast, the patients of group 1 administering sitagliptin-metformin combination reported weight loss. Both groups showed significant improvement in the efficacy profile by the end of the study period. Moreover, no serious side-effects were associated with the drugs administered.

Keywords: Type 2 Diabetes Mellitus, Blood Glucose, Sitagliptin, Glimepiride, Metformin.

INTRODUCTION

T2DM is the most common chronic public health disease, it has affected vast population worldwidewith increased incidence rate among the developing countries [1]. Which is leading towards serious health consequences, reduced quality of life and increased associated mortality rate. Previously,it was considered as a disease of affluence but due to lifestyle modification in urban areas, the disease risk is now more commonin middle to lowincome countries for e.g. Pakistan [2]. Based on the statistics provided by the International Diabetes Federation (IDF) in the year 2017, a total of 7.5 million Pakistanis were affected with T2DM [3]. It is a slow progressive disease, remains asymptomatic and undiagnosed for years. Locally it is observed that the suspects of T2DM carry this silent killer for almost 4-7 years prior the diagnosis with no symptoms, which highlights the fact that we are having several silent undiagnosed diabetic cases [4]. Due to which the frequency of underdiagnosis is increasing readily, delayed diagnosis results in chronic hyperglycemia which assures complications [5].

American Diabetes Association (ADA) prefer fasting plasma glucose (FPG) or 75 gm OGTT for diagnosis of diabetes [6]. While a new criterion has been proposed by the International Expert Committee for diagnosis, which include HbA1c [7]. Although the threshold cut-off is not validated for Pakistani population but globally 48 mmol/mol (6.5%) HbA1c is considered as the cut-off value for diagnosis [8]. The most common first line antihyperglycemic medicine prescribed to every T2DM patient isMetformin (biguanide) [9], it acts by activating adenosine monophosphate-activated protein kinase (AMPK). It reduces the glucose production in the liver, increases the uptake of glucoseand insulin sensitivity leading to glycemic control in T2DM patients [10]. However, in the cases where metformin monotherapy is not sufficiently effective in controlling hyperglycemia, the combination therapy is preferred as it effectively controls and maintain glucose concentration among diabetic patients [9]. Although the efficacy profile of metformin is well-established but the results obtained from the use of single antihyperglycemic agent are sub-optimal in comparison to the combination therapy [11].

Sitagliptin and Glimepiride are the two most common second line drugs used in combination with metformin for the treatment of T2DM. Where, sitagliptin is an oral hypoglycemic medicine that blocks the dipeptidyl peptidase-4 (DPP-4) [12]. It controls the glucose metabolism by stimulating the release of incretin hormones like glucagon like peptide-1 (GLP-1) and glucose dependent insulinotropic peptide (GIP) [13]. Under hyperglycemic condition, these incretin hormones function by lowering the glucagon release and boosting the insulin activity in order to control glucose surge [14]. However, preventing the inactivation of GLP-1 and GIP through DDP-4 aids in effective glycemic control [14]. Glimepiride, on the other hand belongs to the class of sulfonylureas, often recommended in the form monotherapy or in combination with metformin [15]. It is responsible to target both, beta cell dysfunction and insulin resistance [15,16]. Furthermore, it is angiogenic, anti-inflammatory and antioxidative in nature, assures safety profile for cardiovascular cases as well [17].

Several randomized clinical trials and comparative studies had been conducted to evaluate the effectiveness of these therapeutic agents in the management of T2DM both nationally and internationally with minor adverse reactions [18-20]. But a conclusive evidence in favor of a specific combination therapy for the treatment of T2DM, in terms of effectiveness, improved quality of life and decreased adverse effects (AE'S) is yet to be drawn [21,22]. Therefore, it isn't easy to generalize a specific combination therapy or monotherapy for all healthcare settings and population, as the therapeutic approach might vary according to the patient's economic status and personal satisfaction as well. This study aims to assess the comparative safety and efficacy of the combination therapy; sitagliptin and metformin vs glimepiride and metformin among T2DM patients with respect to glycemic control.

MATERIALS AND METHODS

Study design, Setting and Sample size

This observational, comparative, single center study was conducted at department of medicine and endocrinology of Lady Reading Hospital (LRH), Peshawar. The study continued for duration of 1 year from January 2018 to January 2019.

Primary outcomes

The primary aim of the study was to evaluate the alterations in the HbA1c, FPG and PPG level after treatment with Sitagliptin vs Glimepiride in combination with Metformin among T2DM patients. Moreover, the AE's were also monitored and managed accordingly.

Patients Inclusion / Exclusion Criteria

- Inclusion Criteria

All the uncontrolled type 2 diabetics and the newly diagnosed cases (FPG ≥ 126 mg/dl) aged between 18 to 70 years irrespective of gender, taking metformin for the past 3 months, with HbA1C levels >7% and <10% were included in the present study.

- Exclusion Criteria

All T2DM patients exceptthe mentioned age limit and those with type 1, gestational or secondary DM were excluded. Pregnant or lactating females were also ineligible, patients with certain diseased conditions like myocardial infarction, end stage renal failure, hepatic diseases, other terminal illnesses and those with previous history of surgical procedure (4 weeks prior the study conduction) and hypersensitivity were also excluded from the study.

Proposed intervention

Sitagliptin (50-100 mg) and Glimepiride (1-2 mg) were used as the interventions for a period of 24 weeks. The patients were randomly divided into two group, with 90 T2DM patients in each group. With reference to the prescription from the physician and the previous glycemic records, patients in group 1were treated with the combination ofSitagliptin (50-100 mg) plus Metformin (500 -1000 mg) per day, whereas group 2 was given Glimepiride (1-2 mg) plus Metformin (500 -1000 mg) per day (Figure I).

Follow-up Visits, Data management and statistical analysis

The study continued for 24 weeks, FBS, PPG and HbA1c was assessed at baseline, 12 weeks and 24 weeks. The interventional safety, efficacy and tolerability was checked at each follow-up visit. The demographic details including age, gender, BMI and weight were recorded for each patient. Data was analyzed via SPSS version 22.0 and all continuous variables were reported as mean and standard deviation (SD), while frequency (n) and percentage (%) was used for presenting categorical variables. The difference in the efficacy parameters between the groups was compared through paired sample t-test and ANOVA, where $p < 0.05$ was considered statistically significant.

Ethical considerations

Before initiation, the protocol of the study was approved by the ethical review board of Lady reading Hospital, Medical teaching Institution (Reference no. 1888/MA; dated 5th December' 2017). The study was conducted in

accordance to Declaration of Helsinki. The overall study conduct was thoroughly explained to each patient and written informed consent was obtained prior to the enrollment of patients.

RESULTS

There was no statistical difference in the demographic and clinical characteristics among the patients of both groupsat the baseline visit as shown in table I.

Efficacy

The results showed that significant improvement was observed in HbA1c levels, FPG and PPG among the patients of both groups ($p<0.05$). Mean HbA1c levels (%) changed from 10.11 ± 1.62 to 7.85 ± 1.31 (Group 1) and 10.45 ± 1.71 to 8.06 ± 1.46 (Group 2) i.e. 2.26% and 2.39% reduction between baseline and follow-up visit (after 24 weeks) respectively ($p<0.05$). Similarly, the mean FPG decreased from 164.34 mg/dl to 114.27 mg/dl (Group 1) and 163.36 mg/dl to 116.60 mg/dl (Group 2). Moreover, the mean difference observed in the PPG level was -110.78 mg/dl for group 1 and -115.51 mg/dl (Table II).

Safety

Minor adverse reactions were observed after treatment with both Sitagliptin and Glimepiride, where nasopharyngitis was the most reported adverse drug reactionin group 1 and joint pain in group 2. Moreover, it can be seen that more patients from group 2 reported these adverse drug reactions as compared to those in group 1, increased number of hypoglycemicincidences were observed among the patients treated with Glimepiride as compared those treated with Sitagliptin (3% vs 10%) (Table III). The body weight was altered in both groups, moreover variations in body mass index were also present but these alterations were minor and controllable as shown in table IV.

DISCUSSION

Over the years there has been a massive shift in the management and treatment of T2DM, drugs dealing the basics to specificities had been discovered ensuring the efficacy and safety among patients with widespread non-communicable diseases. Much research is now being conducted on the disease specifications and newer antidiabetic agents including dipeptidyl peptidase-4 inhibitors (DPP4i), sodium-glucose co-transporter 2 inhibitors, GLP-1 analogs, and insulin analogs. The modern Sulfonylureas i.e. Glimepiride are being used in combination with metformin in clinical settings [23]. Although the efficacy outcomes are in favor of glimepiride in comparison to sitagliptin according to the literature [24], but due to the associated adverse reactions like hypoglycemia and weight gain its use is limited [25].

The comparative safety and efficacy of sitagliptin and glimepiridein combination with metformin (week 0-24) was assessed in this comparative, observational study on T2DM patients displaying inadequate glycemic control. The combination therapy provided statistically significant reduction in HbA1C, FPG and PPG levels after 24 weeks of treatment, improvements in diabetic control was observed in both groups after treatment i.e. mean HbA1c reduction, 2.26% in group 1 and 2.39% in group 2 ($p < 0.05$) (Table II). Similarly, a study reported significant decline in all three efficacy parameters including HbA1c, FPG and PPG ($p < 0.001$) by the end of 24 week [26], which is supported by other parallel studies as well [27,28].

Minor adverse drug reactions including nausea, headache, nasopharyngitis, joint pain and dizziness etc. were observed among the patients of both group with slight increased number of such incidences in patients treated with

Glimepiride (Table III). These AE's were mild and did not require any treatment modifications or discontinuation. Patients administering sulfonylurea as usually report hypoglycemia and weight gain after treatment [23, 29]. Similar outcomes were observed in our study after treatment with Glimepiride, weight gain and hypoglycemic incidences were more commonly observed among patients of group 2 as compared to the counterparts i.e. the mean weight 72.56 ± 14.10 kg at baseline increased to 73.31 ± 13.94 kg after 24 weeks, the case was reversed for those treated with Sitagliptin as they reported weight loss (Table III & IV). Other than weight gain, the difference in the incidence rate of other adverse experiences was minor between the two groups.

Based on our results both the therapies displayed comparable safety and efficacy profile and were well tolerated. Although the results are in support of the hypothesis but the study holds several limitations indicating the need for a descriptive clinical trial. The sample size of the study groups was small, to strengthen our conclusions further studies on large samples are required in order to check the comparative efficacy of the two drugs on large-scale. More pronounced results are expected if these combination therapies are administered in comparison to control group furthermore, a third additional drug might also help.

CONCLUSION

In conclusion, the combination therapy Sitagliptin plus Metformin is efficacious and well tolerated. Both the drugs showed comparable glycemic control i.e. parameters such as FPG, PPG, and HbA1c decreased during the study period. Hence providing better efficacy, decreased hypoglycemic risk, weight maintenance and safety profile Sitagliptin can be preferable choice as an add therapy with metformin in T2DM patients. Further studies with large sample size and diverse study population are required in support of the study hypothesis.

ACKNOWLEDGMENT

The authors are thankful to Medical Affairs Department of Getz Pharma for their assistance in the research and manuscript writing.

FINANCIAL SUPPORT AND SPONSORSHIP

None.

CONFLICTS OF INTEREST

None.

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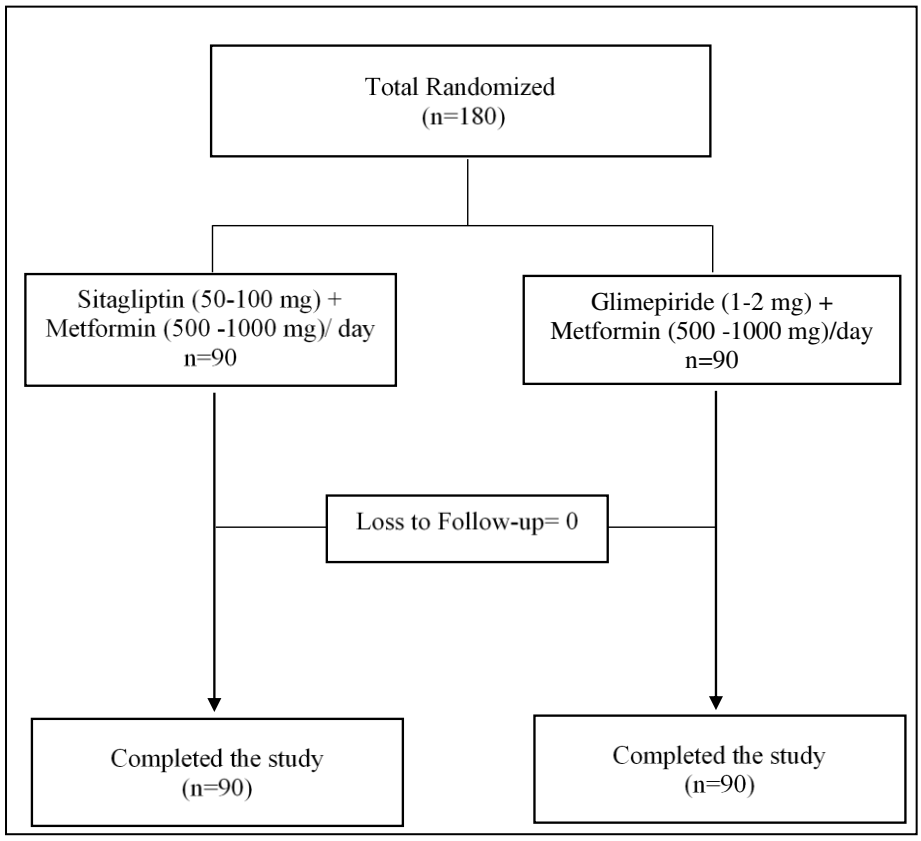

Figure 1: Disposition of Study participants.

Table 1.Demographic and clinical characteristics of the study population at the baseline visit

Baseline Characteristics		**Study Groups (n=180)**		**P-value**
		Group 1 (n=90)	**Group 2 (n=90)**	
Gender	Male	***n(%)*** 34(37.8)	***n(%)*** 40(44.4)	0.363
	Female	56(62.2)	50(55.6)	
		Mean±SD	**Mean±SD**	
Age (years)		48.69±9.57	46.20±10.34	0.096
Weight (kg)		73.27±14.04	72.56±14.10	0.735
BMI (kg/m^2)		27.25±4.63	27.69±4.95	0.541
HbA1c (%)		10.11±1.62	10.45±1.71	0.174
FPG (mg/dl)		164.34±31.41	163.36±28.07	0.84
PPG (mg/dl)		271.16±64.68	300.66±60.50	0.002*

**Values are given as n(%) and Mean±SD*

**Group 1- Sitagliptin/Metformin; Group 2- Glimepride/Metformin*

**BMI- Body mass index, HbA1c-Glycosylated hemoglobin, FPG-Fasting plasma glucose, PPG-Postprandial plasma glucose; SD- Standard deviation.*

**Significant (p-value<0.05).*

Table 2.Comparison of efficacy parameters between the groups both at baseline and after 24 weeks.

Parameters	**Group 1**			**Group 2**			**P-value**
	Baseline	***After 24 Weeks***	***Mean Difference***	***Baseline***	***After 24 Weeks***	***Mean Difference***	
HbA1c (%)	10.11±1.62	7.85±1.31	-2.26±0.31	10.45±1.71	8.06±1.46	-2.39±0.25	<0.05
FPG (mg/dl)	164.34±31.41	114.27±15.65	-50.07±15.76	163.36±28.07	116.60±17.03	-46.76±11.04	<0.05
PPG (mg/dl)	271.16±64.68	160.38±37.91	110.78±26.77	300.66±60.50	185.15±49.40	-115.51±11.1	<0.05

**Values are given as Mean±SD*

**Group 1- Sitagliptin/Metformin; Group 2- Glimepride/Metformin*

**HbA1c-Glycosylated hemoglobin; FPG-Fasting plasma glucose; PPG-Postprandial plasma glucose; SD- Standard deviation.*

**Significant (p-value<0.05).*

Table 3.Adverse drug reactions observed in the groups after drug intervention

Adverse Drug Reaction	**Group 1**	**Group 2**
Nasopharyngitis	46(51.1)	25(27.7)
Headache	7(7.8)	11(12.2)
Nausea	17(18.9)	2(2.2)
Hypoglycemia	3(3.33)	9(10)
Dizziness	3(3.33)	5(5.55)
Joint Pain	3(3.33)	38(42.22)
Jaundice	11(12.2)	0(0)

**Values are given as n(%)*

**UTRI- Urinary Tract Infection*

**Group 1- Sitagliptin/Metformin; Group 2- Glimepride/Metformin*

Table 4.Weight and BMI changes in the groups after drug intervention

Parameters	Group 1		Group 2		P-value
	Baseline	*After 24 Weeks*	*Baseline*	*After 24 Weeks*	
Weight (kg)	73.27±14.04	72.77±13.53	72.56±14.10	73.31±13.94	<0.05
BMI (kg/m^2)	27.25±4.63	27.31±4.41	27.69±4.95	27.74±4.76	<0.05

**Values are given as Mean±SD*

**BMI- Body mass index*

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Comparative Studies on Mineral and Biochemical Analysis of *Penaeus vannamei* and *Macrobrachium rosenbergii*

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Received: 05 Feb 2020 Revised: 07 Mar 2020 Accepted: 11 Apr 2020

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ABSTRACT

The current study was aimed to determine the biochemical composition of fresh water prawn *Macrobrachium rosenbergii* and white leg shrimp *Penaeus vannamei*. The investigation of biochemical composition of the species showed that in *Penaeus vannamei* the amount of protein, carbohydrates, minerals and moisture content were 1.126 ± 0.164, 2.333 ± 0.043, 870500 ± 2120 PPM and 76.955% respectively and in *Macrobrachium rosenbergii* the amount of protein, carbohydrates, minerals and moisture content were 0.523 ± 0.017, 1.502 ± 0.042, 297100 ± 40.8 and 41.911% respectively. The analysis of the tabulated data was performed by using statistical tool to evaluate the mean and standard deviation.

Key words: Prawn, protein, carbohydrates, minerals, moister content.

INTRODUCTION

Aquaculture is a profitable business with better scope for job opportunity and foreign exchange for the nation. Aquaculture is an alternative solution at this crunch, hence this sector boomed globally including India (Joseph *et al.*, 2017). Global commercialization and business involving fish and fish products largely depend on shrimp (Bhaskar *et al.*, 1995). The white leg shrimp *Penaeus vannamei* belongs to family Penaeidae is world most popular shell fish and is mainly consumed in Europe and Asian countries (Puga-lopez, 2013). Crustacean such as shrimps are an important source of protein worldwide (*Heu et al.*, 2003). Along with this it contains calcium, various extractable compounds and minerals for human body, while low in calorie and fat and some elements, such as copper, zinc, manganese, iron and chromium have useful biological function and are found in shrimp at acceptable levels are very useful for human health (Oksuz *et al.*, 2009). Lipid of shrimp contains mostly polyunsaturated fatty acids (essential fatty acids). This essential fatty acids present in shrimp provides health benefits for human such as, eye (retina) and brain development and function (Connor *et al.*, 1992). Fresh water prawn *Macrobrachium rosenbergii* belongs to the family

Palaemonidae which includes the brackish and fresh water grass shrimp and larger liver shrimp (*Eglal et al*., 2011). *Macrobrachium rosenbergii* is a commercially important species of crustacean and has always been concerned as a suitable species for aquaculture because it can be grown in both fresh and low salinity water with good growth and survival rate (Balazs and Ross, 1976). Aquaculture of the fresh water prawns *Macrobrachium rosenbergii* is an emerging industry in the pacific island region (Nandlal and Pickering, 2015). Increasing demand and rising prices for seafood are raising the profile of freshwater prawns as an important aquaculture commodity in pacific island region (Nandlal and Pickering, 2005). However, freshwater prawn products are limited in the markets because their quality is degraded quickly through autolytic, microbial and oxidative spoilage (Haider *et al*., 2013). The prawn *Macrobrachium rosenbergii* contain greater amount of proteins, lipids and unique taste, less fat and has great demand in national and international markets. Shellfish contains potent source of nutrients required for the maintenance and growth of human body (Dong, 2001). Due to low price and efficient availability, the prawns and shrimps have good source of animal protein for low income earners (Adeyeye, 1996). Many previous reports were available on the growth and nutritional quality of *Macrobrachium rosenbergii* under different culture conditions (Gomez *et al*., 1988; Reed and Abramo, 1989; Sheen and Abramo, 1991; Hossain *et al*., 2007; Habashy, 2009). Saravana *et al*., (2010) and Reddy and Reddy (2014) were analysed that the proximate composition of biochemical constituents in the muscle of adult male and female prawns of *Macrobrachium rosenbergii* collected from natural culture site. Khalique *et al*., (2010) were studied the biochemical proteins, lipids, carbohydrates, aspects of shell fish nutrition. Protein is essential for the sustenance of life exists in large quantity of all nutrients as a component of human body (Okuzumi and Fugi, 2000). Shrimps are known to be a source of protein rich in essential amino acids, such as lysine, methionine, cystiene, threonine and tryptophan (Sikorski, 1994). Carbohydrates are major energy sources in human diet. The ratio of carbohydrates is less as compared to other nutrients like proteins and lipids in animal tissues of aquatic species. Essential amino acids (EAAs) play an important role in human nutrition and health promotion. Aquatic animal fats are good sources of essential fatty acids that cannot be synthesized in the human body and they are required for the maintenance of growth, reproduction and synthesis of vitamins. Carbohydrates are major energy source in human diet. The ratio of carbohydrate was less than compared to other nutrients like proteins and lipids in animal tissues of aquatic species.

MATERIALS AND METHODS

Collection of specimens

Fresh water prawns *Macrobrachium rosenbergii* and shrimps *Penaeus vannamei* were collected from the market of Aul, Kendrapara district, Odisha.

Experimental site

The experiment was carried out in Zoology laboratory of Centurion University of Technology and Management, BBSR.

PREPARATION OF SAMPLE

Preparation of muscle extract

Both the specimens were first washed properly, measured and then the flesh was ground and the weight of the ground flesh was measured by weighing machine. It was then stored in the oven for 24 hours at 100◦C. Then the dried flesh was ground to form powder by the help of mortar and pistle.

PROXIMATE ANALYSIS

Protein, carbohydrates, moisture and mineral of the specimen were determined according to Association of Official Analytic Chemists (AOAC) methods (AOAC, 1995).

Estimation of protein

The concentrations of total protein present in muscles of the prawns were estimated by using Lowry's method (Lowry, 1951). This experiment was done by taking 0.5gram of sample and mixed with 10ml of distilled water. Then the mixed sample centrifuged in 8000 RPM for 20min. After centrifugation 1ml of supernatant was collected. In the supernatant 5ml of reagent C (Fehling's A and Fehling's B in the ratio 5:1) was added and incubated for 15min in room temperature. After incubation 0.5ml Fehling's D was added. Then OD (Optical Density) was observed in spectrophotometer at 660nm.

Estimation of carbohydrate

The total carbohydrate content was estimated by the method (Hedge and Hofreiter, 1962). 0.1 gram of sample was taken into a boiling tube, hydrolysed by keeping it in a boiling water bath for three hours with 5ml of 2.5N HCl and cooled to room temperature. Then the sample was centrifuged at 8000 RPM for 3mins. After centrifugation 0.2ml of supernatant was collected. Then standard solution was prepared by taking 0.2ml of working standards. 1ml of water served as a blank made up the volume up to 1ml in all the tubes with distilled water and then 4ml of Anthrone reagent was added, heated for eight minutes in a boiling water bath, cooled rapidly and after that OD was measured by spectrophotometer at 630nm.

Estimation of elemental composition

Estimation of element composition was carried out by XRF method.

Estimation of moisture

Moisture content was measured by the weight difference before and after oven drying at 100◦C for 24 hours. The moisture content was calculated by the following formula:

Moisture (%) = (weight loss / original weight of sample taken) × 100

Moisture content of *Penaeus vannamei*

Original weight = 38.486 gm

Powder weight = 8.869 gm

Moisture (%) = 29.617/38.486 × 100 = 76.955 %

Moisture content of *Macrobrachium rosenbergii*

Original weight = 86.352 gm

Powder weight = 50.161 gm

Moisture (%) = 36.191/86.352 × 100 = 41.911 %

RESULTS AND DISCUSSION

From the data is was observed that the total protein, carbohydrate, elements and moisture content are maximum in Penaeus vannamei as compared to Macrobrachium rosenbergii (as mentioned in table). The proximate composition of fish varied widely from species to species and even within the same species from one individual to another (Stansby, 1962). The difference in the individual variation in terms of proximate composition was aided by sex,

season, reproductive behaviour, food sources availability, capture period and hydrologic level (Oliveira, 2003; May-Ku *et al*., 2006; Nargis, 2006)

CONCLUSION

The meat of freshwater prawn is a lean seafood with high protein, carbohydrates lipids etc. Biochemical analysis of shrimp and prawns proximate composition, the percentage of protein, carbohydrate, moisture, elements content in the muscle of prawn and shrimp are more or less. It is seen that the nutritional values of *Penaeus vannamei* is higher than *Macrobrachium rosenbergii*. Shell fish is an important source of food containing high quality of proteins, vitamins, minerals, carbohydrates etc. It has widely being accepted as dietary sea food due to substantial increase in beneficial effect of consumption.

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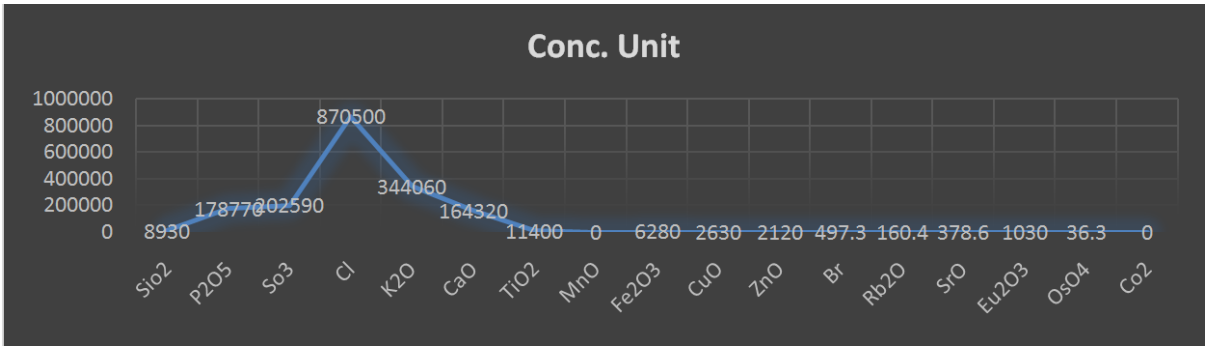
Table 1. Elements content in both the species

SL.No	Compound	Conc. Unit (*P. Vannamei*)	Conc. Unit (*M. rosenbergii*)
01	SiO_2	8930	8350
02	P_2O_5	178770	174080
03	SO_3	202590	232770
04	Cl	870500	80400
05	K_2O	344060	297100
06	CaO	164320	194600
07	TiO_2	11400	782.6

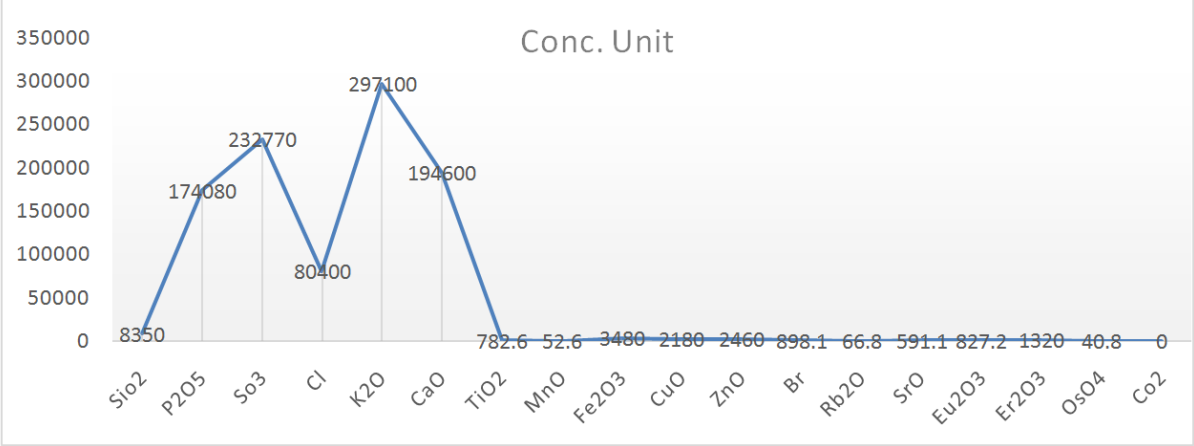
08	MnO	0	52.6
09	Fe_2O_3	6280	3480
10	CuO	2630	2180
11	ZnO	2120	2460
12	Br	497.3	898.1
13	Rb_2O	160.4	66.8
14	SrO	378.6	591.1
15	Eu_2O_3	1030	827.2
16	Er_2O_3	NA	1320
17	OSO_4	36.3	40.8
18	CO_2	0	0

Table.2

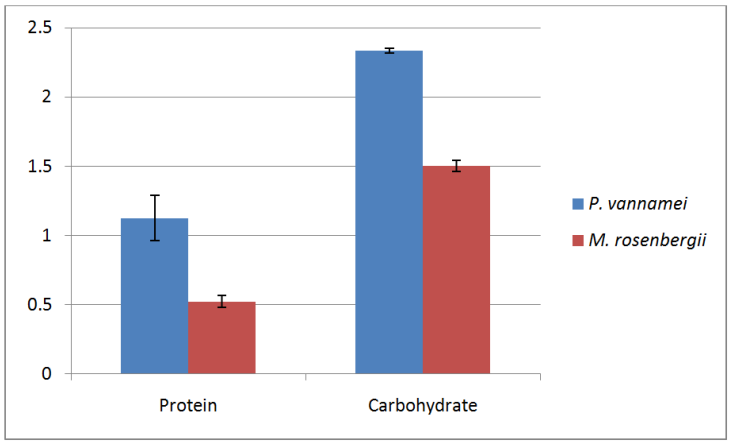
Species	Protein	Carbohydrate	Compounds in ppm	Moisture in %
P. vannamei	1.126 ± 0.164	2.333 ± 0.042	870500 ± 2120	76.95
M. rosenbergii	0.523 ± 0.017	1.502 ± 0.042	297100 ± 40.8	41.91



Graph-1 showing the concentration of compounds in *Penaeus vannamei*



Graph-2 is showing the concentration of compounds in *Macrobrachium rosenbergii*



Graph-3 showing the amount of total protein and carbohydrate concentration in *Penaeus vannamei* and *Macrobrachium rosenbergii*

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

REVIEW ARTICLE

A Review on Ethnobotanical, Phytohemical and Pharmacological Significance of *Elaeocarpus serratus* Linn.

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Received: 25 Feb 2020 Revised: 27 Mar 2020 Accepted: 29 Apr 2020

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ABSTRACT

Elaeocarpus serratus L is a used as traditional medication for the therapy of inflammation, looseness of the bowels, haemorrhage, mental illness and rheumatism. The primary goals of this review are to establish a connection between traditional uses and scientific researches by critically evaluating the available fragmented literary works on traditional, phytochemistry and also pharmacology of *E. serratus*. A bibliographic investigation was accomplished by examining recognized books and peer-reviewed papers, globally accepted clinical data sources like Science Direct, PubMed and Google Scholar from the duration of last twenty years. Ethnobotanical uses of *E. serratus* have been actually derived from different countries like India, Sri Lanka, Bangladesh, Indonesia and also Malaysia for four major classes of illness like rheumatism, diabetes, mental illness and gastrointestinal disruption. This review indicated the effectiveness of *E. serratus* in a number of *in-vitro* and *in-vivo* pharmacological properties such as antimicrobial, antioxidant, cytotoxic, antiarthritis and antidiabetic activity. The pharmacological activity of *E. serratus* may be presence of particular courses of phytocompounds including Flavonoids, Cardiac glycosides, Phytosterols, Triterpenoid, Phenol and Tannins & Phenolic compounds. Traditional uses and scientific evaluation of *E. serratus* shows that it is one of the most extensively used medicinal plants in some parts of the world. This review article is an attempt to sum up literature released in the last two decades on the traditional importance, responsible pytochemical for biological activity and also various pharmacological activities of *Elaeocarpus serratus*.

Keywords: *Elaeocarpus serratus,* 2, 2-diphenyl-1-picryl-hydrazyl-hydrate, Gas chromatography-Mass spectrometry, High performance liquid chromatography, Cardiovascular disease, Superoxide dismutase, Glutathione peroxidase, Glutathione S-transferase.

INTRODUCTION

Medicinal plants have undoubtedly been the backbone of traditional system of medicine which is utilized to treat various illnesses prior to the advancement of contemporary system of medication. Traditional knowledge, combined with phytochemical and pharmacological testing, is an impressive method for determining new leads from therapeutically active plants. Traditionally Medicinal plants are used worldwide to treat various illnesses. The peoples of most under developed countries, even now they depend on medicinal plants for their health and wellness. The traditional knowledge is still available and gained from local or tribal healers. Synthetic medicines are always in fame that its having even further benefits over the traditional medicine like low production expenses, simple quality assurance, strict regulation and deliver a quick effect, but their safety and effectiveness was always continued to be doubtful. Due to the easy availability, safety and efficacy of natural products almost 80% of world population still now trust on natural products. Several hit medications being obtained from plants, nearly 11% of the 252 drugs believed as basic and essential by the WHO were exclusively of flowering plant origin [1]. In early medicine exploration ethanopharmacological uses of medicinal plants are the key hint. Nearly 80% of 122 plant derived drugs were connected to their original ethnopharmacological applications [2]. *E. serratus* is the important drug which included in various system of medicine. Taxonomy of the plant tabulated in table 1. All the parts of the plant are used as a medicine especially fruits are widely used. *E. serratus* comes from the family *Elaeocapaceae* which has 11 genera. Throughout the globe, there are 120 species of *Elaeocarpaus* are found, 25 of which are found in India [3]. The current review is focused on summarizing the traditional importance, presence of phytochemical and pharmacological uses of the *E. serratus* so as to provide consolidated data on these features.

Ethanobotanical uses of *Elaeocarpus serratus* L.

Powdered leaf of *E. serratus* is externally used to treat Rheumatism. Fruit Juices are astringent and orally it is used to cure dysentery and diarrhoea. Aerial part of the plant is to manage Central nervous system and Cardiovascular diseases [4]. Decoction of the bark is given to cure haemorrhage and biliousness. Leaves are used as antidote. Paste of the leaves are used to cure ulcers [5]. Fruit is also used to cure tapeworm infections and leaf juices for dandruff treatment [6]. Fruit juice is given to patient for increasing appetite by incentive of taste buds [7]. Leaves and bark of *E. serratus* are used to treat mental disorders, headache, fever, skin diseases, palpitation and infertility. Fruit is used as a constipating agent during diarrhoea [8]. The vernacular names and general descriptions of *E. serratus* enumerate in table 2 and table 3 respectively.

Phytochemistry of *Elaeocarpus serratus*

Phytochemical screening of *E. serratus* shows that it contains wide range of phytchemicals like Flavonoids, Cardiac glycosides, Phytosterols, Triterpenoid, Phenol and Tannins & Phenolic compounds [10, 11]. Leaves of *E. serratus* contain ellagic acid, myricitrin, myricetin and mearnsetin. The average percentage composition of the fruit pulp of *E. serratus* is Moisture - 80.2 g, Energy - 72 kcal, Protein - 1.2 g, Fats - 1.2 g, Carbohydrate - 16.2 g, Calcium - 18 mg, Phosphorus – 29 mg, Iron - 2.1 mg, Carotene - 330 mg, Thiamine - 20 mg, Riboflavin - 110 mg, Niacin - 0.4 mg and Vitamin C - 20 mg [12]. Fruit pulp of *E. serratus* possessing citric acid, D-Galactose and Pectin [4]. Four different compounds are isolated from the leaves of *E. serratus* namely Myricitrin, Mearnsetin 3-O-β-D-glucopyranoside, Mearnsitrin, Tamarixetin 3-O-α-L-rhamnopyranoside [9]. Two dihydrochalcone derivatives are isolated from the leaves of *E. serratus*, namely 2', 4', 6', 4 -tetra hydroxy dibenzoyl methane-enol form and a- butenyl- a'-[2', 4', 5'-trihydroxy phenyl] ketone. Leaves and Seeds of ethanolic extract of *E. serratus* shows presence of thirty different components and identified by GC-MS [13, 14]. GC-MS analysis of fruit pulb specifies the occurrence of stigmasterol,

α-amyrin, β-amyrin and β-amyrin acetate and also HPLC analysis of fruit pulp shows the presence of Vanillic acid, p-coumaric acid, Ferulic acid, Quercetin and Kaempferol [15].

Pharmacological activity of *Elaeocarpus serratus*

Antioxidant activity

A substantial numbers of medicinal plants accommodate flavonoids and phenolic compounds as a phytochemicals. Flavonoids having the ability to minimise the free radical formation and to scavenge the free radicals followed by reducing the oxidative stress. Lalith Jayasinghe *et al.* are isolated and analysed the antioxidant potential of flavonol glycosides from leaves of *E. serratus*. Antioxidant potential of the isolated compounds were analysed against the DPPH radical. Myricitrin is a glycosyloxyflavone that consists of myricetin. The antioxidant potential of Myricitrin was compared with ascorbic acid and butylated hydroxyl anisole using spectrophotometric method. The half maximal inhibitory concentration (IC_{50}) value of Myricitrin was 3.2μgmL^{-1}. The IC_{50} values of ascorbic acid and butylated hydroxylanisole is (3.9 μgmL^{-1}) and BHA (3.6 μgmL^{-1}) respectively [9]. Sekhar shailasree *et al.* evaluated the antioxidant activity of methanolic extracts of leaves of *E. serratus* by measuring the DPPH radical scavenging and ABTS and then compared with ascorbic acid, butylated hydroxytoluene and quercetin. Polyphenolic compounds are readily soluble with polar solvents like methanol. Significant activity was observed from methanolic extracts of *E. serratus*. The experimental outcome concluded that the methanolic extract exhibit the significant IC_{50} value 0.172±0.28mg.mL^{-1}, 0.14±0.11mg.mL^{-1} and 152±0.19mg.mL^{-1} DPPH radical, ABTS scavenging activity and total phenolic content respectively [16]. Most. Nazma Parvin *et al.* are evaluating the antioxidant potential of methanolic extract of stem bark of *E. Serratus*. The methanolic extract of *E. serratus* exhibit notable antioxidant activity in nitric oxide scavenging activity. In nitric oxide scavenging activity, the IC_{50} value of *E. serratus* extract was 89.325μg/mL while the IC_{50} value of ascorbic acid was 47.684μg/mL. The observations from the results of these *in vitro* experiments seem to indicate that methanolic extract of *E. serratus* exhibit antioxidant activity and effectiveness of *E. serratus* may depends on concentration of extract [17]. Prapty Das *et al.* examined the antioxidant activity, total phenolic and total flavonoidal contents of *E. serratus* by DPPH method, Folin-Ciocalteu method and $AlCl_3$ colorimetric assay method respectively. Methanolic extract of *E. serratus* (IC_{50}-64.10mg/mL) shows better antioxidant activity than petroleum ether extract (IC_{50}-26.10mg/mL), where the antioxidant value is compared with the standard ascorbic acid. Among the two extracts, methanolic extracts of *E. serratus* hold considerable amount of phenolic content (400mg/100g) and also the total flavonoid (500mg/100g) content [10].

Antimicrobial activity

Many plants derived chemicals possessing antibacterial activity against gram positive or gram negative. These pytochemicals act in different mechanism such as having the ability to destruct the bacterial membrane, minimise the bacterial infection, suppression of enzymatic activity, bacterial bio-film formation and etc [18]. Flavonoids and phenolic compounds are very good antioxidants and also displayed good antibacterial activity. Fruits, vegetables, nuts, seeds, flowers are the rich sources of flavonoids and phenolic compounds. Naturally occurring phenolic compounds exhibit antibacterial activity with different mechanism like outer membrane permeabilization, capability to prevent DNA gyrase and obstruct the bacterial energy metabolism. Since most of the phytochmical analysis of *E. serratus* report that plant is rich in flavonoids and phenolic compounds [19]. Fernando Freitas de Lima *et al*. is evaluated the effects of fruit pulp of *E. serratus* against various bacterial stains with different concentrations ranges. Results of the experiments clearly revealed that fruit pulp extracts exhibit bactericidal effect that raised inhibition against *Xanthomonas campestris*, moderate inhibition for *Escherichia coli* and *Salmonella choleraesuis* and weak inhibition against *Bacillus cereus* and *Staphylococcus aureus*. Quantitative evaluation of fruit pulp evident that it contains significant amount of flavonoids, condensed tannins and carotenoids. Many antimicrobial studies concluded that flavonids (cell wall breakdown) and condensed tannins (enzyme inhibition) are responsible for antimicrobial activity of plant extracts [15]. Sujogya Kumar Panda *et al.* is studied the multidrug resistant S. aureus in various Indian

medicinal plants. The aqueous extract prepared from flowers of *E. serratus* (yield-29mg/gm) is one among them were tested its antimicrobial potential against MDRSA. The selected medicinal plants are exhibited broad-spectrum activity by inhibiting all tested MDRSA strains. Experimental results conveyed that Minimal inhibitory concentration of *E. serratus* is 2768µg/ml and Bio-film inhibitory concentration is 630µg/ml [20].

Cytotoxicity assay

Cytotoxicity assay is a type of *in vitro* bioassay to conclude the toxicity or response of metabolite to various tissues or cell lines. Cytotoxicity assay methods are helps to calculate the cell viability, to check the cell membrane integrity, metabolic activity and cell proliferation. Flavonoids and phenolic compounds having the potential to prevent the growth or development of various cancerous cell lines. Mechanism action of medicinal plants containing flavonoids and phenolic compounds are preventing Carcinogen metabolic activation, Antiproliferation, Cell cycle arrest, Induction of apoptosis, Promotion of differentiation, Antioxidative activity and Inhibition of angiogenic process [21]. Sekhar Shailasree *et al.* are evaluated the cytotoxic potential of methanolic extract of *E. serratus* was performed by MTT in Vero cell lines. The half maximal inhibitory concentration value of *E. serratus* is 93.31±0.23µg.mL^{-1}. Hence the findings suggest that methanolic extract of *E. serratus* does not show any significant cytotoxic effect on Vero cell line [16]. Most. Nazma Parvin *et al.* was investigated the cytotoxic potential of three different soluble extracts or fractions such as Pet-ether, Carbon tetrachloride and Chloroform soluble extracts are partitioned from methanolic extract of *E. serratus*. Cytotoxic potential of three fractions is examined by brine shrimp lethality bioassay where Vincristine Sulfate is used as positive control in this experiment. Among three extracts chloroform soluble fraction (LC_{50} - 0.831µg/ml) is displayed a meaningful activity against the brine shrimp nauplii [18].

Anti-diabetic activity

Diabetic is known as metabolic disorders. The increasing the level of blood sugar level leads to diabetic complications like retinopathy, neuropathy, CVD and etc. The simplest approach to minimise the diabetic related complications is to control the blood sugar level. A wide range of plant derived chemicals are moderately powerful in fighting or minimising the difficulties associated with diabetes mellitus. Geetha DH *et al.* is assessing the anti-diabetic activity of *E. serratus* fruits by *in vitro*. A set of α-amylase & α-glucosidase inhibition assay methods were attempted to examine the anti-diabetic effects of *E. serratus* fruits. The outcome of the experiment displayed that ethanolic extract of fruit of *E. serratus* indicated that moderate α-amylase inhibitory activity. The α-amylase inhibitory activity absolutely based on dose controlled manner. It does not show the significant effect on α-glucosidase inhibition [22]. Geetha DH *et al.* is evaluating the anti-diabetic potential of alcoholic extract of *E. serratus* in rat diabetic induced by Streptozotocin. For diabetic treatment two different doses of extract at 200 and 400 mg/kg body weight are administered to diabetic induced rats. Administration of higher dose (400 mg/kg body weight) will bring the elevated blood glucose level in to normal, increase in total protein and total albumin levels, normalization of hepatic enzymes and rehabilitate carbohydrate metabolizing enzymes compared with control diabetic rats. All this favourable effect of alcoholic extract of *E. serratus* evidently depends on dose response manner [23].

Anti-arthritic activity

Arthritis is known as inflammation at bone joints. Rheumatoid arthritis is a chronic, inflammatory disorder that may strike readily moveable joints known as synovial joints. Many modern synthetic drugs are used to treat inflammatory disorders; main drawback was continuous intake of synthetic medication which leads to other common complications like gastrointestinal haemorrhage and peptic ulcer lesions. To overcome these issues many medicinal plants play a significant role in the treatment of arthritis. Uses of anti-arthiritis plants are trusted to be relatively short of harmful causes than the modern synthetic drugs. Geetha DH *et al.* studied on the anti-arthritic activity of *E. serratus* by two *in vitro* models known as inhibition of protein denaturation and anti-proteinase

activities. The experimental outcomes are clearly mentioned that both leaf (68.32%) and seed extracts (62.13%) of *E. serratus* are shown a significantly control the denaturation of protein in inflammatory disease at the dose level of 400µg/ml. At two varying doses of both leaf and seed of *E. serratus* extracts are displayed average anti-proteinase activity. The suggested mechanism of action of anti-arthritic activity of *E. serratus* having the capacity to suppress the denaturation of protein and membrane lysis by restricts the production of auto-antigen [24]. Geetha DH *et al.* evaluated the anti-arthritic activity of ethanolic extracts of *E. serratus* by Freund's adjuvant arthritis model in Wistar rats. The most significant observation of this study is that two different doses of ethanolic extracts of *E. serratus* leaf (200 and 400 mg/kg p.o.) are shows the higher level of protection of paw volume than seed extracts. The ethanolic extracts of both leaf and seed significantly reduced the levels of lipid peroxidation and improve ($p<0.05$) the levels of SOD, CAT, GPx, and GST enzymes close to normal level [25].

CONCLUSION

This review highlights the traditional value, presence of phytochemical and pharmacological activities of *E. serratus* are an indicator of its high medicinal value. The available scientific data shows that *E. serratus* signifies its relevance as medicinal plant made used in a broad range of traditional therapies, especially for diabetes, joint inflammation, skin troubles and cancer. The medicinal importances of *E. serratus* are attributed to the presence of wide range of phytochemicals like saponin glycosides, alkaloids, flavonoids and phenolic compounds. The pharmacological studies are confirmed that the *E. serratus* possesses antimicrobial, antioxidant, cytotoxicity, antiartritic and antidiabetic prospective of this particular plant. However, no sufficient data is offered on the toxicity of *E. serratus,* which is essential for a few comprehensive investigations. Interestingly, polar extracts of *E. serratus* reveals powerful antioxidant and antibacterial properties did not show any substantial toxic effect as reported by other researchers. All the above consolidated information shows that *E. serratus* is safe to use and alternative therapy for the cure or prevention of human illness.

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Table 1. Taxonomy of *Elaeocarpus serratus* Linn

Kingdom: Plantae (plants)
Sub kingdom: Tracheobionta (vascular plants)
Super division: Spermatophyta (seed plants)
Division: Magnoliophyta (flowering plants)
Class: Magnoliopsida (dicotyledanae)
Sub class: Dilleniidac
Order: Malvales
Family: Elacocarpaceae
Genus: *Elaeocarpus* L.
Species: *Elaeocarpus serratus* L.

Table 2. Vernacular names of *Elaeocarpus serratus* Linn

S.No	**Vernacular names of *Elaeocarpus serratus* Linn**
1.	**English name:** Ceylon Olive, Indian olive, Tropical olive, Thai olive Wild Olive tree
2.	**Tamil:** Veralikkai, Veralipalam
3.	**Sinhala:** Veralu
4.	**Malayalam:** Kaarakka
5.	**Assamese:** Zolphai
6.	**Bengal:** Jalpai
7.	**Manipur:** Chorphon
8.	**Brazil:** Azeitona do Ceylao
9.	**Malaysia:** Lengkenang

Table 3. General description of *Elaeocarpus serratus* Linn [3]

S.No	**General description of *Elaeocarpus serratus* Linn**
01	**Habit:** Found in Sri Lanka, India, Bangladesh, Indonesia, Malaysia, Australia
02	**Plant:** Used parts are Fresh fruits, Leaves, Seeds; Evergreen small to medium sized tree; height about 10-12 m.
03	**Fruits and Seeds:** A drupe, about 3-4 cm long, Ovoid, smooth dull, greenish yellow, pulp light to dark green, copious acid, stone ovoid-oblong 1 celled, 1-2 seeded; seeds 3-4 cm. Fruiting during the month between July-October.
04.	**Leaves:** Petiole1-1.15cm; rounded apex, crenate senate margin, obovate oval acute base, fresh leaf is pale green, dried leaf in pale brown in colour.
05.	**Flowers:** flower petals white, laciniate, anthers ciliate. Flowering during the month between March-June
05.	**Wood:** Light yellowish white and wood density is low.

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Studies on Heavy Metals Content in Four Different Fish Scale

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Received: 07 Feb 2020 Revised: 10 Mar 2020 Accepted: 13 Apr 2020

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ABSTRACT

Heavy metals are one of the most widespread contaminants in the environment .They cause environmental pollution from sources such as industrial effluents, mine tailings, land application of fertilizers, atmospheric deposition and leaching of metal ions from the soil into lakes and rivers by acid rain. The most common water pollution comes from mining companies. The pollution of water with heavy metals has been a great concern due to their toxic nature and adverse effect Various techniques were used over the period of time to remove heavy metals namely physical ,chemical and biological treatment. The most common heavy metal pollutants are arsenic, cadmium, chromium, copper, nickel, lead and mercury and zinc. The general purpose of this study is determining the concentration of heavy metal accumulation in scales of *Labeo rohita, Catla catla, Cirrhinus mrigala, Notopterus notopterus.* Four samples of fish scale were collected from the local fisherman of NALCO fish market (Angul). The samples were examined by using the XRF method to determine the concentration of different heavy metals. The different heavy metal and their concentration are analysed. The CaO concentration is maximum and concentration of Br is minimum in four different fish scale.

Keywords: Heavy metal, Fish scale, Toxic, XRF-method, CaO, Br.

After decades of rapid urbanization, population growth and industrialization developing countries are now home to many of the world's most critical air, water and solid waste problems (Hashim *et al.,* 2014). Heavy metal has high density and is toxic or poisonous at low concentration (Koleleni and Haji, 2014).heavy metals are the natural components of earth's crust (Koller and Saleh, 2018). Some heavy metals like copper, selenium or zinc are responsible for maintaining the metabolism of the body (Mertz, 2018). Heavy metals are the environmental pollutants because they adversely affect the plant ,animal and human being (Raja and Namburu, 2014).The impact of heavy metals on aquatic organism is due to the movements of pollutants from different sources results in coincidental mixtutre in ecosystem (Rajeswari *et al.,* 2014)

Heavy metals are the metallic chemical elements that have a high density and a high atomic weight (Fergusson, 1990). The multiple use of heavy metals in the industrial, domestic, agricultural, medical and technological applications have leads to it extending availability worldwide . When these heavy metals concentration increases from their normal value that toxic effects adversely affect the human health and the environment (Tariq *et al.,* 1996). Because of their high degree of toxicity, arsenic, cadmium, chromium, lead and mercury rank among the priority metals that are of public health significance (Koleleni and Haji, 2014). Heavy metals constitute an indistinct group of inorganic chemical hazards ,and those most commonly found at contaminated sites are lead, chromium, arsenic, mercury, zinc, cadmium, copper, nickel (Wuana and Okeimen, 2011).The increasing pace of urbanization and industrialization is the major source of heavy metal contamination (Rai, 2008).

In aquatic ecosystem water contamination by trace metals is one of the main types of pollution that may stress the biotic community. Heavy metals are commonly found in natural waters and some are essential to living organisms yet they may become highly toxic when present in high concentration. Water can be polluted by heavy metals which are accumulated and concentrated by fish therefore they show the degree of environmental pollution (Czedli *et al.,* 2014). All heavy metals are non-biodegradable and persistent in the environment. Therefore removal of heavy metals from wastewater is most important for the safety of environment. Biosorption play a vital role in removal of heavy metal. Biosorption is an easy process. One of the biosorbent for heavy metal removal is fish scale. A number of fish scale of *Labeo rohita*, *Catla catla* give successful result world widely (Zayadi and Othman ,2013). The release of heavy metals into aquatic ecosystem brings out various changes in the aquatic species diversity and ecosystem due to the toxicity and bioaccumulation behavior. Aquatic organism like fish accumulate metals in higher concentration than that present in water (Alweher, 2008).Fish is a major food source for most people (Koleleni and Haji, 2014).

Fish are the aquatic organism and rich in, protein minerals, vitamins and omega 3 fatty acid. Fish can accumulate heavy metal from water. So that fish is a good indicator of heavy metals contamination in water. The fish accumulation the heavy metal, the presence of toxic metal can invalidate their beneficial effects. Some unfavorable effects of heavy metals to human health have been known for long time. This include several threats like liver damage, renal failure, cardiovascular disease and even death .Factor like age, sex, size, reproductive cycle, swimming pattern, feeding behavior and geographical location are responsible for bioaccumulation in fish (Bawuro *et al.,* 2018). Marine diversity hampers a lot due to the influence of heavy metal. Among other organism, fishes are inhabitants that cannot escape from the adverse effect of these pollutants. These aquatic organisms have the ability to accumulate heavy metals from various sources such as soil erosion and runoff, industrial effluents, discharge of waste water, pesticide (Brraich and Jangu, 2012).

MATERIALS AND METHODS

For the study of heavy metal concentration 4 samples of fish (*Labeo rohita, Catla catla , Cirrhinus mrigala, Notopterus notopterus*) were collected from the local fisherman of NALCO market, Angul. They were brought to the laboratory and the scales were removed. Then they were allowed to dry for two days. After drying it under the sun it was further dried in the oven. The dried scales were powdered in crushed machine. Then the crushed samples were collected in a glass container. Then the fine powders were allowed to filter through tea strainer. The powdered fish scales were collected in a plastic bottle. The collected samples were taken for heavy metal analysis through XRF machine.

RESULTS

Figure 1 showing the concentration of Al_2O_3 in 4 species of freshwater fishes. Among them the concentration of Al_2O_3 is highest in *Notopterus notopterus* and lowest in *Cirrhinus mrigala*. But the threshold limit in the fish is 0.05-0.20mg per kg. Figure 2 give that the concentration of P_2O_5 was found to be highest in *Catla catla* and lowest in Notopterus

notopterus. Figure 3 showing that the concentration of Cl is more in Notopterus notopterus and less in *Catla catla* where as threshold limit in fish is 250mg per kg.Figure 4 showing that CaO Concentration is highest in *Cirrhinus mrigala* and lowest in Notopterus notopterus.But the threshold limit is 0.364mg per kg. Figure 5 showing that ZnO degree is more in *Labeo rohita* and less in *Cirrhinus mrigala*. Figure 6 showing that SrO concentration is highest in *Catla catla*. similarly the concentration of SiO2 is highest in *Notopterus notopterus* and lowest in *Catla catla*,SO_3 is more in *Notopterus notopterus* and less in *Catla catla*,K_2O more in *Labeo rohita* and less in *Catla catla*,TiO_2 concentration is more in *Labeo rohita* and less in *Catla catla*, MnO is more in *Labeo rohita* and less in *Cirrhinus mrigala* , CuO is more in *Cirrhinus mrigala* and less in *Labeo rohita*, Br is more in *Notopterus notopterus* and less in *Cirrhinus mrigala*, ZrO_2 is highest in *Labeo rohita* and lowest in *Cirrhinus mrigala*, Fe_2O_3 concentration is highest in *Notopterus notopterus* and lowest in *Cirrhinus mrigala*, Cr_2O_3 concentration is more in *Labeo rohita* and lowest in *Notopterus notopterus*. The permissible limit of MnO, CuO, Fe_2O_3, Cr_2O_3, P_2O_5, SrO, SiO_2, K_2O, TiO_2, Br, is 5 mg per kg, 3mg /kg, 0.3 mg/kg, 0.1mg/kg, 0.005-0.05mg/kg, 013-0.21mg/kg, 5-25mg/kg, 228.8-500.8mg/kg, 1.2-1.3mg/kg and 0.5mg/kg respectively.

This plot show that the correlation of Al_2O_3 of four different species of fish scale shows a positive correlation with ,Cl, Cr_2O_3, Fe_2O_3, ZnO, SiO_2, SO_3, K_2O, TiO_2, MnO, Br, ZrO_2 except with P_2O_5,CaO,SrO,CuO.The correlation of P_2O_5 of four different species of fish scale shows a positive correlation with Al_2O_3, CaO, Cr_2O_3, SrO,CuO except with Cl,Fe_2O_3,ZnO,SiO_2,SO_3,K20,TiO_2,MnO,Br,ZrO_2.The correlation of Cl of four different species of fish scale shows a positive correlation with Al_2O_3, P_2O_5, , Fe_2O_3, ZnO, SiO_2, SO_3, K_2O, TiO_2, MnO ,Br, ZrO_2 except with CaO, Cr_2O_3, SrO,CuO.The correlation of CaO of four different species of fish scale shows a positive correlation with Al_2O_3, P2O_5, Cl, , SrO, , CuO except with Br, ZrO_2, SiO_2, SO_3, K_2O, TiO_2, MnO, Cr_2O_3, Fe_2O_3, ZnO.The correlation of Cr_2O_3 of four different species of fish scale shows a positive correlation with Al2O3, P_2O_5, Cl, CaO, Fe_2O_3, ZnO, SrO, SO_3, K_2O, TiO_2, MnO, , ZrO_2 except with CuO ,Br, SiO_2.The correlation of Fe_2O_3 of four different species of fish scale shows a positive correlation with Al_2O_3, P_2O_5, Cl, CaO, Cr_2O_3,Fe_2O_3,SrO,SiO_2, SO_3, K_2O, TiO_2, MnO,Br, ZrO_2 except withZnO, CuO. The correlation of ZnO of four different species of fish scale shows a positive correlation with Al_2O_3, P_2O_5, Cl, CaO, Cr_2O_3, Fe_2O_3, SrO, SO_3, K_2O, TiO_2, MnO, CuO except with Br, ZrO_2, SiO_2.The correlation of SrO of four different species of fish scale shows a positive correlation with Al_2O_3, P2O5, Cl, CaO, Cr_2O_3, Fe_2O_3, ZnO, CuO except with Br, ZrO_2, SiO_2, SO_3, K_2O, TiO_2, MnO.

The correlation of SiO_2 of four different species of fish scale shows a positive correlation with Al2O_3, P_2O_5, Cl, CaO, Cr_2O_3, Fe_2O_3, ZnO, SrO, SO_3, K_2O, TiO_2, MnO, Br except with ZrO2, CuO. The correlation of SO_3 of four different species of fish scale shows a positive correlation with Al_2O_3, P_2O_5, Cl, CaO, Cr_2O_3, Fe_2O_3, ZnO, SrO, SiO_2, K_2O, TiO_2, MnO,Br, ZrO_2 except between K_2O and CuO.The correlation of K_2O of four different species of fish scale shows a positive correlation with Al2O3, P_2O_5, Cl, CaO, Cr_2O_3, Fe_2O_3, ZnO, SrO, SiO_2, SO_3, TiO_2, MnO,Br, ZrO_2 except between TiO2 and CuO. The correlation of TiO_2 of four different species of fish scale shows a positive correlation with Al_2O_3, P_2O_5, Cl, CaO, Cr_2O_3, Fe_2O_3, ZnO, SrO, SiO_2, SO_3, K_2O, MnO, Br, ZrO_2 except between MnO and CuO.The correlation of MnO of four different species of fish scale shows a positive correlation with Al_2O_3, P_2O_5, Cl, CaO, Cr_2O_3, Fe_2O_3, ZnO, SrO, SiO_2, SO_3, K_2O, TiO_2,Br, ZrO_2 except between CuO and CuO.The correlation of CuO of four different species of fish scale shows a positive correlation with Al_2O_3, P_2O_5, Cl, CaO, Cr_2O_3, Fe_2O_3, ZnO, SrO, SiO_2, SO_3, K_2O, TiO_2, MnO except with Br, ZrO_2.The correlation of Br of four different species of fish scale shows a positive correlation with Al_2O_3, P_2O_5, Cl, CaO, Cr_2O_3, Fe_2O_3, ZnO, SrO, SiO_2, SO_3, K_2O, TiO_2, MnO ,CuO, ZrO_2 .The correlation of ZrO_2 of four different species of fishscale shows a positive correlation with Al_2O_3, P_2O_5, Cl, CaO, Cr_2O_3, Fe_2O_3, ZnO, SrO, SiO_2, SO_3, K_2O, TiO_2, MnO ,CuO,Br .

The result show the correlation of fish scale sample of four different species, it is important to calculate the one way anova of different heavy metal in four different species .It gives the significant value and insignificant value. Out of the four species taken for heavy metal analysis, the degree of Al_2O_3, P_20_5, CaO, SiO_2, SO_3, K_2O of *Labeo rohita* is significant and the concentration of Cl, Cr_2O_3, Fe_2O_3, ZnO, SrO, TiO_2, MnO of this species is insignificant. Similarly the concentration of P_2O_5, CaO, SiO_2 of *Catla catla* is significant and the concentration of Al2O_3, Cl, Cr_2O_3, Fe_2O_3, ZnO, SrO, SO_3, K_2O, TiO_2, MnO, CuO, Br, ZrO_2 of this species is insignificant. Also the concentration of P_20_5, CaO, SiO_2, SO_3

of *Cirrhinus mrigala* is significant and the degree of Al_2O_3, Al2O_3, Cl, Cr_2O_3, Fe_2O_3, ZnO, SrO, SO_3, K_20, TiO_2, MnO, CuO, Br, ZrO_2 of this species is insignificant. From the above study it is found that the degree of Al2O_3, P_20_5, CaO, SiO_2, SO_3 of *Notopterus notopterus* is significant and the concentration of Cl, Cr_2O_3, Fe_2O_3, ZnO, SrO, SO_3, K_2O, TiO_2, MnO, CuO, Br, ZrO_2 of this species is insignificant. Because from the above study it is observed that the significant (p value) is >1.

DISCUSSION

In the study level of heavy metal Cd, Cu, Zn in three fish species collected from the Northen Jordan vally,Jodan, it has been observed that the concentration of Cd and Cu metals was lowest in muscle bone, scale and skin except gills of *Oreochromis aureus.* In *Clarias lazera*, the level of Cd and Cu showd that lowest in muscle and highest in gills. *Cyprinus corpio* showed that Cu level is highest in bone, skin, scales and gills except the muscle. In *Oreochromis aureus*, the level of Zn is more in skin except other organ. According to FAO, the level of Cd, Cu and Zn are varies and the main causes of it, due to the increase of agricultural influx and anthropogenic activity in that area (Al-where, 2008).
The main purpose of study of the application of fish scale as bioindicator of Heavy metal pollution (Pb, Zn) in the *Cyprinus carpio* of the Caspian sea is to identify the degree of heavy metal accumulation in scales, livers and gonads of *Cyprinus carpio*. Results of this study show that fish scale is a better index of lead and zinc content than gonad and liver respectively. It is concluded that the food consumption of *Cyprinus Carpio* gonad should he avoided in study area of Gilan and Mazandaran (Cobasohan *et al.,* 2005).This is due to human activity and other organic and mineral pollutants (Darafash *et al.,*2008).

In distribution of metals in tissue of *Cyprinus carpio*, the aims of the study was to analyse the distribution of selected metals (As, Cd, Pb, Hg, Cr, Cu and Zn) in the tissue of common carp (*Cyprinus carpio*).The collection of fish was done in Czech Republic. The concentration of toxic elements in muscle of carps from Czech Republic is very low. That means the consumption of this carp is safe. (Celechovska, 2007). In The Trace Element Analysis in Freshwater Fish Species, water and sediment in Iyidere stream (Rize-Turkey) the trace element was analysed by Energy Dispersive XRF. In this study P, S, Cl, K, Ca, Ti were observed from ten different freshwater fish samples. In this study, it can be concluded that fresh water fishes of Iyider stream does not contain any toxic metals which are (Rize, Turkey) harmful for consumers (Verep *et al*., 2012). Based on the correlation between heavy metals in fish and sediment in Sakumo and Kpeshie Lagoons, Ghana, it was concluded that the heavy metals levels in fish were low and it did not hazardous to health when consumed by a person (Klake *et al.* 2012).

Various studies were carried out on the different species of fish from different parts in the world. The present study based on the level of heavy metal in fish scale of four different species. The analysis of different heavy metals into fish scale of four different species is done by XRF analysis. Through which different heavy metals were analysed (Verep *et al*., 2012). Comparison of heavy metals content between the fish scale of four different species shows that, concentration of CaO is more and concentration of Br is less in four different fish scale. The concentration Al2O3, P_2O_5, CaO, SiO_2, SO_3, K_2O of *Labeo rohita* is significant. The concentration P_2O_5, CaO, SiO_2 of *Catla catla* are significant.The concentration of P2O5, CaO, SiO_2, SO_3 of *Cirrhinus mrigala* are significant. The concentration of Al_2O_3, P_2O_5, CaO, SiO_2, SO_3 of *Notopterus notopterus* are significant.

CONCLUSION

The result from this study suggested that there were several heavy metals suspected to be present in four different fish scale. The level or concentration of heavy metal present in four different fish scale is identified. According to the result of present study the fish scale of four different species it has proven to the different fish scale contain low amount of heavy metal. Then concentration varies from their natural values. Even these are freshwater fishes, they also contain small amount of heavy metal (Darafsh et *al*., 2005). This is due to the increase of industrial influents and

agricultural activities and other anthropogenic activity. But these fish are can be consumable as a food source (Kalke *et al*., 2012).

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Table-1 show that the different heavy metal concentration of 4 different species

Compounds	*L.rohita*	*C. catla*	*C. mrigala*	*N. notopterus*
Al2O3	1.02	0.91	0.82	1.06
P2O5	30.33	31.44	30.76	29.62
Cl	0.29	0.18	0.22	0.36
CaO	62.60	63.99	64.40	62.45
Cr2O3	0.01939	0.00718	0.00638	0.003
Fe2O3	0.60	0.39	0.33	0.68
ZnO	0.127	0.07804	0.06422	0.0665
SrO	0.14	0.22	0.17	0.07
SiO2	1.82	1.08	1.38	2.97
SO3	1.80	0.99	1.09	1.95

K2O	1.03	0.58	0.65	0.61
TiO2	0.12	0.06656	0.0721	0.08142
MnO	0.3908	0.02802	0.016	0.03591
CuO	0.00521	0.01741	0.01789	0.01207
Br	0.00197	0.00135	0.00123	0.00303
ZrO2	0.0096	0.0137	0	0.00042

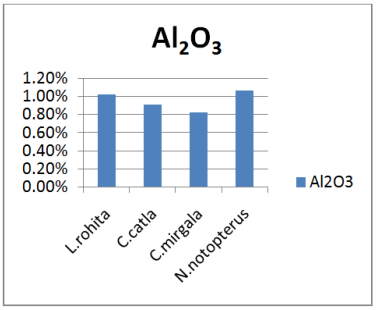

Fig.1.Conc of Al_2O_3 in four different fishscale

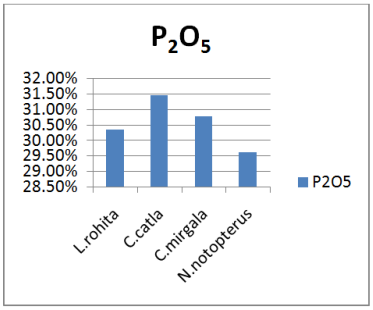

Fig.2. Conc of P_2O_5 in four different fishscale

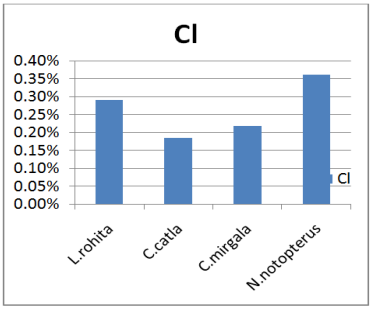

Fig.3. Conc of Cl in four different fishscale

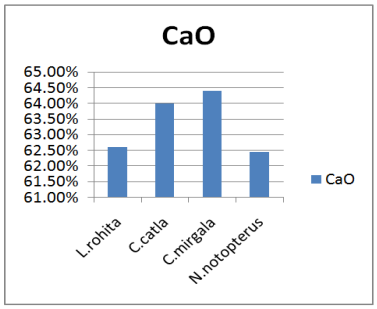

Fig.4. Conc of Cao in four different fishscale

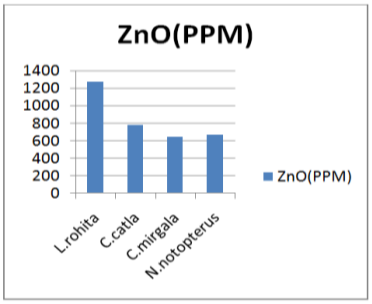

Fig.5. Conc of ZnO in four different fishscale

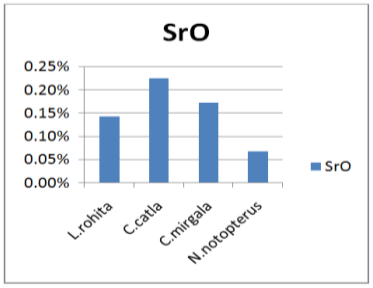

Fig.6. Conc of SrO in four different fishscale

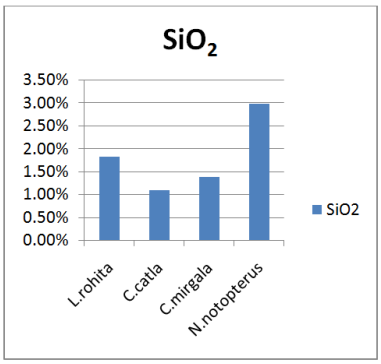

Fig.7. Conc of SiO_2 in four different fishscale

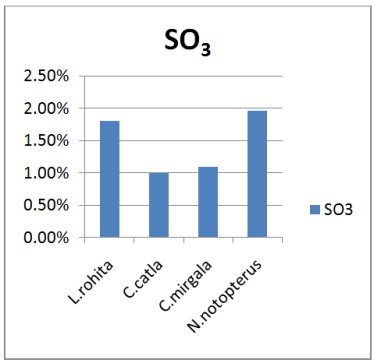

Fig.8. Conc of SO_3 in four different fishscale

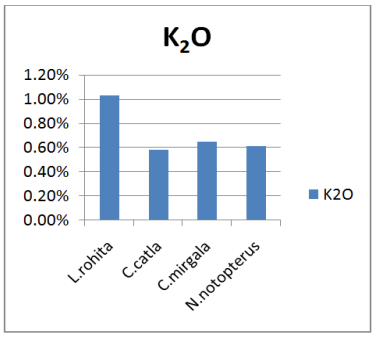

Fig.9. Conc of K_2O in four different fishscale

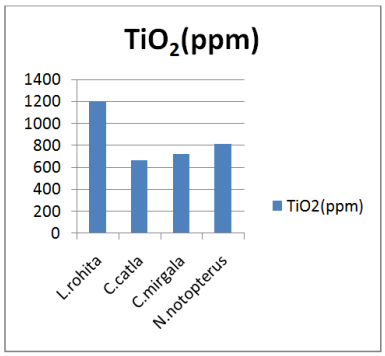

Fig.10. Conc of TiO_2 in four different fishscale

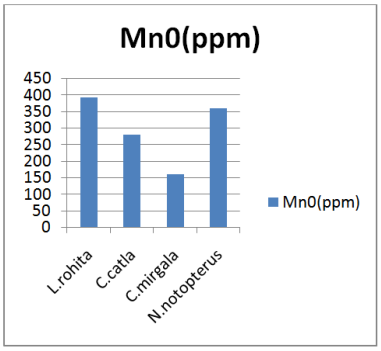

Fig. 11. Conc of MnO in four different fishscale

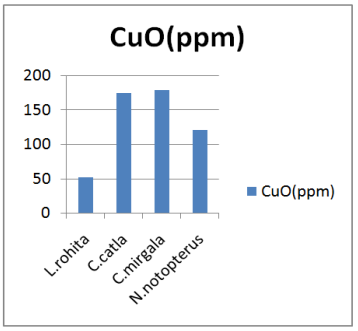

Fig.12. Conc of CuO in four different fishscale

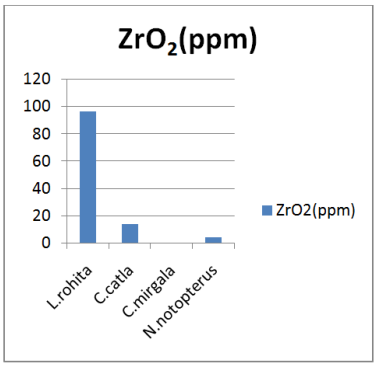

Fig.13. Conc of ZrO_2 in four different fishscale

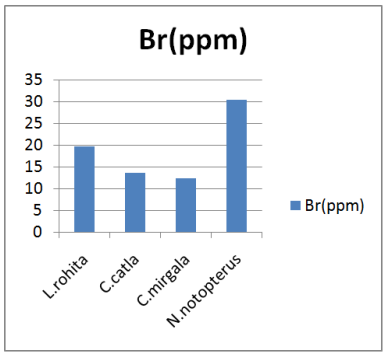

Fig.14. Conc of Br in four different fishscale

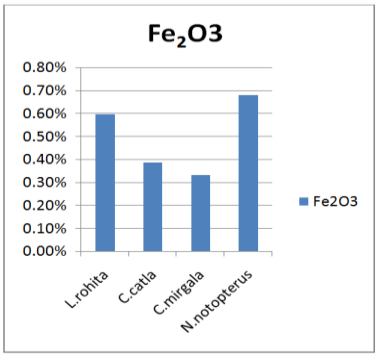

Fig.15. Conc of Fe_2O_3 in four different fishscale

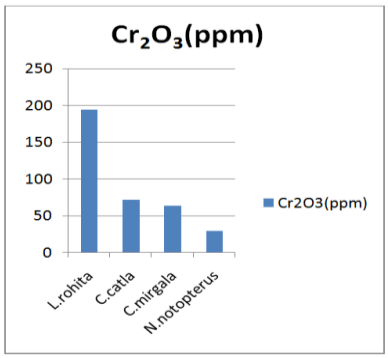

Fig.16. Conc of Cr_2O_3 in four different fishsacle

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Knowledge and Clinical Practice Pattern Related to Hypothyroidism Management of the Physicians from Different Specialties; A KAP Survey

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Received: 19 Feb 2020 Revised: 21 Mar 2020 Accepted: 23 Apr 2020

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ABSTRACT

Due to numerous diagnostic complexities and inappropriate disease management practices, it is essential to provide evidence-based research highlights. This study aims to provide a documented summary regarding the knowledge, attitude and practices (KAP) of the physicians in managing hypothyroidism and also to assess the clinician's insight upon the specific cases including pregnancy and old age. The study was conducted upon the physicians attending the current medical education (CME) programs and meeting conducted by Pakistan Endocrinology Society. Data was collected through a questionnaire, designed to assess the knowledge and practices pattern regarding hypothyroidism including thyroid diagnosis, treatment options, follow-up frequency and associated risk factors influencing the decision making in different case scenarios was also acquired. Out of 760 physicians, 291(38.3%) physicians recommended Anti-thyroid peroxidase (Anti-TPO) as the diagnostic test for middle aged females presented with hypothyroidism symptoms. 82.6% physicians preferred a dose increment of 25 mcg/day (Levothyroxine), follow-up after every 6 weeks was preferred by 79.1% physicians and Levothyroxine (12.5 mcg) daily was recommended during follow-up visit by majority of the physicians. Thyroid Stimulating Hormone (TSH) target ranges were evaluated for different case scenarios, where the most preferable target was <1 mU/L in case of a 25-year-old female (hypothyroidism and trying to conceive), and same as in case of an 85-year-old female. Based on the current practicing patterns and knowledge, awareness programs and campaigns highlighting the practices and management of thyroid imbalances is very important in order to avoid maladministration and treatment failures.

Keywords: Knowledge, Attitude, Practicing Patterns, Hypothyroidism, Physicians Decision Making.

INTRODUCTION

The underactive thyroid gland, Hypothyroidism is among the most common condition diagnosed in routine clinical practice by the family physicians, obstetricians, general physicians, endocrinologists, surgical and reproductive specialists [1,2]. It is evident that 9.4% of the overall adult population has been affected by hypothyroidism of which 0.4% of the cases are of overt- hypothyroidism and 9% are of sub-clinical hypothyroidism [3]. In Pakistan the prevalence of this clinical condition is 4.1% with increased frequency among females [3-5]. Based on the previous evidences the risk of hypothyroidism increases with age, 1.7% of overt-hypothyroidism cases and 13.7% of subclinical hypothyroidism cases recorded among the adults of 65 years of age where the upper limit for normal TSH was 4.5 mIU/L [6]. The theory that the normal TSH range among older adults which is used as base for treatment is relatively higher and may be associated with better and long survival rate [6-9]. Therefore, the risk and chances for complications increases with the growing age as lowering the TSH levels in such cases is unwarranted and results in overtreatment [10,11]. TSH overtreatment increases the risk for cardiac arrhythmias, osteoporosis, fractures and also results in cardiovascular mortality [12-14].

Despite of the fact that guidelines and recommendations for management of hypothyroidism has been developed but still there are a number of differences in the management and treatment practices and beliefs among the healthcare providers. These controversies are related to TSH targets for specific cases i.e. patients with mild subclinical hypothyroidism and elderly patients, etc. [15,16]. Currently the TSH ranges are specified for females of childbearing age (trying to conceive) and pregnant females [17]. Appropriate screening and management of hypothyroidism is very important for patient health and wellbeing. Therefore, the assessment of physician's knowledge, attitude and practices related to hypothyroidism is essential for improvisation in the local clinical practices and to initiate programs directing physicians for appropriate hypothyroid management. Through current study our aim was to provide evidence regarding current clinical management of hypothyroid cases among physicians of different domains in Pakistan and to evaluate the physician perception regarding strategies and treatment modifications for specific circumstances like subclinical hypothyroidism, pregnancy and old age. It will provide a clear picture to the Pakistan Endocrine Society regarding the current statistics of the knowledge and practices of the physicians and will assist in the development of proper guidelines for the management of Hypothyroidism.

METHODOLOGY

This KAP survey on hypothyroidism was conducted on a total of 760 physicians who attended the CME programs on the thyroid management and also the meetings conducted by Pakistan Endocrine Society from October to December 2019. The doctors were provided with a questionnaire at the beginning of the session, with 10 multiple choice questions and limited time allotted for completion (15 mins). The questionnaire was designed with the help of a survey conducted by Burch et al. (2013) [15]. The questionnaire was designed for evaluation ofphysician's knowledge, perceptions and practices for a hypothyroidism index case.

Study Tool

Case Scenario: A 48-year-old women presents with a 9-month history of fatigue, cold intolerance, poor concentration and constipation. She is otherwise healthy, takes no medication and is non-smoker. She has a blood pressure of 135/90, a pulse rate of 55 beat per minute and weight 60 kilogram. She has a firm diffuse goitre, approximately twice normal size. Serum TSH is 50 m U/L (normal 0.4-4.5 mU/l) and free T4 (FT4) is 0.6 ng/dL (normal 0.8-1.8 ng/dL). Questions 1–6 were regarding diagnostic evaluation, preferred treatment and follow-up frequency of the above-mentioned index case. Questions 7–9 were regarding physician's perception for TSH target ranges for three distinct groups including (1) a case of 25 year old hypothyroid woman who is trying to conceive, (2) a 52 year old

hypothyroid man who is having dyslipidemia and (3) a 85 year old hypothyroid woman who has fracture her left hip few years ago. *Case Scenario:* A 32 years old lady with hypothyroidism is taking 100 mcg levothyroxine once daily, her TSH is 2.2 mU/L (normal 0.4–4.5 mU/L), she is now 5 weeks pregnant and is asking about what to do with her levothyroxine. Questions 10 & 11 were regarding hypothyroidism management.Question 12 was based on the strategies involved in correcting the hypothyroidism case. *Case Scenario:* 30-year-old lady referred by her gynaecologist with deranged thyroid profile. She has history of recurrent miscarriages in the past and her TSH was found to be high on 2 occasions i.e. 5.6 and 6.2 mU/L (normal 0.4–4.5 mU/L), her Anti-TPO antibodies are positive and her FT4, Total T4 and Total T3 are all normal. Question 13 was based on the common influencing factors that might alter the decision for recommending levothyroxine replacement to someone who has subclinical hypothyroidism i.e. TSH between 4.5-10 mU/L (normal 0.4-4.5 m U/L), and FT4 normal.

Study participants

The study participants included practitioners from diverse expertise including family physicians, general practitioners, internal medicine specialists, surgeons, obstetricians and endocrinologists practicing in Pakistan, involved in hypothyroidism management and attended the CME programs and meetings held by Pakistan Endocrinology Society.

Ethical Consideration

The study proposal was approved by Ethical Review Board (ERB) of Lady Reading Hospital [Reference # 218-A/LRH; Dated 20th October 2019].

Statistical analysis

The statistical analysis was performed via Statistical Package for the Social Sciences (SPSS) Version 22. Frequency and percentages were used for presenting all categorical variables while mean and standard deviation was used for all discrete variables.

RESULTS

Responses from 760 practitioners were recorded out of which majority family physicians (n=300) followed by general practitioners (n=246), Internist (n=111), Endocrinologist (n=25) and others (n=57). 580 male physician and 180 female physicians were enrolled in this study. Hypothyroidism encounters managed on monthly basis were <5 for 453 (59.6%) physicians, 6-10 for 168 (22.1%), 11-20 for 38 (5.0%), 21-20 for 61 (8.0%) and >30 for 22 (2.9%) physicians (Table I).

Diagnostic evaluation, therapy & follow-up frequency

The diagnostic parameters were inquired, Anti-TPO, total T3, free T3, lipid profile and repeat free T4 were mostly recommended by 291(38.3%), 136(17.9%), 102 (13.4%), 46 (6.1%) and 60 (7.9%) physicians respectively. Anti-Thyroglobulin (Anti-Tg) and Technetium-99m (Tc-99m) thyroid scan were rarely preferred. Regarding the treatment regime, levothyroxine (25 mcg)/day was recommended by 628 (82.6%) physicians while only 24 (3.2%) preferred levothyroxine (125 mcg)/day. According to 555 (73.0%) preferred 2-4 weeks interval for retesting thyroid hormone replacement adequacy and 371 (48.8%) preferred FT4 test to ensure its adequacy at follow-up visit. 502 (66.1%) physicians suggested an increment of 12.5 mcg levothyroxine dose/day at follow-up visit when the patients TSH level is 8 mU/L and FT4 is 0.93 ng/dL. 601(79.1%) physicians recommended follow-up after every 6 weeks after initial recovery (Table II).

Out of 760 respondents, 74.70% physicians recommended <1 mU/L TSH target for a 25 years old female with hypothyroidism trying to get pregnant while only 6.40% suggested TSG target in between 2.5-4.5 mU/L for such a case. TSH target for a 52-year-old hypothyroidism man with dyslipidemia was <1 mU/L was recommended by 73.20% physicians followed by 8.70% physicians who suggested TSH in between 2.5-4.5 mU/L. 77.60% physicians suggested <1 mU/L TSH target for 85-year-old hypothyroid women with left hip fracture (Figure I).

The physician's perception regarding the management and practice in case of a 32 years old woman with hypothyroidism taking levothyroxine (100 mcg)/day, with TSH level 2.2 mU/L, currently 5 weeks pregnant was inquired. Around 562(73.9%) preferred to stop levothyroxine while 55(7.2%) preferred dose increment of 50 mcg/day. Recommended frequency of TSH testing was also inquired 610 (80.3%) preferred a follow-up after every 2-3 weeks. 581(76.4%) physicians recommended retest in 3 months for 30-year-old women with deranged thyroid profile with history recurrent miscarriages, high TSH on 2 occasions i.e. 5.6 and 6.2, positive Anti-TPO antibodies and normal FT4, Total T4 and Total T3 (Table III).

Decision making in subclinical hypothyroidism

Around 315(41.4%) physicians suggested that the risk for Coronary Artery Disease (CAD) is the most common influencer for decision making among hypothyroid patients, followed by elevated LDL cholesterol 109(14.3%), hypothyroidism symptoms 13(1.7%), known CAD 30(3.9%). Treatment without justification was selected by 94(12.4%) physicians while goiter and obesity were also selected by a few (Table VI). According to the results in figure 2 fracture (suggested by 39.9% physicians) was the most significantly reported influencer for decision making regarding TSH target among hypothyroid patients followed by fatigue (36.4%), cardiac arrhythmia (35.0%), pregnancy (28.3%), age (26.4%), weight (24.3%), osteoporosis (20%) and interest in conception (11.3%). While among other rarely suggested were obesity, patient preferences and multiple falls and only 1.1% physicians believed that none of the above-mentioned factors were responsible for influencing decision making.

DISCUSSION

The evaluation of knowledge and practices of practitioners is necessary in this era of globalization in medicine field. In favor of the above-mentioned objective our study assessed the clinical practice patterns and knowledge regarding hypothyroidism management by the healthcare providers in Pakistan under diverse case scenarios. The study included physicians from diverse specialties including family physicians, general practitioners, internal medicine specialists, surgeons, obstetricians and endocrinologists. During the diagnostic evaluation of the index case of a 48-year-oldfemale with the clinical historyand biochemical report suggestive of hypothyroidism, Anti-TPO was the most preferred test by the physicians followed by total T3, free T3, repeat free T4 and lipid profile while Anti-Tg and Tc-99 m thyroid scan were preferred by a very few physicians (Table 2). Similarly, a study reported Anti-TPO antibodies as the most reported diagnostic investigation for hypothyroidism 218 (70.78%), followed by thyroid ultrasonography, anti-thyroglobulin, lipid profile, free T3 and total T3 [18]. Around 82.6% physicians recommended 25 mcg/day while 3.2% were in favor of 125mcg/day (Table 2) which is comparable to a similar study in which 96.1% physicians recommended the initiation of levothyroxine in the index case with hypothyroidism and also the global observed rate (98.9%) supported the findings [15].

The dose adjustments in levothyroxine during the treatment of hypothyroidism in the index case is common with an increment of 12.5mcg followed by 25 mcg. Moreover, decrement of 12.5 mcg was also recommended by a number of physicians 74(9.7%). In consistent to our results, a study reported that 61.1% physicians preferred an increment of 25 mcg, followed by 50 mcg, and 12.5 mcg [15]. Although there has been progressive development in several national and international guidelines in support of management of hypothyroidism, still there exist several controversial issues which are to be resolved in regards to patients age, pregnancy and other rare complications [19,20].

In the current study, the physicians were questioned regarding the TSH targets for different case scenarios, the most preferred TSH target for a 52-year-old hypothyroidism man with dyslipidemia was <1 mU/L (73.2%) which was lower in comparison to a study reporting the preferred target range in between 2.5–4.99 mU/L for a similar case by majority of the physicians [18]. While based on a global survey conducted in 2013, the recommended TSH target was 1.0 to 1.9 mU/L for 408 respondents (47.7%) followed by 2.0 to 2.9 mU/L, 0.5 to 0.9 mU/L and 3.0 to 3.9 mU/L [15]. In addition, a study conducted in Scotland to evaluate the age and gender specific TSH target ranges indicated that the TSH references ranges for normal adult population is not same for the different age groups. Moreover, utilizing age-specific TSH target ranges (especially in elderly patients, 70 years old) would help to avoid misclassification and overtreatment which further leads to complications including hyperthyroidism, atrial fibrillations and osteoporosis [16,21,22].

Neurodevelopmental impacts of maternal hypothyroidism are much of concern and it must be noted that the hypothyroid management during pregnancy is an area where further research and evidence-based references are required in order to provide a verified target range. The pre-pregnancy TSH target among young females trying to conceive was<1 mU/L (74.70%) (Figure 1), while in comparison 95.1% physician preferred TSH target < 2.5 mU/L (p<0.001) in similar case [16]. According to the International guidelines of Endocrine Society (USA), levothyroxine is recommended in all subclinical hypothyroid females whereas levothyroxine is only recommended for the subclinical hypothyroid females presented with positive anti-TPO antibody, or TSH >10 mIU/L [23]. Moreover, the TSH screening among pregnant females is not recommended by any national or international guideline. Influencing factors involved in the decision making regarding subclinical hypothyroidism were also inquired and risk factor for CAD was the significant influencer during the decision making to start levothyroxine replacement in subclinical hypothyroidism (Table 4). In references to this, positive TPO antibodies was reported as the most common influencing factor affecting decision making (62.3%), followed by hypothyroidism symptoms, LDL cholesterol elevation, etc [15].

Among the limitations of this study were unequal representation of physicians Limitations of this study include the lack of equal representation of doctors with diverse specializations. Moreover, the physicians included in the current sample were limited and might not be representing the whole community of physicians in Pakistan as we included only the members attending the CME and meetings held by Pakistan Endocrine Society. However, the current study generated useful data regarding knowledge, attitude and practices of the physicians regarding hypothyroid dismmanagement, and also provided the significant details for improvement in the management guidelines highlighting the current core issues in clinical practice.

CONCLUSION

In conclusion, the study provided with the current standings of the clinicians regarding the knowledge and practices for managing hypothyroidism. Significant positive approach was observed in relation to age and pregnancy specific TSH targets. Moreover, with support of case scenarios with pregnant females, physicians preferred strategies for correcting hypothyroidism in such circumstances were also highlighted. The perceptions regarding follow-up frequency, diagnostic tests and treatment strategies were also evaluated and the responses were quite similar to the international global trends followed for the management of hypothyroidism.

CONFLICTS OF INTEREST

The authors have no conflicts of interest.

ACKNOWLEDGEMENT

We are thankful to the Medical Affairs department, Getz Pharma for providing technical support throughout the study.

SOURCE OF FUNDING

None.

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Table I: Demographic characteristics of the study practitioners

Variables	Sub-Categories	(n=760)
Age group	Less than 30 years	117 (15.4)
	31-40 years	169 (22.4)
	41-50 years	194 (25.2)
	51-60 years	190 (25.0)
	61-70 years	36 (4.7)
	No response	54 (7.1)
Gender	Male	580 (76.3)
	Female	180 (23.7)
Specialties	Family Physician	300 (39.5)
	Internist	111 (14.6)
	Endocrinologist	25 (3.3)
	General practitioner	246 (32.4)
	Other	57 (7.5)
	No response	21 (2.8)
Professional grade	Consultant/Attending	150 (19.7)
	Specialist/Fellow	190 (25.0)
	Resident in training	155 (20.4)
	Others	208 (27.4)
	No response	57 (7.5)
City of Practice	Lahore	750 (98.7)
	Peshawar	10 (1.3)
Practice locality	Small town or rural practice	79 (10.4)
	Large city-based practice	613 (80.7)
	No response	68 (8.9)
Hypothyroidism encounters managed per month	Less than 5	453 (59.6)
	6-10	168 (22.1)
	11-20	38 (5.0)
	21-30	61 (8.0)

	More than 30	22 (2.9)
	No response	18 (2.4)
Practicing Institute	Private Multispecialty Hospital	68 (8.9)
	Government General Hospital	276 (36.3)
	Government Primary Health Care	152 (20.0)
	Private Physicians' Clinic	240 (31.6)
	No response	24 (3.2)

Values are given as n(%)

Table II. Diagnostic evaluation, preferred treatment and frequency of follow-up of the index patient.

Case Scenario: A 48-year-old women presents with a 9-month history of fatigue, cold intolerance, poor concentration and constipation. She is otherwise healthy, takes no medication and is non-smoker. She has a blood pressure of 135/90, a pulse rate of 55 beat per minute and weight 60 kilogram. She has a firm diffuse goiter, approximately twice normal size. Serum TSH is 50 m U/L (normal 0.4-4.5 mU/l). and free T4 is 0.6 ng/dL (normal 0.8-1.8 ng/dL)		
Questions	**Options**	**n (%)**
Further recommended test	Anti-TPO	291 (38.3)
	Total T3	136 (17.9)
	Free T3	102 (13.4)
	Lipid profile	46 (6.1)
	Repeat free T4	60 (7.9)
	Anti-Tg	7 (0.9)
	Tc-99 m thyroid scan	5 (0.7)
	No response	113 (14.9)
Dose of levothyroxine initiated	25 mcg once daily	628 (82.6)
	125 mcg once daily	24 (3.2)
	No response	108 (14.2)
Preferred time interval for retesting adequacy of thyroid hormone replacement	2-4 weeks	555 (73.0)
	14-16 weeks	37 (4.9)
	6-8 weeks	5 (0.7)
	No response	163 (21.4)
Preferred test to ensure adequacy of thyroid hormone replacement at follow-up	Free T4	371 (48.8)
	Anti-TPO	101 (13.3)
	Free T3	197 (25.9)
	TSH	305 (40.1)
	Total T4	38 (5)
	Anti-Thyroglobulin	38 (5)
	Total T3	82 (10.8)
	Thyroid Ultrasound	25 (3.3)
At follow-up visit, the TSH is 8 mU/L and free T4 is 0.93 ng/dL and the condition is symptomatically better but minor fatigability reported.		
Change in levothyroxine dose (with reference to above mentioned detail)	Increment by 12.5 mcg daily	502 (66.1)
	Decrement by 12.5 mcg daily	74 (9.7)

	Increment by 25 mcg daily	32 (4.2)
	No response	152 (20.0)
Upon further follow-up visit the patient achieves Euthyroidism (TSH 3.2 mU/L)		
Frequency of follow-up	Every 6 weeks	601 (79.1)
	Every 9 months	11 (1.4)
	Every 3 months	12 (1.6)
	Every 6 months	7 (0.9)
	No response	129 (17.0)

*TSH-Thyroid Stimulating Hormone; Anti-TPO- Anti-thyroid peroxidase; FT4- Free T4; Anti-Thyroglobulin (Anti-Tg) and Technetium-99m (Tc-99m) thyroid scan

Table III. Strategy for correcting hypothyroidism in 32-year-old female with hypothyroidism (index case)

Case Scenario: A 32 years old women with hypothyroidism is taking 100 mcg levothyroxine once daily, her TSH is 2.2 mU/L (normal 0.4–4.5 mU/L), she is now 5 weeks pregnant and is asking about what to do with her levothyroxine		
Preferred Advice	Stop levothyroxine	562 (73.9)
	Make a dose increment of 50 mcg daily	55 (7.2)
	Continue Same Dose	10 (1.3)
	No response	133 (17.5)
Preferred frequency of TSH testing during pregnancy	Every 2-3 weeks	610 (80.3)
	Every 12-14 weeks	9 (1.2)
	No response	141 (18.6)
Case Scenario: A 30-year-old women is referred to you by her gynecologist with deranged thyroid profile. She has history of recurrent miscarriages in the past and her TSH was found to be high on 2 occasions i.e. 5.6 and 6.2 mU/L (normal 0.4–4.5 mU/L), her Anti-TPO antibodies are positive and her FT4, Total T4 and Total T3 are all normal.		
What will you do next?	Retest in 3 months	581 (76.4)
	Start low dose levothyroxine	10 (1.3)
	Start high dose levothyroxine	5 (0.7)
	No response	164 (21.6)

*Values are given as n(%)

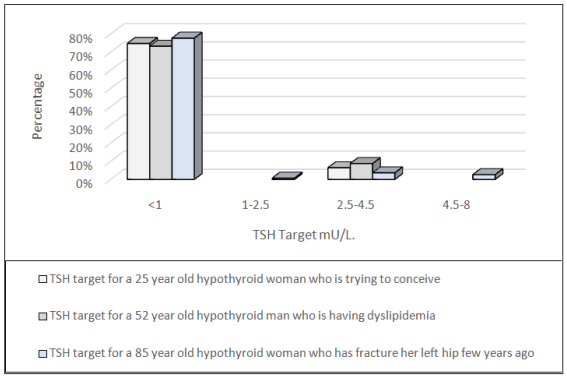
*TSH-Thyroid Stimulating Hormone; Anti-TPO- Anti-thyroid peroxidase; FT4- Free T4.

Table IV. Factor influencing the decision to start levothyroxine replacement in subclinical hypothyroidism(TSH between 4.5-10 mU/L and FT4 normal)

Influencers	n(%)
Risk factor for CAD	315 (41.4)
LDL cholesterol elevation	109 (14.3)
Treat without justification	94 (12.4)
Hypothyroidism symptoms	13 (1.7)
Known CAD	30 (3.9)
Goiter	7 (0.9)
Obesity	11 (1.4)

*Missing data not included

*CAD- Coronary Artery Disease; LDL-Low Density Lipoprotein



*TSH-Thyroid Stimulating Hormone

Figure I: Percentage responses regarding TSH levels for KAP evaluation through different case scenarios

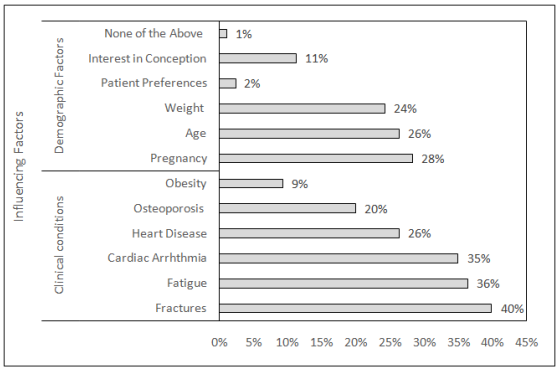


Figure II: Influencers affecting decision making regarding TSH range when treated with thyroid hormone replacement therapy

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Information Retention in Different Methods of Oral Hygiene Instruction Delivery among Orthodontic Patients

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Received: 29 Feb 2020 Revised: 31 Mar 2020 Accepted: 02 May 2020

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ABSTRACT

The aim of this study is to compare the information retention in three different methods of oral hygiene instruction (OHI) delivery. A cross-sectional study was undertaken with a total of 30 participants (7 males and 23 females). They were divided into 3 groups (n = 10) which were the verbal, written and audiovisual (AV) methods. All groups received similar content of information in their preferred languages (Malay or English). After 5 minutes, subjects were asked to answer a pre-tested questionnaire to assess their information retention. The questionnaire comprised of three parts (demographic details, usage of oral health product and retention of information). The answers from all groups were compared. Data collected was analyzed using SPSS version 22. Most of the subjects were able to answer the questions given correctly (verbal=92, written=97, AV=95). Comparison for each method of oral hygiene instruction given shows no significant differences on recall of information between all groups. There is no difference in retention of information between the verbal, written and audiovisual methods of oral hygiene instruction in orthodontic patients.

Keywords: oral hygiene instruction delivery, orthodontic patient, information retention.

INTRODUCTION

Orthodontic treatment with fixed appliances complicates the oral hygiene care as the presence of bands, wires and ligature will promote plaque retention. Patients wearing orthodontic appliances have difficulties in maintaining good oral hygiene (1) and are more susceptible to dental caries and periodontal disease. Clinician plays an important role in educating and motivating patient to perform proper oral hygiene care thus helps in maintenance of optimal oral health (2). Mechanical methods such as tooth brushing and flossing technique require time, motivation and manual skill. Even patients who are properly trained and instructed to maintain satisfactory oral hygiene often see their compliance falter unless constant health education reinforcement is provided (3). The effective motivation of individuals and effective of delivery oral hygiene will promote oral hygiene care among patients (4). A good oral hygiene is very important as a study done among healthy Indonesian found that those with poor oral hygiene have higher risk of getting cardiovascular disease (5). A few studies have shown that dental health care education (6) and oral health literacy (7,8,9,10,11,12,13) has a role in altering individuals' behavior related to oral health care. The oral hygiene instruction should be simple and easy to be understood by the patients. Patients that are able to understand and practice the instructions or information given to them will reflect the professionalism and good skill of the clinicians. Thus, this study is conducted to compare the information retention in three different methods of oral hygiene instruction (OHI) delivery i.e. the verbal, written and audiovisual (AV) methods.

MATERIAL AND METHODS

A cross-sectional study was undertaken from a university Orthodontic clinic. Ethical approval was obtained from The UniversitiKebangsaan Malaysia (UKM) Institutional Review Board for Research and Ethics and consent from the patients were obtained prior to the start of this study. The questionnaire used was pre-tested to a group of 15 subjects. All patients aged 12 years and above who had received orthodontic treatment were recruited. They were divided into 3 groups (n = 10) based on 3 different OHI delivery methods i.e. Verbal (verbally with demonstration using tooth model), Written (illustrated pamphlet) and Audiovisual (video). All content of informations were similar in their preferred languages (Malay or English). After 5 minutes, subjects were asked to answer a questionnaire to test for their information retention. The questionnaires comprised of three parts i.e.; demographic details, usage of oral health kit and retention of information. The answers from each group were compared. Statistical analysis was conducted using Statistical Package for the Social Sciences (SPSS) version 22. Chi-Square test been used to compare between groups.

RESULT

Demographic data

A total of 30 subjects were included in this study. 23 of the subjects were female and 7 were male. Sample comprised mostly of Malays (86.7%), followed by Chinese (10.0%) and Indians (3.3%).

Usage of Oral Health Product

All subjects used a manual toothbrush. Majority of subject brushed three times a day (73.3%) at around 3 minutes (46.7%).When assessing the usage of other oral hygiene aids, all subjects used interdental brush to help them in maintaining oral hygiene. 90% of the subjects used mouthwash and only 56.7% of them used floss (Table 1).

Retention of Information

All subjects (n=30) answered correctly for question 1,4,6 and 10. 29 subjects answered correctly in question 2,8 and 9 while 5 subjects who had wrongly answered question 3 and 7. In question 5, each of the group had one subject who answered the question incorrectly (Table 2). In comparison between the three methods of OHI i.e. verbal, written and audiovisual (AV), the written group has the highest subjects of correct answers (n= 97), followed by AV group (n=95) and verbal group (n=92). However, the differences were insignificant ($p>0.05$). Majority of the correct answer were between 10 subject and 9 subject for each of the group except in question 3 and 7. Three subjects answered wrongly for question 7 in the AV groups while only 1 subject answered it wrongly in each of the written and verbal groups. However, the differences were not significant ($p>0.05$). The lowest correct answer was in question 3 by the verbal group (n=6).

DISCUSSION

Manual toothbrush was preferred by all subjects and they brushed thrice daily which was slightly higher than once a day brushing found by other researchers (1,14). Based on the finding for other types of oral aids usage, our subject used mostly of inter dental brush and mouthwash for daily cleaning and is supported by a Malaysian study (15). Regarding retention of information, most of the subjects were able to retrieve back the information given via all the different methods except in question of 'duration of brushing' and 'flossing technique'. This maybe reflects the lesser use of floss in many orthodontic patients (16) and people usually did not time for their brushing time. In comparison to the three groups, subjects in the written group performed insignificantly better than the subjects in the verbal and AV groups. Patients has been shown to respond slightly better in written instruction than verbal method (17,18,19,20). However, there were other studies found that verbal instruction improved patients' knowledge levels (16,21,22). In our study, reading the catalogue on oral hygiene instruction before answering a written questionnaire with the similar presentation makes it easier for them to answer the questions. Our study used convenient sampling as there were no proper patient's list that will attend the orthodontic clinic prior to the patient's appointment. Further studies can be done to compare short and long term recalls in order to reflect the retention of the information through time.

CONCLUSION

Most of the subjects were able to answer the given questions correctly regarding the compliance and usage of oral health products. Although the written group had the most correct answer to the questions, however, there were no significant differences when comparing the information retention in the three different methods of oral hygiene instruction delivery.

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Table 1: Usage of Oral Health Product

		n (%)
Tooth Brushing Habit		
Frequency	Twice	8 (26.7%)
	Three times	22 (73.3%)
Duration	1 minute	2(6.7%)
	2 minutes	10(33.3%)
	3 minutes	14(46.7%)
	Not sure	4(13.3%)
Oral Hygiene Aids		
Floss		17(56.7)
Interdental toothbrush		30 (100)
Mouthwash		27 (90)

Table 2: Frequency and Percentages of Correct Answer by the Subjects on Assessment of Retention of Information

Question No.	Frequency & Percentages of Correct answer	Written	Verbal	Audiovisual	⚜-value
1	Importance on cleaning of bracket	10(100.0%)	10(100.0%)	10(100.0%)	0.076
2	Interdental toothbrush usage	10(100.0%)	10(100.0%)	9(90.0%)	0.355
3	Duration of tooth brushing	9(90.0%)	6(60.0%)	10(100.0%)	0.09
4	Good tooth brushing	10(100.0%)	10(100.0%)	10(100.0%)	0.861
5	Importance of using floss	9(90.0%)	9(90.0%)	9(90.0%)	0.391
6	Flossing time	10(100.0%)	10(100.0%)	10(100.0%)	0.486
7	Flossing technique	9(90.0%)	9(90.0%)	7(70.0%)	0.383
8	Importance of interdental toothbrush	10(100.0%)	9(90.0%)	10(100.0%)	0.102
9	Function of interdental toothbrush	10(100.0%)	9(90.0%)	10(100.0%)	0.099
10	Importance of mouthwash	10(100.0%)	10(100.0%)	10(100.0%)	0.056

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Studies on Some Biochemical Compositions of Flesh Tissues of Chinese White Shrimp (*Fenneropenaeus chinensis*)

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Received: 10 Feb 2020 Revised: 12 Mar 2020 Accepted: 14 Apr 2020

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ABSTRACT

Shrimps are a good source of protein, carbohydrate, minerals and very low in fat and calories. The present study investigated the procimate and mineral compositions of *Fenneropenaeus chinensis*. The samples were collected from the fish market of Jatni. The moisture content of the flesh was 45.90gm and the protein content was 2.162 ±0 .02 and the carbohydrate content was 2.506 ± 0.002. The CaO had the highest mineral content that was 360250ppm. The results of the present study reported that *Fenneropenaeus chinensis* is a good source of protein and gives metabolic energy and average mineral supplies.

Keywords: *Fenneropenaeus chinensis*, mineral, energy, carbohydrate,

INTRODUCTION

The *Fenneropenaeus chinensis* belongs to the Order: Decapoda and Family: Penaeidae of Phylum: Arthopoda. These have elongated bodies and a primarily swimming mode of locomotion. These are covering stalk-eyed crustaceans. They contain long and narrow muscular tails, long antennae and slender legs. They swim forward by using paddling with swimmerets for swimming. The swimmerets present on the underside of their abdomens. They use their tails for defending themselves, which help in driving them backwardly. These have thin and fragile legs. They use hem primarily for perching (Rudloe and Rudloe, 2009). The approximately life span of shrimp is one to seven years. They usually live solitary, but during the spawning season they form a large school (Gracia, 1996). These are cultivated at an individual level of mainland China. The *Fenneropenaeus chinensis* species was described by Pehr Osbeck in 1765. Shrimps are an extremely good source of protein. Shrimps are very low in fat and calories , making them a very healthy choice of food .Shrimps have high cholesterol content, but they are low in saturated fat (Bligh and

Dyer,1959). The saturated fatty acid have single bond between molecule in fatty acid chains . It is in solid form at room temperature, which is very difficult to soluble. In other hand the unsaturated fatty acid have double bond between molecules in fatty acid chains. It is in liquid form at room temperature, which is easy to soluble. Again minerals are essential in shrimp nutrition. Minerals have essential roles in osmotic regulation and moulting (Vijayan and Diwan, 1996). It is also components of many biological compounds such as enzymes, hormones and high energy compounds. There are many inorganic elements in the body of shrimp with the skeletal structure and biochemicals involved in vital physiological function. Dietary requires important mineral elements (Deshimaru and Yone, 1978). Carbohydrate is also considered in the shrimp nutrition. The sample is valuable in the diet, because apart from supply of good quality proteins and carbohydrates, it also contains several dietary minerals which are beneficial to man and animals. This work was undertaken to determine the protein, carbohydrate and mineral composition of the flesh tissues of *Fenneropenaeus chinensis* collected from the Jatni fish market. The protein (Lowry, 1951), carbohydrate (Morris, 1948) and mineral (Fitton, 1997) content of the samples are determined.

MATERIALS AND METHODS

The samples *Fenneropenaeus chinensis* (n=5) were collected from Jatni fish market. Then the samples were washed with deionized water to remove any adhering contamination. Then these were drained under folds of filter paper. Samples were then put in crushed ice in insulated containers. Then these were brought to the laboratory for preservation to analysing the samples (Ravichandran *et.al*, 2009).

Preparation of samples for analysis

The shrimps were wrapped in aluminium foil and frozen at -4^{o} c for two days before samples were prepared for analysis .After defrosting, the shrimps were separated into the exoskeleton (head and the outer body shell) and the endoskeleton (flesh) .Then the flesh were ground by the help of mortar pestel .Then these were kept in oven for drying at 95-105^{o} C untill dried. Then it was again ground into fine powder by the help of mortar pestle (Ravichandran *et al.*, 2009).

Determination of biochemical composition
Estimation of protein

The protein was estimated by Lowry method (Lowry, 1951) .The stock solution was prepared the day before the experiment from the egg white. A mixture was prepared by adding 10ml of egg albumin with 10ml of distilled water. Then 10ml solution was taken from this mixture and got converted into standard solution by adding 50ml distilled water. It was stirred well and kept for 1day .The buffer solution was prepared by adding one buffer capsule ad 100ml of distilled water .Then 6 tubes were taken . In 1st ,2nd ,3rd ,4th and 5th tube, 1ml,0.8ml,0.6ml,0.4ml,0.2ml of distilled water and 0ml, 0.2ml, 0.4ml, 0.6ml, 0.8ml of stock solution was taken respectively . In 6th tube 1ml mixture from the solution (0.5gm sample powder and 10ml buffer solution) was taken .The mixture of 6th tube was taken in centrifuge tube and then it was centrifugation for 20minute at 8000rpm .Then the supernatant was taken in an experimental tube .Then Fehling's solution was prepared .Total 60ml solution was prepared by adding 58.95ml of Fehling A with 1.05ml of Fehling B. Then the mixture was stirred well .5ml from this mixture was added to each tube .All tubes were kept in oven for 15minutes at 37c. Then the FCR was prepared by adding 2ml of Regent D and 2ml distilled water. Then 0.5ml from mixture was added each tube .All tubes were again kept in oven for 30 minutes at 37^{o} c .Then optical density was measured of each tube by spectrophotometer at 640nm.

Estimation of minerals

The mineral content was estimated by XRF method (Fitton, 1997) .XRF stands for X-ray fluorescence. An X-ray fluorescence spectrometer is a x-ray instrument used for routine , relatively non-destructive chemical analyses of rocks , minerals , sediments and fluids .Various minerals like SiO_2 , P_2O_5, SO_3 , Cl, K_2O, CaO, TiO_2, MnO, Fe_2O_3, CuO, ZnO, Br , ZrO_2, Eu_2O_3, OsO_4 , CO_2 were determined .

Estimation of carbohydrate

The carbohydrate was estimated by Anthrone method .The stock solution was prepared from glucose. A mixture was prepared by adding 100mg of glucose with 100ml of distilled water. Then 10ml solution was taken from this mixture and got converted into standard solution by adding 50ml distilled water. It was stirred well .Then 7 experimental tubes were taken .In 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th}, 6th tube , 1ml, 0.8ml, 0.6ml, 0.4ml, 0.2ml and 0.0ml of distilled water and 0.0ml, 0.2ml, 0.4ml, 0.6ml, 0.8ml and 1ml of stock solution was taken. In 7^{th} tube, 5ml of 2.5N HCl and 0.1gm of sample powder were taken. Then all the tubes were hydrolysed by keeping these in a boiling water bath for three hours and after three hours, these were cooled down at room temperature. Then the solution were neutralized with sodium carbonate .Then the sample solution was taken in a centrifuged tube and was centrifuged .Then the supernatant was collected in experimental tube .Then the Anthrone reagent was prepared by adding 0.2gm Anthrone powder with 100ml of concentrated H_2SO_4 .Then 4ml of Anthrone reagent was taken in each tubes and again heated for eight minutes in a boiling water bath .Then these were allowed to cool down .Then the optical density was measured in spectrophotometer at 640nm.

RESULTS

Table1. Mineral content of the shrimp sample *Fenneropenaeus chinensis* in ppm

Sl. No.	Compound Name	Concentration Amount (ppm)
01	SiO_2	9020
02	P_2O_5	149220
03	SO_3	195520
04	Cl	51180
05	K_2O	216030
06	CaO	360250
07	TiO_2	1240
08	MnO	1100
09	Fe_2O_3	8620
10	CuO	3820
11	ZnO	2010
12	Br	839.7
13	ZrO_2	157.8
14	Eu_2O_3	974.4
15	OsO_4	11.7
16	CO_2	0.0

Fig.1. Flesh of shrimp

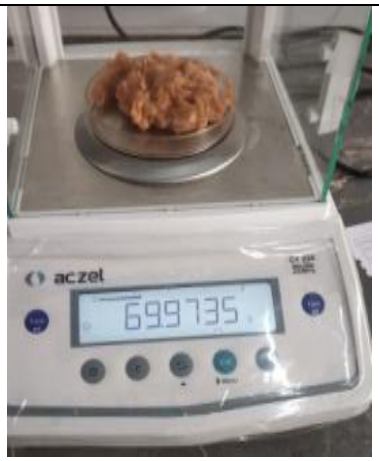

Fig.2.Weight of flesh before dried

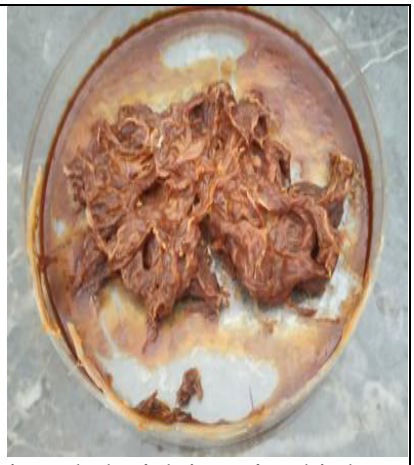

Fig.3. Flesh of shrimp after dried

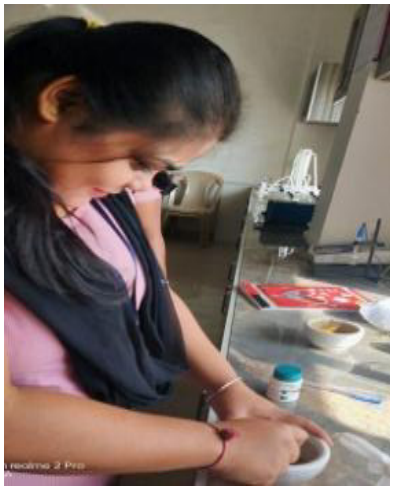

Fig.4.Ground of flesh of shrimp

Fig.5.Powder of flesh of shrimp

Fig.6. Weight of powder of flesh

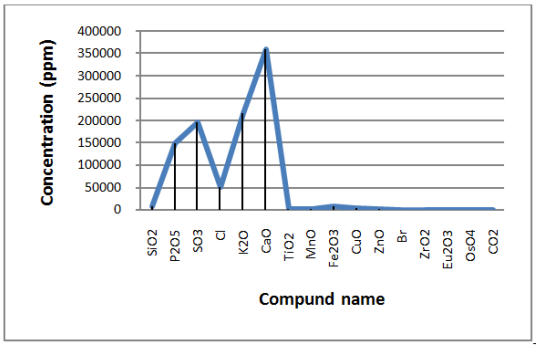

Fig.7: Mineral compostion in flesh of *F. chinensis*

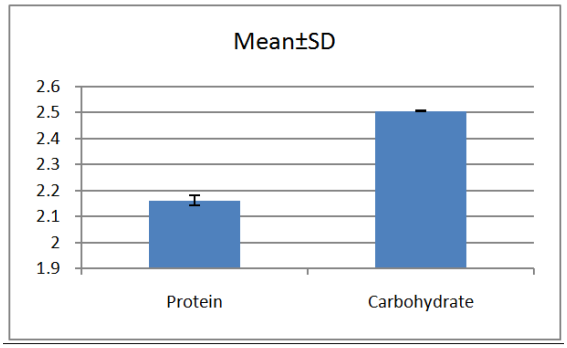

Fig.8: flesh of protein and carbohydrate *F.chinensis*

The result showed that the protein content in *Fenneropenaeus chinensis* was 2.162 $\pm$ 0.02 and the carbohydrate content is 2.506 ± 0.002. The moisture content of the sample recorded was 45.90gm. The mineral contents in *Fenneropenaeus chinensis* are shown in (table no.1 and fig. No 7).

DISCUSSIONS

The findings of the current study was different from the study on *Penaeus indicus* (Ravichandran, 2009) where the protein content was 41.3±0.3 and the carbohydrate content was 2.4±0.6 and the study on *Macrobranchium rosenbergii* (Reddy and Reddy, 2014) where the protein content was 74.24 ± 0.49 and the carbohydrate content was 5.50 ± 0.34. The difference in composition was due to different species and different environmental conditions.

CONCLUSION

The flesh of Chinese white shrimp contains protein, minerals and carbohydrate content in a good amount. Carbohydrate is the main source of energy in our bodies. Protein helps in repairing and builds up tissues. Minerals helps in growth and development of the body and it helps in maintaining a healthy life .As the shrimp contains these contents which are very essential for the body, it is a very good source of food.

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Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Regeneration, Callus Formation and HPLC Determination of Some Cardiac Glycosides of *Digitalis lanata* Ehrh

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Received: 27 Feb 2020 Revised: 30 Mar 2020 Accepted: 02 May 2020

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ABSTRACT

In the present study, the seeds of *Digitalis lanata* Ehrh. were surface sterilized, germinated on hormone-free Murashige and Skoog's culture media supplemented with 30 g/l sucrose and solidified with 5 mg/l Phytagel. Leaves and roots of the obtained 21 days old seedlings were used for callus induction while the leafless dwarf stems and the shoot apical tips were used for regeneration of plantlets. The number of shootlets which could be obtained from single apical shoot tip explant increased from 1.00 ± 0.00 in control group up to 5.00 ± 0.00 with 3 mg/l BA + 3 mg/l Kin treatment and reached up 8.33 ± 0.58 shoots/stem explant in response to the treatment with 3 mg/l BA+ 6 mg/l NAA. Both results are significantly positive increases in comparison to their corresponding controls. Growth curves of calli from both leaf and root explants were plotted over a period of 8 weeks. At the end of growth period, callus from root explants which was compact and yellowish green reached up 7.803 g compared to 3.383 g leaf callus which was friable green. HPLC have shown that callus cultures exceeded the mother plant in biosynthesis of digoxin and digitoxin and that the root callus (445.158 µg/g) was more active than the leaf callus (228.247 µg/g) in comparison to the corresponding control (100 µg/g).Type of explant and growth regulators used affected cardenolides biosynthesis both. The results obtained in this study indicate the promising role of using plant biotechnology in the propagation and secondary metabolite production by *D. lanata.*

Keywords: Foxglove – Micropropagation – Callus – HPLC – Digoxin – Digitoxin.

INTRODUCTION

Digitalis lanata is a plant species belonging to the family plantaginaceaceae. *Digitalis* species are generally native to the Balkans; Hungary, Italy, Germany, Spain, Lebanon, Romania, Transcaucasia, Turkey, Japan and India (Olmstead

et al 2001). Agrawal *et al*. (2012) mentioned that *D. lanata*, which is adopted in this study, has a high economic value due to the many phytopharmaceutics like the famous constituent digoxin in addition to many other cardenolides. It is well-known that *Digitals* cardenolides have extensive therapeutic uses especially to treat heart ailments; a fact that was first reported by Willion Withering in 1885 as mentioned by Withering (2014). Verma *et al*. (2016), observed gradual depletion of the number of the wild population of *Digitalis* species in addition to limited cultivation in the field. The same authors added also that natural propagation of *Digitalis* through seeds is not satisfactory due to low germination frequency. *Digitalis* plants require a period of about two years for optimal development and maximizing accumulation of the important biologically active substances.

Due to the above mentioned facts, tissue culture was strongly suggested to play a role in solving the limited propagation of *Digitalis*. Micropropagation of same *digitalis* spp. through tissue culture was previously reported by (Verma et al. 2016; Patil et al. 2013; Ghanem et al. 2010). It has been mentioned by Vaisree and Tsay (2004), Bosila et al. (2003), Verpoorte and Memelink (2002), Vanek et al. (1986) and Hagimori et al. (1982), that different plant tissue culture techniques including callus and cell suspension cultures can be used as alternative ways for the production of *Digitalis* medicinally important metabolites. The main objectives of the present study included the *in vitro* micropropagation and callus production from seedling explants with evaluation of callus potential for digoxin and digitoxin biosynthesis because *D.lanata* does not grow naturally in Egypt (not included in the flora of Egypt) and its traditional seed-propagation is associated with some problems although the active ingredients obtained from it are commonly used in Egypt to treat heart diseases.

MATERIALS AND METHODS

The present study was conducted at the Plant Tissue Culture Laboratory, Botany and Microbiology Department, Faculty of Science (Girls), Al-Azhar University, Cairo, Egypt during 2018-2019. The seeds of well identified *Digitalis lanata* Ehrh. were obtained from the Experimental Station of Medicinal Plants, Faculty of Pharmacy, Cairo University, Egypt. HPLC of cardiac glycosides of D. *lanata* was carried out using the facilities of Central laboratory at the Desert Research Center. El-Matarya, Cairo, Egypt.

Pretreatment

Due to the very low frequency of seed germination, the seeds of *D. lanata* were subjected to vernalization (kept at 1-3 ^{0}C for one month).

Seeds surface sterilization and germination

Surface sterilization of seeds was carried out by using 20% commercial bleach for 15 minutes followed by washing with sterile distilled water for 3 successive times. After 21 days of sowing the seeds, apical meristems, leaves, roots and the short dwarf stems were excised from the healthy seedlings and used as explants (Figure 1).

Culture media, growth regulators and cultural conditions

Murashige and Skoog's (1962) basal salts was used, fortified with vitamins and 30 g/L sucrose, solidified with Phytagel ™ (5gm/l) and the PH was adjusted to 5.6-5.8 prior to sterilization with 0.1 M KOH. Seed germination was carried out using hormone-free MS culture media. For micropropagation and/or callus formation various plant growth regulators were used in concentrations illustrated in table (1). Jars were kept in plant growth chamber with the following cultural conditions: 16/8 light to dark photoperiod provided by cool-white fluorescent lamps at a photosynthetic photon flux density of 70 μ mol m^{-2} S^{-1} and at 60 $\pm$ 10% relative humidity, and incubation temperature $25 \pm 2^{\circ}C$.

HPLC of cardiac glycosides

The quantitative assay of *D. lanata* cardiac glycosides (digitoxin and digoxin) using HPLC were carried out according to Kelly *et al.* (1995). Extraction of cardiac glycosides from mother plant and callus culture was carried out as

described by Hagimori et al. (1982) where lyophilized callus and seedling cells (0.1gm) were homogenized with 50 ml ethanol in a glass homogenizer. The homogenate was heated at 74 ^{0}C for 4 hours and filtered. The filtrate was dried in vacuum, and the residue was taken up in 2 ml of ethanol and diluted with 18 ml H_2O.

Statistical analysis

Data were statistically analyzed using SAS 9.1 (SAS "Institute INC. Cary, NC 27513, USA).

RESULTS AND DISCUSSION

Regeneration

With respect to the effect of different plant growth substances treatments on apical meristem and short dwarf stems explants, the results illustrated in table (2) and figure (2) may show that there are some promising results regarding the *in vitro* micropropagation of *D. lanata*. The apical meristem explants 10/10 responded to the treatment with 3 mg/l BA+ 3 mg/l Kin in comparison to the control group which only 80/10. The number of shootlets per explant increased from 1.00 ± 0.00 in control group up to 4.00 ± 0.00 in response to the treatment with 6 mg/l BA and up to 5.00 ± 0.00 in response to the treatment 3mg/l BA+ 3 mg/l Kin. Both results are statistically significant in comparison to the corresponding control. It seems that the combination of both BA and Kin was much more effective than BA alone in multiplication of shoot bud. It is to be noticed (from the same table) that the increase in the number of shoot buds induced was accompanied by a general decrease in the length of the shoots obtained. It seems that the general growth of the adopted explants was distributed between bud multiplication and shoot elongation. However, the number of leaves per explant was significantly increased from 5.00 ± 1.00 up to 7.33 ± 0.58 as a result of the treatment with 3mg/l BA+ 3 mg/l Kin.

The results obtained with respect to the dwarf short stems did not differ so much from the results obtained with apical meristem explants. Table (2) has revealed that 10 out of 10 explants responded to the treatment with a combination of BA and Kin which is equal to the response of the control group explants, while on the other hand, the best number of shoots was obtained in response to the treatment with 3 mg/l BA+ 6 mg/l NAA, which reached up 8.33 ± 0.58 shoots/explant, a value that is considered double the number of shoots of the control group explants. However, the general observation that the increase in the number of shoot buds was accompanied by a general decrease in the length of the shoots obtained has been repeated also with dwarf stem explants. The shoot lengths had decreased from 0.40 ± 0.10 cm (control) to 0.23 ± 0.06 cm in response to the treatment with 3 mg/l BA+ 6 mg/l NAA. The results obtained indicate and confirm the previously obtained results that *in vitro* tissue culture techniques might be used successfully for micropropagation of *D. lanata* and accordingly make use of the many advantages of such technology.

Earlier reports showed that cytokinins as BA was the most efficient for shoot regeneration in many plant species (Stefaan *et al*1994). Also *Hagimori et al.* (1982; 1984) reported that shoot culture of *D. purpurea* on MS medium containing 1 mg/l BA + 1 mg/l IAA. Similarly, Perez-Albnso *et al.* (2009) also investigated that the best medium for shoot forming cultures was MS supplemented with 1mg/l BA + 0.1 mg/l IAA in *D. purpurea.* Pèrez-Alonso *et al*. (2009), mentioned a method could be used effectively for obtain direct shoot organogenesis from *D. purpurea* explants by optimizing the concentration of BA and IAA added to MS culture media. Patil *et al*. (2013), working with *D. purpurea*, also obtained some positive and promising results with micropropagation by addition of BA, Kin and TDZ alone to the MS culture Media. The authors reported that the best number of shoot buds which could be obtained per explant reached up to 12.7 ± 0.00 cm. The used hormonal treatment also affected shoot regeneration frequency and the length of the shoots obtained. These results are considered more or less similar to the result in the present study. Verma *et al*. (2014), after their experiment with *D. ferruginea* came to the result that BA in combination with NA A and not 2, 4-D could successfully induce shoot buds from hypocotyl derived callus.

It is to be remembered that in agreement with the results obtained in this study, Zhao *et al*. (2014) reported that in most cases the combination of auxin and cytokinin induces morphogenesis better than auxins or cytokinins alone

and previous studies have shown also a positive response of several digitalis species to BA and NAA combination.as mentioned by Cacho *et al.* (1991).

Callus induction and growth curve

The results illustrated in table (3) shows the effect of different growth regulators applied on the induction of callus formation from both root and leaf explants. With respect to root explants, it has been observed that two of the growth regulators combination could successfully induce callus formation. Medium callus growth was obtained using 6 mg/l BA and vigorous callus growth (+++) was obtained using 3 mg/l BA and 6 mg/l NAA. Weak callus induction from leaf explants was obtained using 0.5 mg/l Kin + 5 mg/l 2,4-D while the use of 3 mg/l BA + 6 mg/l NAA resulted in a medium callus growth. From the obtained growth curve of calli from both root and leaf explants was carried out for 8 weeks on MS culture media supplied with 3mg/l BA+ 6 mg/l NAA as illustrated in table (4) and figure (3). The callus from root explants was compact and yellowish green colored while the callus from leaf explants was friable green.

The results obtained in this study may agree and confirm the results obtained by other authors who previously observed that the combination between various auxins and cytokinins could induce callus formation in other Digitalis species successfully e.g Hagimori *et al.*(1982) working with *D. putrpurea*; Herrera *et al.*(1990) working with *Digitalis thapsi* L. Fatima *et al.* (2009) working with *D. lanata*; Verma *et al*. (2011) and (2012) working with *D.. ferruginea* and *D. lamarckii* respectively. And it has been reported also that callus properties depend on the hormonal combination used for its induction, for example Chow *et al.*(1990) reported compact dense and green calli by impregnating into the culture media NAA/BA, versus yellow white, friable calli induced when NAA was replaced by 2,4-D. The results illustrated in table (4) and figures (3), (4) show the growth rate and curve of calli induced from both root and leaf explants of *D. lanata* over a period of 8 weeks using the combination of growth regulators which previously resulted in the best vigor of calli growth.

In general, there was no big difference in calli fresh weight in the first couple of weeks and then the rate of growth of callus from root explants (7.803 gm) exceeded that of leaf explants (3.383 gm). At the end of the incubation period, callus from root explants exceeded double the fresh weight of the callus produced from leaf explants. However, it is to be remembered also that callus of root explants was compact while the callus from leaf explants was more or less friable. It seems that the nature of the callus depends not only on the growth regulators combination used but also the type of explant used. Palazon *et al.* (1995) cleared that pieces of callus obtained from seedlings *D. purpurea* were grown on solid Murashige-Skoog's medium supplemented with 1 mg/l BA and 0.1 mg/l IAA or NAA and that the replacement of the natural auxin (IAA) by the synthetic one (NAA) increased callus growth, a result that more or less agree with the results obtained in this study.

HPLC analysis of digoxin and digitoxin

From the results illustrated in table (5) and figure (5), it can be seen that calli whether from leaf or root explants exceeded the mother plant in the biosynthesis of (Digoxin +Digitoxin) as measured by HPLC. The sum of Digoxin and Digitoxin in the mother plant was 100 µg/g. The maximum amount of the sum of the two cardenolides (445.158 µg/g), which is more than 4 folds the control one was obtained using 3 mg/l BA + 6 mg/l NAA. It is to be mentioned that callus initiated from root explants showed better activity in synthesizing cardiac glycosides than its corresponding callus from leaf explants 228.247 µg/g. The results illustrated in the same table shows that there were some qualitative and quantitative differences in the cardiac glycosides contents in comparison to the corresponding control. For example, of the 100 µg/g cardenolides of the control sample, Digoxin reached 11.530 µg/g while the Digitoxin content reached up 88.470 µg/g. This difference may come back to the nature of the explant used or the qualitative and/or quantitative contents of internal sugars. From the same table it can be observed also that the type and concentration of plant growth regulators used play a role in the biosynthesis of digoxin and digitoxin. The above mentioned factors (type of explant, plant growth regulators and the internal phytochemical composition of the cultured tissues) play a role in cardiac glycosides biosynthesis on both quantitative and qualitative levels.

Previous studies of Bosila *et al*. (2003) have proved that calli from leaf explants of *D. lanata* could successfully synthesize cardiac glycosides, as observed in our study, and that the best digitoxin content was obtained in response to the treatment with 18 hours light/day. On the other hand, calli induced from root explants in the same work, gave the lowest glycosides content. These results are somewhat in contrast to the results obtained in the present study. Hagimori *et al*. (1982) came to the conclusion that chloroplasts are not essential for the synthesis of Digitoxin by *Digitalis* cells an observation that was also noticed in the present study. Probably, the interaction between type of explant and growth regulators is the main factor that affect biosynthesis of cardiac glycosides. Some more or less similar results were obtained by Grave *et al*. (1980) working with *D. lanata.* Cingoz *et al*. (2014) reported that callus derived from hypocotyl explants from four *Digitalis* spp. were cultured on MS medium supplemented with IAA and TDZ. After a month of culture, callus was transferred to MS medium containing 10 mM H2O2 as elicitor and then incubated for 6 hours. Quantitative analysis for five cardenolides (Lanatoside C, Digitoxin, Digoxigenin, Gitoxigenin and Digoxin), showed that no digoxin was detected in all treatments and control groups while the total cardenolides estimated were in the order of *D. lamarckii* (586.65 µg/g dw), *D. davisiana* (506.79 µg/g dw), *D. cariensis* (376.60 µg/g dw) and *D. trojana* (282.39 µg/g dw). The results obtained in this study strongly indicates that plant growth regulator is the key to enhance the plant cell to manufacturing secondary metabolites in*D. lanata*. Moreover a note is sparked in this study with the previous studies that, the amount of secondary metabolites increased not due to using high concentration of plant growth regulator but by using suitable plant growth regulator in critical concentration with meet the need of the specialized explant. This lead to say that, more cytological and physiological studies on the action mechanism of these compounds still needs further studies.

Declaration

The authors declare that they have no conflict of interest in the publication and that the submitted manuscript is originally, unpublished and not under simultaneous consideration by another journal.

Abbreviations

BA	6-Benzylaminopurine
GA_3	Gibberellic acid
HPLC	High Performance Liquid Chromatography
IAA	Indole-3-acetic acid
IBA	Indole-3-buteric acid
NAA	α-Naphthalene acetic acid
TDZ	Thidiazuron
2, 4-D	2, 4-Dichlorophenoxy acetic acid
MS	Murashige and Skoog

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Table (1): Concentrations of different growth regulators added to MS culture media to induce micropropagation and /or callus formation from *D. lanta* different explants *in vitro*

Number of treatment	Growth substances used (mg/l)			
	BA	Kin	NAA	2,4-D
1 (control)	-	-	-	-
2	3	-	-	-
3	6	-	-	-
4	3	3	-	-
5	3	6	-	-
6	3	-	3	-
7	3	-	6	-
8	-	0.5	-	3
9	-	0.5	-	4
10	-	0.5	-	5

BA: Benzyl adenine, Kin: Kinetin, NAA: Naphthalene Acetic Acid, 2,4-D: 2,4-Dichlorophenoxy Acetic Acid

Table (2): Effect of some growth regulators treatment on *D. lanata* explants cultured *in vitro* on MS culture media

explant	Criteria measured	control	3 mg/l BA + 6 mg/l NAA	6 mg/l BA	3 mg/l BA + 3 mg/l Kin
Apical meristem	Response (out of 10 replicates)	8	---	4	10
	Number of Shoots per explant	1.00±0.00 c	---	4.00±0.00 b	5.00±0.00 a
	Shoot length (cm)	0.43±0.06 a	---	0.23±0.06 b	0.30±0.10 ab
	Number of leaves per shoot	5.00±1.00 b	---	3.67±1.15 b	7.33±0.58 a
Short dwarf stem	Response (out of 10 replicates)	10	7	6	10
	Number of Shoots per explant	3.67±0.58 c	8.33±0.58 a	4.67±0.58 c	6.00±1.00 b
	Shoot length (cm)	0.40±0.10 a	0.23±0.06 b	0.23±0.06 b	0.37±0.06 ab
	Number of leaves per shoot	4.67±0.58 b	5.00±1.00 b	6.33±0.58 b	10.3±1.53 a

Table (3): Effect of different growth regulators treatments on callus induction and growth after 3 weeks of incubation at 25 °C and 16/8 light to dark photoperiod

Growth substances used (mg/l)				Response observed					
				Root callus			Leaf callus		
BA	Kin	NAA	2,4-D	growth	color	texture	growth	color	texture
-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-
6	-	-	-	++	Bale yellow	compact	-	-	-
3	3	-	-	-	-	-	-	-	-
3	6	-	-	-	-	-	-	-	-
3	-	3	-	-	-	-	-	-	-
3	-	6	-	+++	Yellowish green	compact	++	green	friable
-	0.5	-	3	-	-	-	-	-	-
-	0.5	-	4	-	-	-	-	-	-
-	0.5	-	5	-	-	-	+	Yellowish brown	lose

No callusing (-), poor callus growth (+), medium callus growth (++), vigorous callus growth (+++)

Table (4): Growth of callus induced from root and leaf explants of *D. lanata* (fresh weight in grams) on MS culture media supplemented with 3 mg/l BA + 6 mg/l NAA over a period of 8 weeks

Age of callus in weeks	Callus from root explants	Callus from leaf explants
0	0	0
2	0.546	0.500
3	1.830	1.386
4	3.223	2.113
5	5.710	2.611
6	6.233	2.821
7	6.921	3.100
8	7.803	3.383

Each value is a mean of 5 determinations

Table (5): Effect of explant type and growth regulators treatments on the contents of digoxin and digitoxin in intact plants and callus cultures of *D. lanata* (µg/g) as determined by HPLC

Serial	Plant material analyzed	Growth regulators used (mg/l)				Digoxin and Digitoxin µg/g	Digoxin µg/g	Digitoxin µg/g
		kin	2,4-D	BA	NAA			
Control	Intact seedlings	----	----	----	----	100	11.530	88.470
1	Leaf callus	0.5	5	----	----	143.657	102.190	41.467
2		----	----	3	6	228.247	199.606	28.641
3	Root callus	----	----	3	6	445.158	291.726	153.432
4		----	----	6	----	236.096	208.636	27.460

Fig (1): Germination of surface sterilized seeds after 21 days

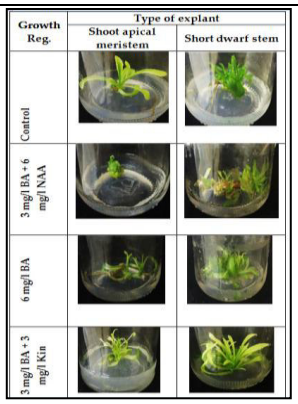


Figure (2): Effect of some growth regulators treatment on *D. lanata* explants cultured *in vitro* on MS culture media.

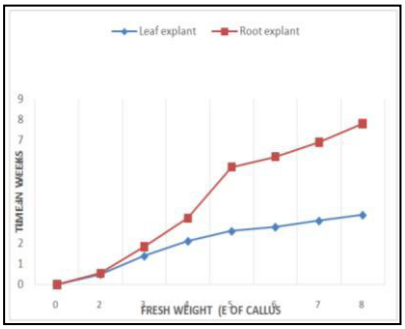
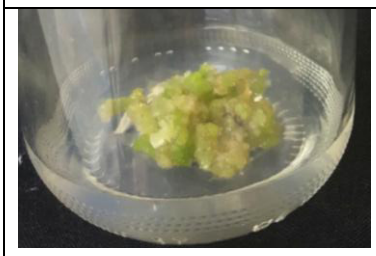
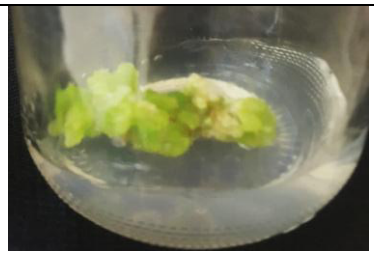


Figure (3): growth curve of calli induced from root and leaf explants of *D. lanata*.

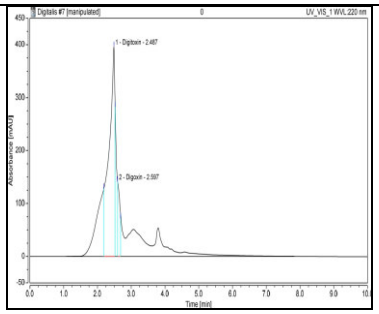


a

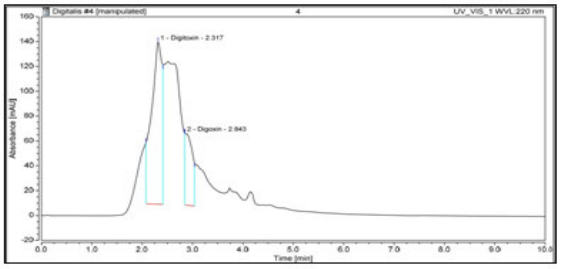


b

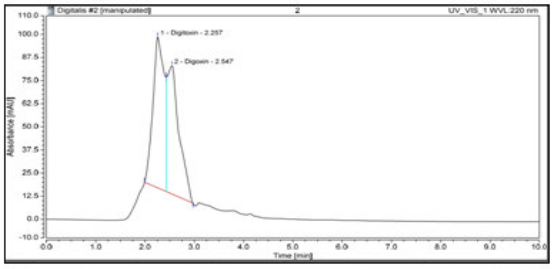
Figure (4): calli obtained from root explants (a) and leaf explants (b) of *D. lanata* after 5 weeks of growth on MS supplemented with 3 mg/l BA plus 6 mg/l NAA



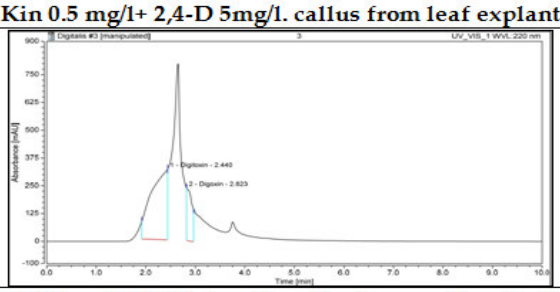
Control



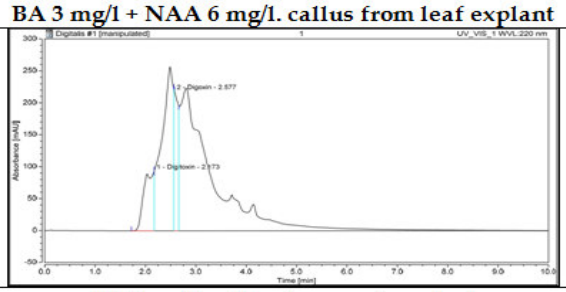
Kin 0.5 mg/l+ 2,4-D 5mg/l. callus from leaf explant



BA 3 mg/l + NAA 6 mg/l. callus from leaf explant



BA 3 mg/l + NAA 6 mg/l. callus from root explant



BA 6 mg/l. callus from root explant

Figure (5): HPLC charts of digoxin and digitoxin of *D. lanata* intact plants and calli.

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Studies on the Feeding Behaviour of Scorpion in Captivity

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Received: 12 Feb 2020 Revised: 13 Mar 2020 Accepted: 15 Apr 2020

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ABSTRACT

Scorpions are an ancient group of nocturnal terrestrial arthropods and referred as living fossil. Scorpion could even be a nocturnal animal foraging during the night. Scorpions found in hot dry desert show combination of behavior, morphology and physiological adaptations to regulate to their habitat. Scorpions use their bodies to help them have a natural defense against predators. They will bite and inject venom once they are threatened. Young scorpion attain maturity very slowly and some of scorpion species require five years to reach their maturity. Scorpion feeds on small arthropods that is cannibalism or other small organisms like insects, rodents, and other small animals. Studies on scorpion of India have received very little attention as compared to other animal groups. This may due to their poisoning much specialized habitats, nocturnal habits and other difficulties for collecting them. This study can help naturalists and researchers for exploring more of this phylum in some extent from different countries of the planet.

Key-words: Scorpion, feeding, arthropods, captivity, India.

INTRODUCTION

Scorpions are belonging to the phylum Arthropods and subphylum Chelicerata and being considered as the most ancient in between terrestrial animals [1].Scorpions are iconic arachnids and referred as small group of with two thousand living species distributed across eighteen different familiesof nocturnal terrestrial arthropods [2].They are classified under the most ancestral arthropods through in origin and morphology of body. During the Silurian they

became first appeared as an aquatic organism and went through small morphological changes [3]. They are defined them as 'living fossils' because of their apparent conservative nature. In scorpions most certainly developed significantbehavioral, physiological, biochemical and ecological adaptations which have been combined which ensure their continued success for about the past 450 million years [4].

In terms of digging burrows, scorpions are being classified as non-burrowing and burrowing.Non-burrowing scorpions can live under the objects laid on the ground, camelthorn, underneath the stones, inside the wall cracks, inside the gaps and cracks in the trees and small pieces of wood and leaves, or can enter human dwellings like wardrobes, shoes or boots to take shelter. Burrowing scorpions have capabilities of digging and dig up to 25 to 50cm deep burrows[5].During the day, they seek shelter beneath rocks, in cracks or burrows that they dig in to the substrate, or beneath the loose outer layers of the many plants shrubs and trees. They are solitary and rather stationary in nature [6]. Scorpions found in hot dry desert show combination of behavior, morphology and physiological adaptations to regulate to their habitat. Mostly they are burrowing and nocturnal [7].

All species of scorpions are poisonous. Scorpion stings can cause morbidity and mortality significantly [8]. Venom Scorpion shows variability by subspecies with a complex structure composed of neurotoxic and acidic proteins, organic compounds and salts, thereby having cardiovascular, renal side effects, hematologic and neurologic with local effects like swelling, pain, redness and burning [9]. Scorpions belongs to family Buthidae are most deadly; though, of families, Hemiscorpiidae and Scorpionidae also threatening for humans [4]. The scorpion was appeared first as aquatic species. According to the history of evolution, scorpions evolved from water scorpions or Eurypterida group as these two groups have many morphological features which are common. In Carboniferous (359–299 MYA), marine and amphibious scorpions persisted, out of which some species may reached toTriassic (251-200MYA) and Permian (299–251 MYA) periods. During the early Carboniferous or late Devonian (416–359 MYA), the first air-breathing or terrestrial scorpion seen on land [4].

Scorpions first appeared in the Silurian period and are the ancient arthropod lineage, and as per fossil records, since the Paleozoic period, their body plan remained largely unchanged.The internal evolutionary relationshipsand the placement of scorpions within Arachnida referred by contentious long morphological characters. For their effective conservation theirtaxonomy and phylogenetic reconstructions are critical [10]. Scorpion remains are often found in coal deposits that are believed to be of carboniferous period (230-600 MYA). The scorpions of carboniferous and descendants show anatomical differences. The actual life span of scorpion is not known. Age ranges in between 4 to 25 years. They live in areas where temperature ranges between 20-35°C and may survive at temperatures ranging from below freezing to desert heat [11]. Cannibalism known to be an important interaction among species of scorpions. Intraguild predation (by other scorpion species) is referred as primary cause of mortality, while predators like other large invertebrate and vertebrate and are secondary cause. The main cause of mortality of smaller immature scorpions are cannibalism [12].

Climbing of shrub by immature classes of large scorpion species and small scorpion species areknown to be a strategy of foraging which can maximize capture of prey and to reduce risk of predation, mainly cannibalism by larger scorpions or can be considered as a predator-avoidance behavior. Shrub selection by this scorpion depends upon availability of smaller and suitable prey and abundance of prey on the different shrubs [6]. Sit-and-wait for foraging is common in nocturnal scorpions, as they sit in one place and wait till the prey to come close to capture it. If scorpions are unsuccessful in capturing preythey move to a different place. Adult females showdoor keeping behavior which means they forage at the entrance or near their burrows [12]. Scorpion could even be a nocturnal animal foraging during the night. They ambush their prey, rarely pursuing them. They are very aggressive when it involves foraging. They sense vibrations around them and strike with what they hear. They search prey like insects, rodents, and other small animals. They need a bent to eat only live food but within the scarcity of food they're getting to consume dead insects. Many species of scorpions sleep in burrows. It's observed that scorpions begin during the day from holes for feeding. This is often in season. Heavy feeding is common within the Scorpion. Once they need

little or no food their metabolism slows down. While this does help them to conserve energy it makes them more vulnerable to predators. Insect's structure is important for their overall diet [11]. Scorpions use their bodies to help them have a natural defense against predators. They will bite and inject venom once they are threatened. Capturing prey for the scorpion involves having the ability to surprise them. They inject venom and paralyze the prey if it is large or to kill it instantly if it is small. This venom will run through the body of the prey and it'll start to decompose and liquefy the insides. This then allows the Scorpion to be able to consume it [11]. Scorpions are shy animals, normally plan to run away and conceal when disturbed but sting if molested. Young scorpion attain maturity very slowly and some of scorpion species require five years to reach their maturity [13].

Scorpions are gendered with males and females. In the spring season mating occurs that is with suitable climatic condition like rise in temperatures and long days get. Their gestation period lasts for about three or four months which mostly depending on the food resources and the temperature. Parthenogenesis seen in some species, like in species of the genera Tityus and Buthacus, rarely males can be found. The Pullus hatches,having some morphological differences as compared to adults. They climb up to the back of their mother to molt just after birth. After this they possess miniature adultcharacters except sexual maturity. After a series of seven molts they attend adulthood with ability to reproduce [1]. In the activity season that is during the reproductive period of scorpion, males are mobile and vagrant, apparently searching for mates [14]. Scorpion populations can be sensitive to environmental changes due to a low reproductive rate (long generation time, long gestation time, small litter size) and high mortality of immature females [10].

Under UV lightwhole body of all the scorpions are fluoresce. There are two fluorescent pigments that is a derivative of coumarineand a derivative of carboline. Prior to common molt of pullus,they do not produce fluorescence. These nocturnal animals can be easily collected due to their fluoresce nature [15]. In the late 18th century scorpion studies begun and for almost 150 years, researchers focused primarily on general anatomy, very rudimentary biogeography, descriptive taxonomy, and venom biochemistry. Since the 1970s, expansion in basic research on scorpions seen focusing on ecology evolutionary, physiology, behavior, and reproductive biology [12]. Studies on scorpion of India have received very little attention as compared to other animal groups. This may due to their poisoning much specialized habitats, nocturnal habits and other difficulties for collecting them [13]. During the same period the other parallel and well-established aquatic group was Silurian Scorpions. Both of these had many common characters such as compound eyes, external gill books and similar chewing structure on the first segments of the first pair of appendages. The main evolution which made the Silurian aquatic scorpion to migrate from water to land was an evolution to external gills in to an indoor book lungs [13].

The main aim of the study is to analyse the behaviour and ethogram of the scorpions in captive condition.The objective of the present work will help the researchers and scientists to take appropriate decision and to know the value of captive behaviour of scorpion. This study is an attempt for guidance along with preference to their ethogram and also can provide basic knowledge.

MATERIALS AND METHODS

The specimens were collected randomly and scorpions were searched under rocks, gap of soil, leaf litter and under bark. Thirty numbers of scorpions were captured and kept inside a laboratory testing ground. The laboratory testing ground was a plastic tray. During captivity, natural conditions were provided to the scorpions. There were some stones were fixed with gum on its surface and by pouring some soil, some grasses were planted on the surface. Water was filled on the surface of the tray till half of the stones submerged inside water. A piece of net made up of steel was covered the whole tray. The samples were collected in two phases. In the month of August and September the first phases of samples were collected from the different villages of western Odisha, they are included M.Srigida, Bijepur, Sarandapali, and Sohela. A number of 23 scorpions were captured in the first phase of collection. The second

phase of sample collection was occurred in the month of October and November in Khandagiri, Bhubaneswar, Odisha, India and 7 scorpions were collected in this phase. For identification, the samples were named and marked. Data were collected form daily observation. Regular movement of the scorpions were captured opportunistically usinga mobile phone (Redmi Note 7s) camera. Several video clips were also captured for further detailed study purpose. The activities and behavior were noted on a note pad. All the scorpions were captured for their behavior and ethogram analysis. Each and every scorpion were released in to the nature after 40 days of their data collection.

RESULTS AND DISCUSSION

The first activity of scorpions during foraging was exploration of the Laboratory Testing Ground (LTG) environment by touching the substrate with pedipalps to find the food source. Observations shows that telson does not influence on this behaviour. Ethogram of Feeding in case of scorpion was observed that, theyseized the prey and held it by chelate and pedipalp while paralyzing by the sting. The paralyzed food is transferred to preoral cavity and turn to pieces by chelicerae. The process of feeding is very slow. During feeding pedipalp pass the killed prey to chelicerae which tear it to pieces. The coxae of pedipalp & first two pairs of legs now press upon the prey so that the entire body fluid is squeezed out into preoral cavity. The liquid comes out from the body of prey is sucked and driven into oesophagus and stomach.

I) Calculations for *Reported Total feeding active time of Scorpions: (Table- 1)

Total feeding active time = $\frac{\{\text{feeding time(in minutes)} \times 0.000694\}\ \text{day}}{\text{days of having food}} \times 100$

The sum of total feeding active time = 211.4%

The average total feeding active time = $\frac{211.4}{30} = 7.04\%$

II) Calculations for average day of feeding (days) and percentage (%):

i) Let x = The average of total days of feeding by the samples during captivity

x = the sum of days of feeding / total numbers of sample

$\Rightarrow x = {}^{449}/_{30}$ = 14.96 days

ii) Let x^1 = The average percentage(%) of total days of feeding during captivity

$x^1 = {}^{x}/_{\text{total days of captivity}} \times 100$

$\Rightarrow x^1 = {}^{14.96}/_{40} \times 100$ = 37.4%

III) Calculations for average feeding time (min) and percentage (%):

i) Let y = the average of feeding time of all scorpions during captivity

$y = {}^{\text{the sum of feeding time}}/_{\text{total numbers of samples}}$

$\Rightarrow y = {}^{42801.1}/_{30}$ = 1426.70 minutes

ii) Let y^1 = the average percentage of feeding time of all the scorpion during captivity

$$y^1 = {}^{y}/_{\text{total times of captivity}} \times 100$$

$$\Rightarrow y^1 = {}^{1426.70}/_{57600} \times 100$$

$\Rightarrow y^1$= 2.47%

Table 1. is showing that out of 30 scorpions, sample S4 showing highest 19 (47.50%) feeding days among all. S4 have 2633.09 minutes of feeding time (4.57%) which is quite lesser than the overall feeding time of S12. S12 had highest feeding time with 5.90%. Rather than this S17 had highest total feeding active time (15.4) among all. The average active feeding days is calculated to be 14.96 days. Out of 30 samples, sample S5, S7, S14, S15, S18 show 15 days of active feeding activity. The average percentage of feeding time of all scorpions is about 2.47%. After analysing 30 scorpions, Table 1. showing that sample S2, S9, S13, S22, S25 had feeding time near to the overall average feeding time. The total average feeding active time of 30 samples were calculated to be 7.04%. Out of 30 samples S13, S2, S8 and S25 shown total feeding active time near to average. Whereas S1, S3, S6, S10, S11, S12, S17, S18, S20, S21, S23 and S27 show large margin of difference from the average total feeding active time.

Table 2. showing the feeding status of S24 during the investigation. S24 had the lowest days of active feeding with lowest feeding time (477.28 minute). Many small arthropods like spider, butterfly, grasshopper and moth were given as food. Spider was given for 8 times, out of which on 5th day of captivity it had spider for once. Butterfly was given for 6 times, out of which it intake butterfly as food for 3 times. Grasshopper was given for 3 times, sample S24 intake grasshopper as a food on 7th day of captivity. Moth was given 5 times, but S24 feed on it for 2 times. The food was provided for 21 days out of which it intake 7 times and reject foods for 14 times. The percentage of accepting food out of 52.5% is 33% and rejection of food is 67%.

CONCLUSION

Scorpion is an important living fossil with ancient history among other terrestrial organism. This work represent that scorpion spend one-third of total captivity in feeding. As per the observation the scorpion use their telson to paralyse the given prey very rarely. This present investigation on scorpion will help naturalist and researchers with useful information of its feeding. This paper describe the feeding habit of terrestrial arthropod.

Acknowledgement

The author wish to thank Dr.Siba Prasad Parida (Associate Prof.) for his suggestions on this work and for providing necessary facility, which made this project successful. The authors would like to thank and acknowledge for providing necessary help and support by Centurion University of Technology and Management. The author's heartiest gratitude to the most trusted friend LopamudraSamantaray for her help and motivation.

Conflict of interest

We declare that this investigation have no conflict of interest.

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Table 1. Showing the no. of days of the scorpions had their food and its percentage

Name of Scorpion	Spend days for feeding (in days)	Spend days for feeding (in %)	Feeding time (In Min)	Feeding time (in %)	Total Feeding active time
S1	14	35%	630.42	1.09%	3.12%
S2	13	32.50%	1289.02	2.23%	6.88%
S3	16	40%	860.54	1.49%	3.73%
S4	19	47.50%	2633.09	4.57%	9.62%
S5	15	37.50%	980.36	1.70%	4.53%

S6	13	32.50%	639.23	1.10%	3.40%
S7	15	37.50%	1755.42	3.04%	8.12%
S8	17	42.50%	1897.46	3.29%	7.74%
S9	13	32.50%	1643.45	2.85%	8.76%
S10	13	32.50%	2359.48	4.09%	12.6%
S11	18	45%	448.06	0.77%	1.72%
S12	16	40%	3402.34	5.90%	14.76%
S13	16	40%	1667.25	2.89%	7.23%
S14	15	37.50%	2108.56	3.66%	9.76%
S15	15	37.50%	1110.37	1.92%	5.14%
S16	14	35%	723.3	1.25%	3.58%
S17	11	27.50%	2456.24	4.26%	15.4%
S18	15	37.50%	468.33	0.81%	2.16%
S19	13	32.50%	787.46	1.36%	4.2%
S20	16	40%	860.57	1.49%	3.73%
S21	13	32.50%	2199.53	3.81%	11.74%
S22	16	40%	1322.06	2.29%	5.73%
S23	12	30%	1743.32	3.02%	10.08%
S24	7	17.50%	477.28	0.82%	4.72%
S25	11	27.50%	1246.44	2.16%	7.86%
S26	14	35%	674.41	1.17%	3.34%
S27	16	40%	2998.4	5.20%	13.01%
S28	10	25%	764.25	1.32%	5.3%
S29	14	35%	1896.39	3.29%	9.4%
S30	13	32.50%	758.07	1.31%	4.04%

Table 2. Feeding status of S24 in captive condition

Days	Provided Food	Status
1	No food was provided	
2	Spider	Not had
3	Spider	Not had
4	Butterfly	Had it
5	Spider and Butterfly	Had it
6	Spider	Had it
7	Grasshopper	Had it
8	Butterfly	Not had
9	Spider	Not had
10	Spider	Not had
11	No food was provided	
12	No food was provided	
13	No food was provided	
14	Moth	Had it
15	No food was provided	
16	No food was provided	
17	Butterfly	Not had
18	No food was provided	

19	No food was provided	
20	Butterfly	Not had
21	No food was provided	
22	No food was provided	
23	Spider	Not had
24	Butterfly	Had it
25	No food was provided	
26	No food was provided	
27	Moth	Not had
28	No food was provided	
29	Grasshopper	Not had
30	Moth	Had it
31	No food was provided	
32	Spider	Not had
33	No food was provided	
34	Moth	Not had
35	No food was provided	
36	Moth	Not had
37	No food was provided	
38	Grasshopper	Not had
39	No food was provided	
40	No food was provided	

Table 3. Feeding of S24 (days) and their percentage (%) of food intake

Food	**Total days**		**In percentage**	
Not provided	19		47.5%	
Provided	21	Taken food	52.5%	Taken food
		7		33%
		Not taken food		Not taken food
		14		67%

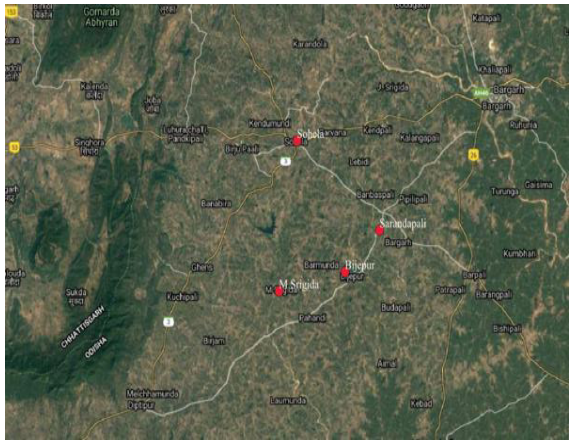


Figure 1. Showing the area of sample collection during phase 1

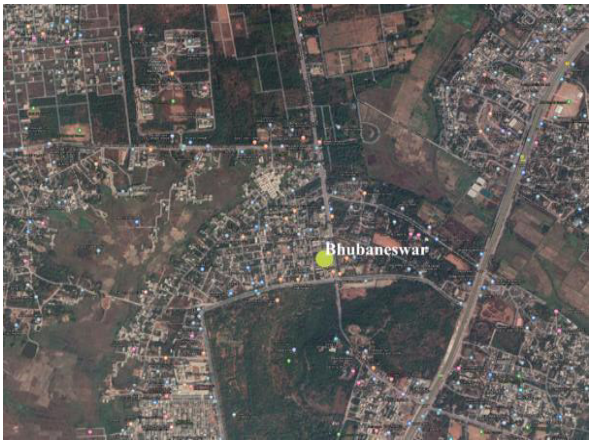
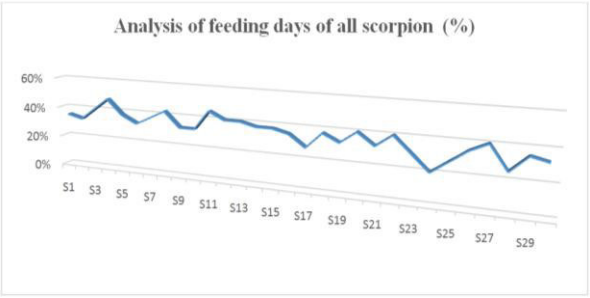


Figure 2 .Showing the area of sample collection during phase 2

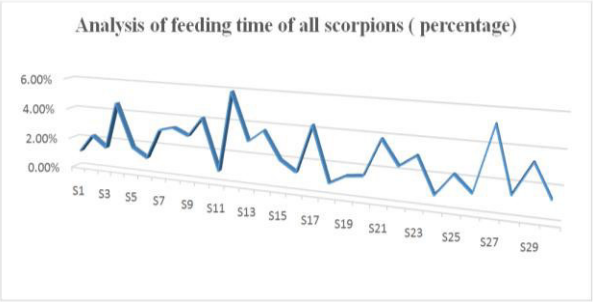
Figure 3. Making small pieces of food with the help of chelicerae

Figure 4. Scorpion having moth

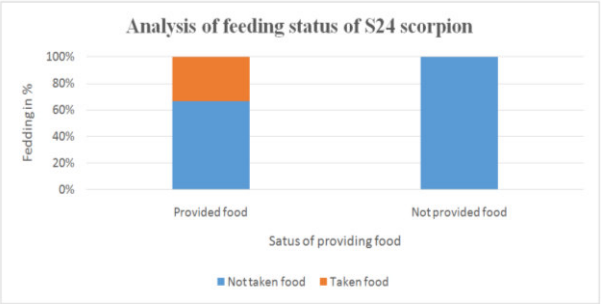
Figure 5. Showing scorpion sample after releasing in to nature



Graph 1. Showing percentage of the days of food having during captivity



Graph 2. Showing percentage of total feeding time of each scorpion sample



Graph 3. Showing analysis of feeding status of S24 scorpion

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Integrated ARIMA-NAR Model to Analyze the Dynamics of Spread of COVID-19

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Received: 11 Feb 2020 Revised: 12 Mar 2020 Accepted: 15 Apr 2020

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ABSTRACT

A key question prevails among all individuals today is how the disease, COVID-19 would propagate in an environment in which it is left unconstrained wherein, assertive efforts to mitigate the disease's adverse effects are in headway. In the present study, Auto Regressive Integrated Moving Average (ARIMA) and integrated ARIMA- Nonlinear Auto Regressive Neural Network (ARIMA-NAR) models were devised to understand the dynamics of time series data of number of COVID-19 cases in the states of Maharastra, Gujarat, Tamilnadu and Andhra Pradesh and subsequesntly execute the short term forecast (fifteen days). Data from 14th March 2020 to 14th April 2020 were used to train the models, data collected from 15thApril 2020 to 25th April 2020 were used to test the model and forecast was done for a period of fifteen days i.e. from 26th April 2020 up to 10th May 2020. The results of forecast indicate that the combined model, ARIMA – NAR can accurately exhibit the short term forecast.

Keywords: ARIMA; NAR; Forecasting; Timeseries; Integrated ARIMA-NAR.

INTRODUCTION

Time series modeling and analysis is one of the dynamic area of research where a lot of attention is paid across the world. Over the years, reserachers have put a lot of effort to collect and study the data rigorously to develop an appropriate model for forecasting and also to improve the efficiency of the model [1]. A time series forecasting tool can serve as an early warning system. In specific, an epidemic forecating tool assists authorities to employ preventive measures to reduce the spread of infectious diseases and adequately deploy the available resources.

Prediction of trend and future of epidemics is a difficult task as the variables differ for each outbreak and availability of genuine real time data. A pragmatic approach based on exponential smoothing was adapted by Fotios and Spyros in establishing the trend of Coronavirus disease (COVID-19)[2]. Mathematical models on epidemic forecast mostly rely on clinical surveillance, data available on the internet, human interactions and biological factors unlike weather forecast that rely on physical systems, data from satellites [3]. With the limited data available, mathematical forecasting model's conclusions vary very widely as the number of assumptions and uncertainities are more. Despite the fact that epidemic forecasts are really hard, still any forecast is anything but reliable [4], they have adapted augmentation of the data available to fine tune the data and redict the forecast of COVID-19. Wu et al., [5] have proposed a nowcast and forecast of COVID-19 based on the data of number of people affected around Wuhan and number of people migrated from Wuhan. Jia et al., [6] used three mathematical models such as Logistic model, Bertalanffy model and Gompertz model to understand and analyze the dynamics of COVID-19 pandemic and could forecast that 49852- 57447 number of people could be infected in Wuhan based on Logistic model.

Toshikazu [7] estimated the epidemic peak based on reproduction number of COVID-19 in Japan adapting SEIR compartmental model using least square method-Poisson noise. Lei et al., [8] adapted subset selection, forward selection, lasso regression, ridge regression, and elastic net methods to estimate the coefficients in social media search indexes to optimize the method based on estimation error for predicting new cases. The transmission route of COVID-19 from person to person is either through respiratory droplets or by fomites. Goh et al., [9] evaluated the transmissibility of COVID-19 adapting comptational tools to predict the intrinsic disorder predisposition of the viral proteins. In India, the first case was reported on 30th January, 2020 [10]. Statistical data provided by [10, 11] shows that by 2nd March 2020, there was a sudden increase of confirmed cases by 40% due to virus hotspotsand further on, the number of cases have been increasing alarmingly. Since the onset of symptoms of the first identified case, the first COVID-19 death was confirmed on 12th March 2020. Owing to the outbreak, The Epidemic Diseases Act, 1897 was invoked and educational institutions, commercial establishments were shutdown to avoid mass gatherings. Exit and entry thermal screening was adapted at airports, seaports and hospitals etc., [12]. A 14 hour voluntary public curfew was observed on 22 March 2020, to break the chain of transmission of disease from person to person, on the call of Mr. Narendra Modi, The Honorable Prime Minister of India. Further, a nationwide lockdown was ordered on 24th March 2020 for 21 days. People with respiratory infections and symptoms of any kind of fever were isolated so as to stop the spread of virus. Despite the stringent measures taken by authorities such as social distancing and maintaining personal hygiene, by 25th April 2020 the number of active cases, number of people recovered and number of deceased were increased and given as 19250, 5938 and 825 respectively [11, 13]. More over, there has been a steep ascent in COVID-19 cases in the sates in Maharashtra, Gujarat, Tamilnadu and Andhra Pradesh [11] which is very much alarming.

In this study, Auto Regressive Integrated Moving Average (ARIMA) and integrated Auto Regressive Integrated Moving Average – Nonlinear Autoregressive Neural Network (ARIMA– NAR) models were devised to to understand the dynamics of time series data on COVID-19 in the states of Maharashtra (MH), Gujarat (GJ), Tamilnadu (TN) and Andhra Pradesh (AP)subsequesntly perform a short term forecast to predict the progression. The number of people could get infected in the near future based on these models were compared and analyzed.

Data collection and model building

Source of data

The data used for this study on prevalence of COVID-19 were collected on a daily basis from 01st February 2020 to 25th April 2020 from the official website of WHO (https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports), Ministry of Health and Family Welfare, Government of India (https://www.mygov.in/covid-19) and https://www.covid19india.org/. The demographic view of distribution of COVID-19 cases across INDIA as

on 25th April 2020 is presented in Figure 1 [11]. Data from 14th March 2020 to 15th April 2020 were used to train the models, data collected from 16th April 2020 to 25th April 2020 were used to test the model and forecast was done for a period of fifteen days i.e. from 26th April 2020 up to 10thMay 2020.

ARIMA model

ARIMA models are in general represented as, ARIMA(p,d,q) where p, d and q stands for order of auto regression, degree of differencing and order of moving average respectively. The general ARIMA equation is given as [14],

$$\hat{Y}_t = \mu + \phi_1 Y_{t-1} + \ldots\ldots\ldots + \phi_p Y_{t-p} - \theta_1 e_{t-1} - \ldots\ldots\ldots\ldots\theta_q e_{t-q} \quad (1)$$

Where $\hat{Y}_t$ is forecasted value, μ is a constant, ϕ is slope coefficient and θ is coefficient of error terms. The series data is subjected to stationarity and seasonality to ensure the statistical equilibrium and this was done by generating a time series plot of number of people affected as shown in Figure 2(a), 2(b), 2(c) and 2(d) for the states of Maharashtra, Gujarat, Tamilnadu and Andhrapradesh respectively between the number of people affected and date (from 14th March 2020 to 25th April 2020).

The Box-Cox transformation was adapted to stationarize the data and checked for standard deviation. The non-seasonal and seasonal difference values were estimated. The autocorrelation function (ACF) and partial autocorrelation function (PACF) were depicted for the series data to confirm the parameters of auto regression and moving average, the same are presented in Table 1. Based on the ACF and PACF plots, the appropriate ARIMA model should be selected. The determination of parameters p and q can be done by conventional method using maximum likelihood estimation [15]. The values of Akaike Information Criterion (AIC) and/or Bayesian Information Criterion (BIC) for standard ARIMA models were estimated. ARIMA(1,2,2)was suitable for MH, ARIMA(1,1,0) for GJ, ARIMA(1,2,1) for TN and ARIMA(2,2,2) for AP. The goodness of models fit was checked and Ljung-Box test [16] was performed to validate the estimated results for 'White Noise' check and found False. Forecasting for the time series data was applied and also, the reverse forecast was executed for the original data and verified. The analysis of data and forecasting was done using IBM SPSS, statistical software [17].

Nonlinear Autoregressive Neural Network (NAR) model

Nonlinear Autoregressive Neural Network can model very complex non linear relationships with in the data [18]. The nonlinear autoregressive model is defined for an order p∈N . Thus, the nonlinear autoregressive model of order p is given as,

$$f(t) = F(f(t-1), f(t-2), \ldots f(t-p)) + \varepsilon(t) \quad (2)$$

Where, $f(t)$ is the present value, $f(t-i)$, i=1,2, ……,p is the ith past value of the time series, F is the non linear function and ε is the error in forecast. NAR is a feed forward network to estimate the non linear function and the feed forward algorithm is given as [19,20],

$$\hat{f}(t) = \hat{F}(f(t-1), f(t-2), \ldots\ldots, f(t-p)) \quad (3)$$

and $$\hat{f}(t) = \alpha_0 + \sum_{i-1}^{n} \alpha_i A(\beta_k + \sum_{k=1}^{p} w_{ik} f(t)) \quad (4)$$

Where, α_i , i-1,2,3,……,N are constants, A is the activation function, w_{ik} are the weights, β_k are the biases.

ARIMA-NAR model

ARIMA is a widely used model for time series data with linear pattern and models on NAR are best suitable for data with non-linear pattern. Time series data on COVID-19 is neither linear nor non-linear but contain both patterns [21]. In this regard, a mathematical model is essential to analyze both linear and non-linear patterns in the time series data. Thus, the integrated ARIMA-NAR model can handle both linear and non linear patterns with overall improved forcasting performance. The integrated model works in two stages, in the first stage ARIMA model is adapted to analyze the linear pattern and then NNAR model is adapted to analyze the non-linear pattern. The general form of integrated model is presented below [20]:
Let the timeseries has a linear and non linear structure,

$$y_i = L_i + N_t \quad (5)$$

Where, Y_i is monthly incidence, L_i is the linear patetrn and N_t is the non linear pattern at a specified time t. The forecast of time series using ARIMA is expressed as,

$$v_t = y_t - \hat{L}_t \quad (6)$$

Where, v_t is the residual from ARIMA model at time *t* and $\hat{L}_t$ is the predicted value from ARIMA. Thus, the residuals from ARIMA model were given as input to NAR model. The NAR model for the given n number of inputs and v_t residuals is given as,

$$v_t = f(v_{t-1}, v_{t-2}, ..., v_{t-n}) + e_t \quad (7)$$

Where, *f*is a non linear function as obtained from neural network. The combined prediction of the given time series is estimated as,

$$\hat{y}_t = \hat{L}_t + \hat{N}_t \quad (8)$$

Where, $\hat{y}_t$ is the final predicted value using the integrated model.

In the present study, ARIMA(1,2,2)was selected for MH, ARIMA(1,1,0) for GJ, ARIMA(1,2,1) for TN and ARIMA(2,2,2) for AP. As described in section 2.2, the standard procedure was adapted to obtain the residuals which were fed as input to NAR.The residual plots of ARIMA models for the four states are presented in Figure 3. Neural network time series tool in MATLAB was used to estimate the forecast. The given time series data contains 43 observations hence the NAR model with order 43 was chosen. 70 % of the data was used to train the model while 15% of the data was used for testing and 15% of the data was used for verification.

Analysis of forecasting

The plots of actual number of people affected till 25th April 2020 and predicted number upto 10th May 2020 for the states of MH, GJ, TN and AP are presented in Figure 4 (a), (b), (c) and (d). The consolidated values of the forecast is presented in Figure 5. Several statistics including Root of Mean Square Error (RMSE), Mean Absolute Percentage Error (MAPE) and Mean Absolute Error (MAE) for all models were calculated to examine the performance of models and presented in Table 2.To come to a better conclusion, MAE values for all models can be taken into account. It can be seen from Table 2 that ARIMA – NAR model exhibits better result as compared with ARIMA as adapted to all states.From this analysis, it can be inferred that the integrated model can perform well in forecasting than single models, as it can handle both linearity and nonlinearity of the time series data [22, 23, 24]

CONCLUSION

The present study demonstrates the analysis on trend of COVID-19 cases in the states of Maharastra, Gujarat, Tamilnadu and Andhra Pradesh. ARIMA and integrated ARIMA-NAR models were devised to perform the short term forecast. In overall, ARIMA models could be very much suitable for data which is linear in pattern and the combination of ARIMA and NAR estimates better results as it could analyze the linear and nonlinear patterns in the data.Mathematical models, such as those that forecast the spread of epidemics must overcome the challenges of integrating incomplete and inaccurate data in computer simulations, estimating the probability of multiple possible scenarios, incorporating changes in human behavior and/or the pathogen, and environmental factors.

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Table 1: ACF and PACF plots for ARIMA models

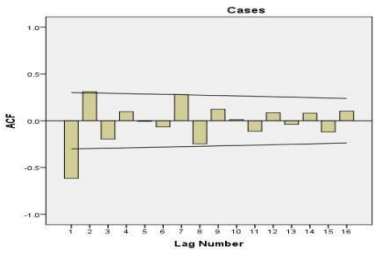
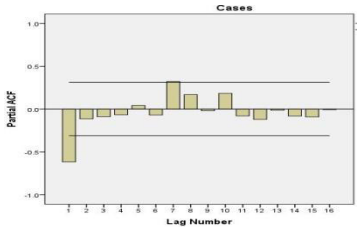
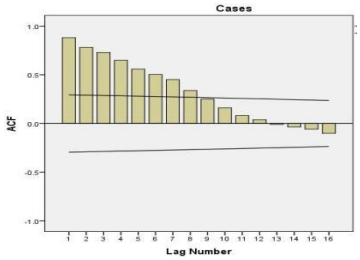
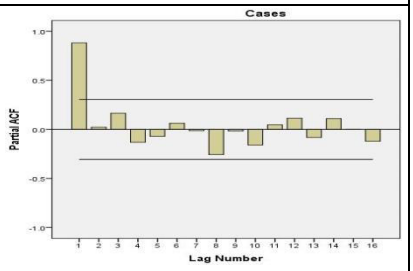
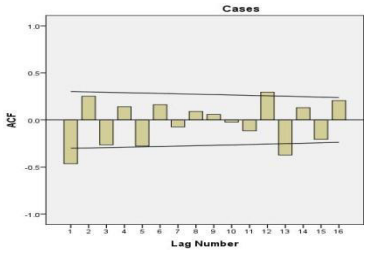
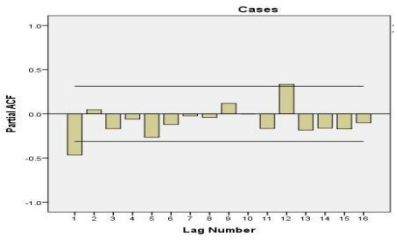
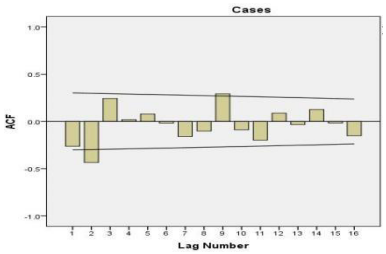
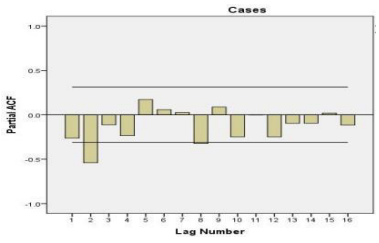
State	Model	ACF	PACF
Maharastra	ARIMA(1,2,2)		
Gujarat	ARIMA(1,1,0)		
Tamilnadu	ARIMA(1,2,1)		
Andhra Pradesh	ARIMA(2,2,2)		

Table 2: Results of statistics

State	Model	RMSE	MAPE	MAE
Maharastra	ARIMA(1,2,2)	91.829	15.699	57.916
	ARIMA – NAR	89.634	13.989	55.903
Gujarat	ARIMA(1,1,0)	40.932	13.714	23.138
	ARIMA – NAR	38.574	12.659	22.471
Tamilnadu	ARIMA(1,2,1)	25.336	20.743	15.489
	ARIMA – NAR	24.293	18.569	13.273
Andhra Pradesh	ARIMA(2,2,2)	15.013	36.601	10.297
	ARIMA – NAR	14.351	33.476	9.479

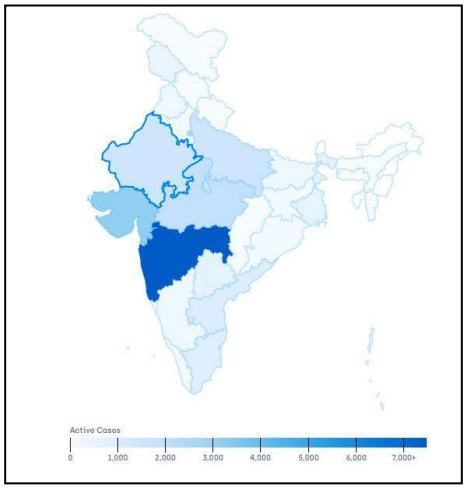


Fig 1. Demographic view of number of people affected [11]

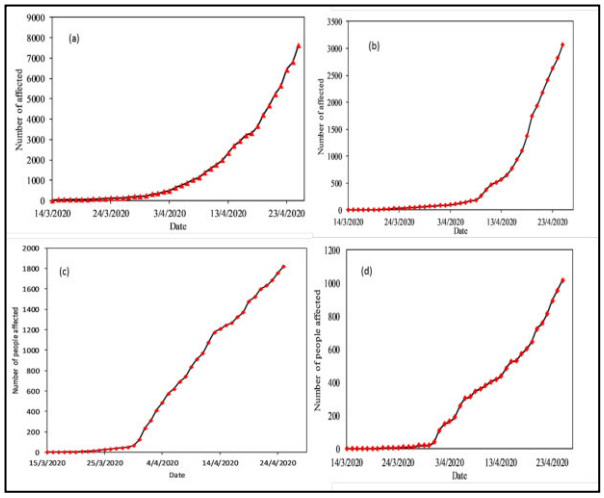


Fig 2. Time series plot of number of people affected in the states of (a) Maharastra, (b) Gujarat, (c) Tamilnadu and (d) Andhra Pradesh

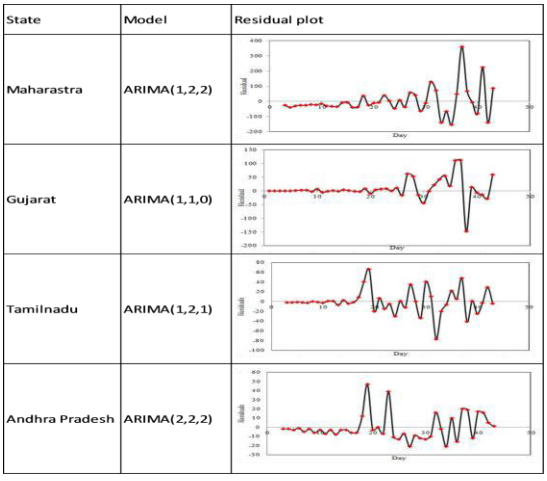


Fig 3: Residual plots from ARIMA models

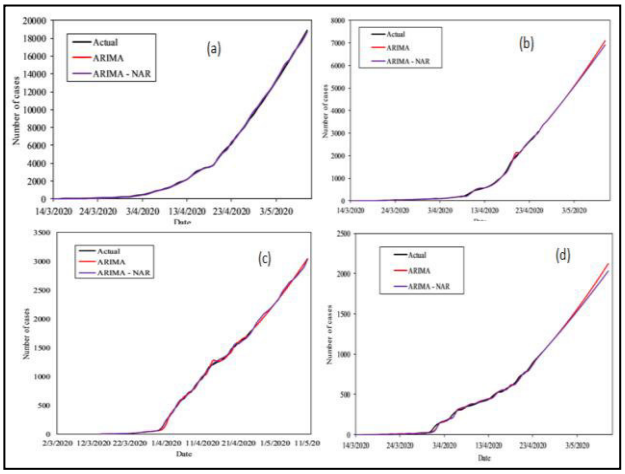


Fig 4: Actual and forecast values for the states of (a) Maharastra, (b) Gujarat, (c) Tamilnadu and (d) Andhra Pradesh

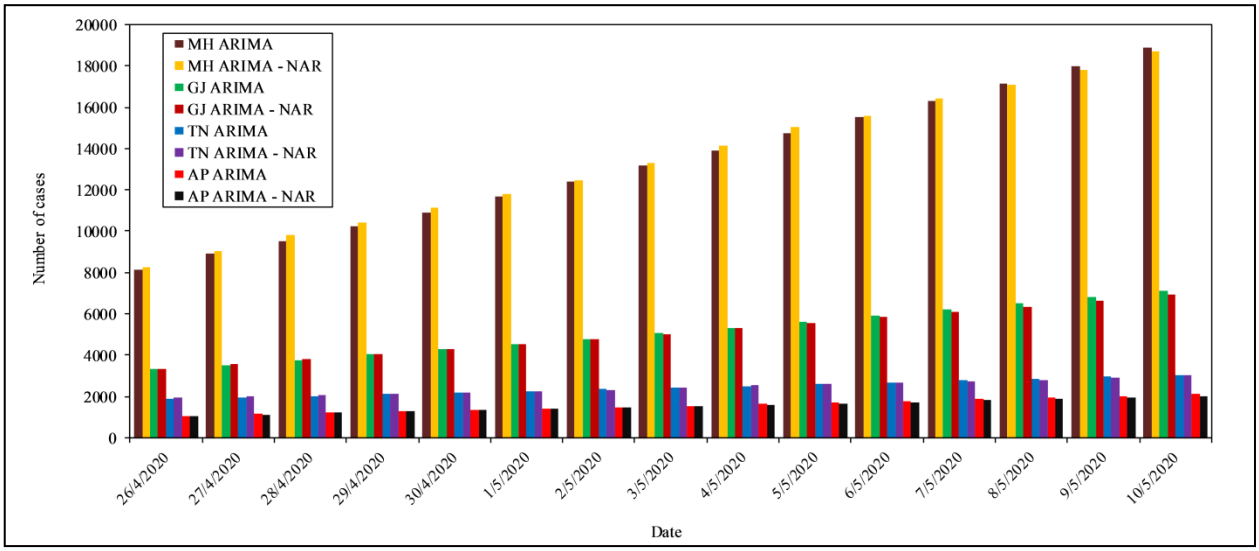


Fig. 5. Consolidated forecast values for fifteen days for the states of Maharastra, Gujarat, Tamilnadu and Andhra Pradesh using ARIMA and ARIMA-NAR

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Effect of Cyclophosphamide on Oxidative Stress Markers in the Mouse Granulosa Cells *In vitro*

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Received: 01 Feb 2020 Revised: 02 Mar 2020 Accepted: 04 May 2020

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ABSTRACT

The actively proliferating granulosa cells are the major targets of cyclophosphamide-induced toxicity in the ovary. Though *in vitro* studies reported cyclophosphamide-induced oxidative stress in the granulosa cells as shown by a reduction in glutathione content and an increase in concentration of reactive oxygen species, it is not known whether cyclophosphamide affects the activities of cellular anti-oxidant enzymes and causes lipid peroxidation by increased reactive oxygen species production. In the present study, incubation of the granulosa cells isolated from the ovaries of normal adult female mice with metabolically activated cyclophosphamide for 2 hours in DMEM/Fl2 medium, resulted in a significant dose-dependent decrease in the activities of anti-oxidant enzymes viz., super oxide dismutase and catalase, and a dose-dependent increase in concentrations of reactive oxygen species and malondialdehyde. The results, for the first time, provide a strong and direct evidence that cyclophosphamide toxicity of the granulosa cells involves a compromise in anti-oxidant defense of granulosa cells due to loss of anti-oxidant enzyme activities and the excess generation of reactive oxygen species, which results in membrane lipid peroxidation.

Keywords: granulosa cell, cyclophosphamide, oxidative stress, lipid peroxidation, *in vitro*.

INTRODUCTION

Antineoplastic, alkylating drugs like cyclophosphamide (CP) cause temporary [1] or permanent [2] amenorrhea in young women treated for cancer and autoimmune diseases. Though menstrual cycles are resumed in these patients,

they might suffer from premature ovarian failure due to depletion of primordial follicle reserve. Studies in animal models report that cyclophosphamide causes premature failure by destroying ovarian follicles [3-13]. It is shown that in mice, primordial follicles are more sensitive to the CP toxicity [4,6-9] where as in rat secondary and antral follicles are more affected[12,14]. However in both mice and rat, CP targets the granulosa cells of large growing and antral follicles [7,8,14-17].

Oxidative stress has been implicated as the mechanism of CP-induced granulosa cell toxicity [18]. Oxidative damage occurs when the delicate balance between the intracellular levels of reactive oxygen species (ROS) and endogenous antioxidants is disrupted. An increased production of ROS and reactive nitrogen species (RNS) can cause oxidative damage to cellular macromolecules (lipids, proteins, and nucleic acids), often leading to cell death. The results of the *in vivo* animal experiments show evidences for CP-induced oxidative stress in the ovary. CP treatment caused an increase in concentration of RNS, nitric oxide[19]and decline in the activity of ovarian anti-oxidant enzyme, superoxide oxide dismutase (SOD) [12,19-23] and concentration of anti-oxidant, glutathione (GSH)[12,21,23-25].When ROS and RNS are not detoxified by endogenous or exogenous antioxidant molecules, these convert unsaturated lipids to peroxides, producing toxic aldehydes like malondialdehyde (MDA) as byproduct. MDA isthe product of lipid peroxidation as well as a marker of oxidative stress. CP treatment causes an increase in concentration of MDA in the rat ovary [12,19-21,23]. Further, CP treatment enhanced the production of cleaved caspase-3 [13,21], apoptosis of granulosa cells [24,26,27] and follicles[11,13] and DNA [28] damage in the ovaries of mice or rat.

In vitro studies help elucidating the mechanisms of cytotoxicity [5]. In rat granulosa cells, CP caused a dose-dependent reduction in the viability, survival, progesterone and estradiol accumulation [5,29] *in vitro*. However, the mechanism of CP-induced damage to the structure and function of ovarian granulosa cells was poorly described in these studies. CP also causes dose-dependent [24] increase in apoptosis of the rat granulosa cells [24] and mouse [16] ovaries following *in vivo* [24] and *in vitro* [16] treatments. The studies, though showed apoptosis of ovarian cells, following CP treatment, the biochemical alterations underlying apoptotic cell death were not revealed. As shown in the *in vivo* experiments described above, CP exposure caused a sudden depletion of anti-oxidant GSH level, increase in reactive oxygen species (ROS), oxidative DNA damage, activation of caspase 3 and apoptosis in human granulosa cell lines (COV434) [41] *in vitro*. However, it is not known whether CP causes any alterations in the activities of endogenous anti-oxidant enzymes, or whether it causes membrane lipid-peroxidation in the granulosa cells of the ovary, *in vitro*. Therefore, the present study was undertaken to find out whether CP induces alterations in the activities of anti-oxidant enzymes and lipid peroxidation in the granulosa cells *in vitro*, to provide a direct evidence for CP-induced oxidative stress in the granulosa cells of mouse.

MATERIALS AND METHODS

The ovaries for harvesting the granulosa cells were collected from normal healthy adult female mice.

Isolation of the granulosa cells (GCs)

The GCs were isolated from the ovaries using the method of Campbell[30], modified by Zareifard*et al*[31]. The ovaries were transferred to DMEM/Fl2 medium containing in 6.8 mM EGTA and 0.2% bovine serum albumin. Using a set of fine needles, the ovaries were gently punctured and the GCs were allowed to ooze out into the medium. The debris were removed and the suspension was incubated at 37º C for 15 minutes. After incubation, the suspension was transferred into a tube and centrifuged for 5 minutes at 100g. The resultant pellet was re-suspended in hypertonic sucrose solution (0.5 M sucrose, 0.2% BSA and 1.8 mM EDTA dissolved in DMEM/Fl2 medium) and incubated for another 5 minutes. After incubating in sucrose solution, the suspension was diluted with 3 volumes of DMEM/Fl2 media and centrifuged again for 5 minutes at 100g. The pellet was re-suspended in DMEM/Fl2 media.

Cell viability assay

Trypan blue test was conducted to determine the viability of the isolated GCs. The cell suspension in DMEM/Fl2 medium was mixed with an equal volume of 0.2% trypan blue in DMEM/Fl2. It was allowed to stand for 5 minutes at room temperature. Then, 10 μL of this mixture was placed on a Neubaur's chamber and the total number of cells was counted. The % of viable cells was calculated by the following formula;
% viable cells = [1.00 – (Number of blue cells ÷ Number of total cells)] × 100.

Preparation of S-9 fraction

The S-9 fraction was prepared from the liver of adult female Swiss albino mouse following the method described by Manson and Simons [32] to be used for metabolic activation of CP and formation of its active metabolites [33]. The liver tissue was homogenized in 0.05 M Tris-KC1 buffer of pH 7.4 (1:4 w/v) containing 0.14 M HCl. The homogenate was centrifuged (-4ºC) twice at 9000g for 15 minutes. The supernatant obtained is termed S-9 fraction. The S-9 fraction was stored at -70ºC until used.

Preparation of S-9 mixture

The S-9 mixture was prepared by adding several cofactors to the S-9 fraction. The S-9 fraction was mixed with 0.4 M $MgCl_2$, 1.65 M KCl, 1 M glucose 6-phosphate and 0.5 M NADPH in 0.1 M phosphate buffer (pH 7.4) to make S-9 mixture containing 10% S-9 fraction[34]. The S-9 mixture was further diluted with phosphate buffered saline (PBS) to get 1% S-9 fraction in the mixture. One percent concentration of S-9 fraction maintains sufficient activity of S9 to generate toxic metabolites of CP [35]. This concentrationof S9 is typically used for the activation of CP.

Metabolic activation of CP

Different concentrations of CP (10 μM, 100 μM and 1 mM) were incubated with 1% S9 mixture at 37°C for 30 minutes [34, 36] for activation (Table 1). The 30 minutes incubation period was set because the concentration of acrolein, a metabolite of CP formed, in presence of liver microsomes was found to be decreasing after 1 h of incubation [36]. In addition, earlier studies show that the metabolite of CP, formed by the activation using liver S-9 is stable for ~50 min [34].

Determination of S-9 concentration for incubating GCs with CP

The S-9 mixture could induce cytotoxicity and limit its effectiveness as metabolizing system in *in vitro* cultures [32]. Hence, for subsequent assays that involve incubation of GCs with activated CP, 1% S-9 concentration might be inappropriate. Therefore, a non-cytotoxic concentration of S9 with reference to GCs, was identified for conducting further *in vitro* assays. GCs suspended in culture media (2 X 10^5 cells, 88-92% viability) were exposed to S9 alone at 37°C in 5% CO_2, 95% air for 2 h at concentrations ranging from 0.02 to 0.2%. The culture medium used was DMEM/Fl2 supplemented with penicillin (31 μg/ml) and streptomycin (50 μg/ml). Trypan blue exclusion test was conducted to determine the cell viability. The dose (0.12%) that showed maximum GC viability (Figure 1) was chosen for treating GCs.

Treatment of the GCs with activated CP

The reaction mixture (CP+S-9 mixture) containing different concentrations of activated CP (Table 1) was added to separate tubes containing GCs suspended in culture medium (2 X 10^5 cells, 88-92% viability) supplemented with penicillin and streptomycin, after adjusting the final concentration of S9 to 0.12% (Table 2). GCs suspended in

medium without CP served as controls (Table 2). Tubes containing GCs in medium and 0.12% mixture was also maintained to find the effect of S-9 on these cells, if any (Table 2). All the tubes were incubated at 37°C in 5% CO_2, 95% air for 2 h. After incubation, the suspension was centrifuged for 5 minutes at 100g. The pellet was re-suspended in media, centrifuged again and the cell pellet obtained was homogenized in 0.32 M sucrose solution. The resultant suspension was centrifuged and the supernatant was used to determine activities of SOD, catalase (CAT) and the concentrations of MDA and ROS.

Biochemical assays

The activities of SOD and CAT in the GCs after incubation were determined following the procedures described by Marklund and Marklund [37] and Aebi [38] respectively. The lipid peroxidation in the GCs was measured by estimating the concentration of MDA [39] whereas the ROS concentration in the GCs was determined by fluorometric assay described by Black and Brandt [40] using DCF-DA as a substrate.

Statistical analysis

The values of each parameter were expressed as mean ± SE. One way ANOVA followed by Duncan's multiple range test were used to determine significant difference among mean values of different treatment groups.

RESULTS AND DISCUSSION

The activities of SOD and CAT in the GCs *in vitro* showed a dose-dependent significant decrease and the concentrations of ROS and MDA showed a significant dose-dependent increase compared to the controls, following exposure to CP (Table 3). The GCs treated with S-9 alone did not show any significant difference in these parameters compared to the controls. Chemotherapy-induced premature ovarian failure in the women of reproductive age is a growing concern in recent years. While chemotherapy is an inevitable priority in female patients undergoing cancer treatment, attempts to rescue the ovary from damage using adjunct therapies bring hope to the survivors. However, it is necessary to understand the mechanism by which the drug induces toxicity in the normal untargeted cells, to rescue them from the damage, without compromising the therapeutic efficacy of the drug. In the present study, the effects of CP on the mouse granulosa cells *in vitro* was studied to understand whether CP induces oxidative stress, as oxidative stress is known to cause apoptosis of the GCs[24].

Anti-oxidant enzyme SOD detoxifies ROS, converting them into water and H_2O_2, whereas CAT converts the peroxides to water. The coordinated defense action of the cellular anti-oxidant enzymes neutralize ROS and provides an optimal oxidative environment for the normal functioning of the cell [18]. An imbalance between the production of free radicals and resistance by anti-oxidant system leads to oxidative stress in a cell. In the present study, there was a significant increase in the production of ROS by the GCs following 2 h of CP exposure. Further, in these cells, the increased production of ROS resulted in an increase in lipid peroxidation, as indicated by a significant increase in MDA concentration, one of the reliable markers of oxidative stress. However, the activities of anti-oxidant enzymes remained low in these cells, indicating a compromised defense system. The results are in agreement with earlier report that CP treatment interferes with anti-oxidant defense as shown by a dose-dependent depletion of GSH, a cellular anti-oxidant and rise in ROS concentration in the granulosa cells [41]. However our results provide direct evidence for inhibition of anti-oxidant enzyme activities, as a cause for oxidative stress in the granulosa cells, as shown by an increase in MDA concentration, a product of membrane lipid peroxidation, an indicator of cell damage.

In vivo animal studies have also shown that CP treatment reduces cell function [5,29], causes apoptosis, [16,24], proliferation inhibition [28] and senescence [42] of the GCs. The findings of the present study provide direct evidence for CP-induced oxidative stress for granulosa cell damage.

Conflict of interests

The authors report no conflicts of interest.

Ethical consent

The experimental protocols were approved by the Institutional animal ethics committee (UOM/IAEC/09/2016) and the guidelines of the committee were followed for the maintenance of the animals.

Acknowledgements

The authors acknowledge University Grants Commission for financial assistance and the University of Mysore and the Institute of Excellence, University of Mysore for providing the facilities for work.

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Table 1: Protocol for Incubation of a Low, Medium and High Dose of Cyclophosphamide with S-9 Mixture for Metabolic Activation.

Tubes	S9 mixture (1%) diluted in PBS	PBS solution	CP dissolved in PBS	
Control	-	+	-	Incubated for 30 minutes at 37ºC
S9	+	-	-	
CP – low dose	+	-	10 µM	
CP- mid-dose	+	-	100 µM	
CP- high dose	+	-	1mM	

CP= cyclophosphamide, PBS= phosphate buffered saline, S-9= S-9 fraction

Table 2: Protocol for *In vitro* Incubation of the Isolated Granulosa Cells with Metabolically Activated Cyclophosphamide at Different Concentrations.

Tubes	Granulosa cells suspended in culture media	Reaction mixture CP + S-9 fraction	S9 fraction (0.12%) alone	
Control	+	-	-	Incubated for 2 h at 37ºC, 5% CO_2
S9	+	-	+	
CP – 10µM	+	+	-	
CP- 100µM	+	+	-	
CP- 1mM	+	+	-	

CP= cyclophosphamide, S-9= S-9 fraction

Table 3: Effect of Cyclophosphamide (CP) on the Activities of Anti-Oxidant Enzymes and Concentrations of Malondialdehyde and Reactive Oxygen Species in the Gcs *In vitro*

	Activity of anti-oxidant enzymes		Concentration of	
Groups and Treatment	**SOD (U/mg protein)**	**CAT (nmol/mg/min)**	**MDA (nmol/mg)**	**ROS (DCF/mg protein)**
Control	2817± 118^{a}	0.145 ± .040^{a}	82.8 ± 12.0^{a}	0.115 ± 0.004^{a}
S-9	2763 ± 243^{a}	0.179 ± 0.07^{a}	75.0 ± 11.5^{a}	0.110 ± 0.015^{a}
CP (10 µM)	1855 ± 144^{ab}	0.048 ± 0.009^{b}	208.6 ± 51.0^{b}	0.162 ± 0.021^{ab}
CP (100 µM)	1628 ± 39^{b}	0.031± 0.004^{b}	200.1 ± 14.4^{b}	0.221 ± 0.059^{bc}
CP (1 mM)	1133 ± 130^{c}	0.020 ± 0.001^{b}	214.1 ± 39.0^{b}	0.285 ± 0.017^{c}
ANOVA, F-value (30,4)	**24.00 P <0.05**	**2.80 P<0.05**	**6.32 P <0.05**	**4.41 P <0.05**

Note: All values are mean ± SE.Mean values in the given column with same superscript letters do not significantly differ whereas those with different super script letters differ significantly ($p < 0.05$) as judged by Duncan's test.CAT = catalase, MDA= malondialdehyde, ROS= reactive oxygen species, SOD= superoxide dismutase.

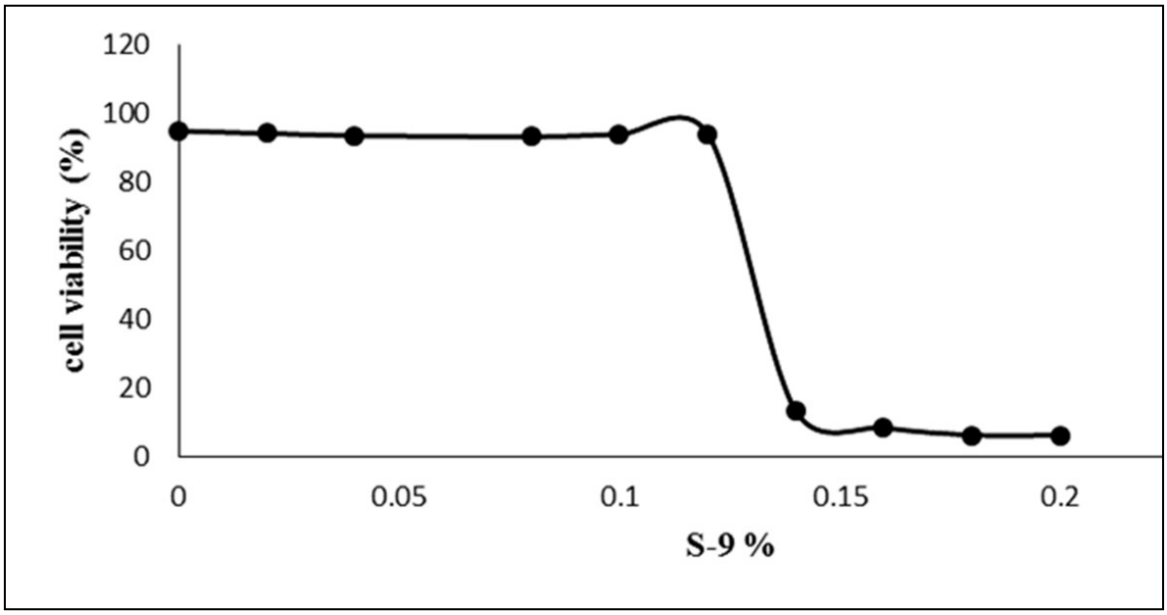

Figure 1. Effect of S-9 on the granulosa cells at 2 h incubation

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

In silico Molecular Docking Reveals the Interaction of Resveratrol with the SARS-CoV-2 Main Protease

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Received: 12 Feb 2020 Revised: 14 Mar 2020 Accepted: 16 Apr 2020

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ABSTRACT

2019 Novel corona-virus (2019-nCoV) also referred to as severe acute respiratory syndrome Corona-virus 2 (SARS-CoV-2) emerged as a global risk and put the entire globe into unrest. Unavailability of specific drug against the virus is more imperative. This challenging situation requires development of biomolecules for efficient treatment against severe acute SARS-CoV-2. The crystal structure of SARS-CoV-2 main protease (M^{pro}) has been released, thus can be used for fast *in silico* docking. This may result into identification of active biomolecules primarily phytochemical. *In silico* Molecular Docking revealed that the phytochemical, Resveratrol effectively binds at the active pocket of the SARS-CoV-2 main protease.

Keywords: 2019-nCoV, SARS-CoV-2, SARS-CoV-2 main protease, docking, phytochemicals.

INTRODUCTION

The pandemic situation caused due to the 2019-nCoV represents a severe public health calamity across the globe. The city of Wuhan was the epicentre where the outbreak of this human pathogen emerged, and resulted to human ailment, termed as COVID-19 [1, 2]. SARS-CoV-2 belongs to the beta corona-virus genus, closely related to the previously identified severe acute respiratory syndrome corona-virus (SARS-CoV) [3, 4]. Public Health Emergency of International Concern (PHEIC) was declared by the World Health Organization (WHO) owing to its fast rate of transmission within the humans [1, 5, 6]. Crystal structure of the SARS-CoV-2 main protease (M^{pro}) proves to be an exceptional ground for screening specific ligands [7]. SARS-CoV-2 main protease can be beleaguered for developing

antibodies, diagnostics and vaccines. Reportedly, M^{pro} and other known viral proteins are defining features paving the path of virus from entry to infection in the host cell [8, 9, 10]. Moreover, M^{pro} can also be an effectual target to diminish the viral replications within the host cells since it facilitates the synthesis of functional viral proteins. The effectiveness of traditional medications on the restriction of COVID-19 growth does not have any scientific back up as of now, since the underlying molecular mechanisms are unclear. The phytochemicals are fundamentally bioactive compounds and has the potential to amend cellular physiology. Here, we report that Resveratrol, a phytochemical mostly enriched in some selected plants binds into the active site of the SARS-CoV-2 main protease as revealed by the *in silico* molecular docking and thus further studies may reveal the effectiveness of resveratrol to be used as COVID-19 therapeutics.

METHODS

Viral Protein Structure and Phytochemical dataset collection

The 3D structure of M^{pro} was accessed from Protein Data Bank accession 6M03. The SDF accession CHEBI:45713 corresponding to the Resveratrol was obtained and consequently both the protein and the ligands were used for *In silico* analysis.

Molecular docking

For the *in silico* molecular docking, BIOVIA's Discovery Studio docking method [11] was used for molecular docking. The catalytic pocket of the M^{pro} protein was specified and targeted for binding of the ligand. CDOCKER Energy and CDOCKER Interaction Energy signify the affinity of the ligands with the protein receptors. Basically, high positive values of the CDOCKER Energy, CDOCKER Interaction Energy and a diminutive difference between the CDOCKER Energy and CDOCKER Interaction Energy are considered to be the most favourable [12].

RESULTS AND DISCUSSION

It was found that resveratrol (Fig. 2), a common phytochemical specifically binds to the active pocket of the SARS-CoV-2 M^{pro} (Fig. 3), as apparent from higher CDOCKER energy and CDOCKER interaction energy. Since, simple active bio molecule like resveratrol effectively binds into the active pocket of the M^{pro} under *in silico* conditions it is quite possible to design pharmacophore molecules based on the structural and functional identity of resveratrol and eventually can be used in the pharmaceutical sector. Chemical synthesis of resveratrol can be cost effective as compared to the isolation process from specific plants.

Conclusion and Future perspectives

The current *in silico* molecular docking based study reveals that resveratrol can target the reported SARS-CoV-2 M^{pro} (Fig. 4). It would be extremely noteworthy being confirmed *in vivo*. It is crucial to develop diagnostic tools, potential therapeutics and antibodies selectively for the COVID-19 proteins. Phytochemicals like resveratrol is commercially available and thus may be effectively prescribed to circumvent the current global scenario. Essentially, this study makes an attempt to reveal simple phytochemicals like resveratrol which can be employed for designing novel therapeutics.

Author contribution statement

KBS, GKP and AS conceived the idea. GKP, AS, SKS, NN performed the experiments. KBS, GKP and AS analyzed the data. All authors have significant contribution in drafting the manuscript.

Funding

The present study was financially supported by Centurion University of Technology and Management, Odisha, India.

ACKNOWLEDGEMENTS

Authors are thankful to the administration and management of Centurion University of Technology and Management, Odisha, India for providing necessary facilities to conduct the experiment.

Conflict of interest

The authors declare that they have no conflict of interest.

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Table 1: CDOCKER ENERGY and CDOCKER INTERACTION ENERGY values generated for the interaction of Resveratrol with the active site of SARS-CoV-2 main protease (M^{pro}).

Ligand		Receptor		Interaction Status		
SDF accession	Phytochemical	Protein	PDB accession	Docking Result	CDOCKER ENERGY	CDOCKER INTERACTION ENERGY
CHEBI:45713	Resveratrol	COVID-19 Main Protease	6M03	POSITIVE	13.04	25.59

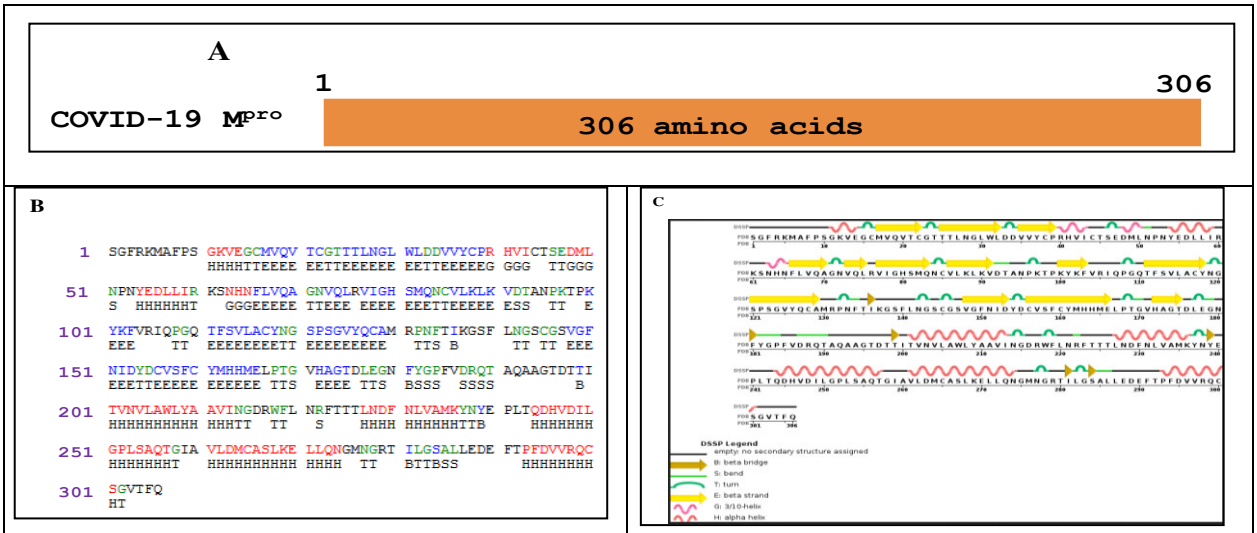

Fig. 1: Amino acid sequence of COVID-19 M^{pro}. (A) M^{pro} contains 306 amino acids. (B) Sequence and Define Secondary Structure of Proteins (DSSP) image of COVID-19 M^{pro}. (C) Sequence chain image of COVID-19 M^{pro}. Images 1(B) and 1(C) were generated from the Protein Data Bank.

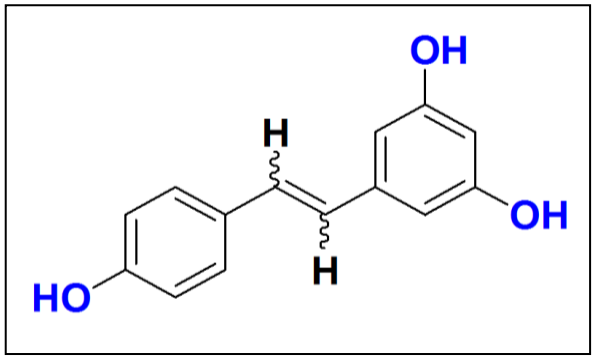

Fig. 2: Chemical structure of Resveratrol

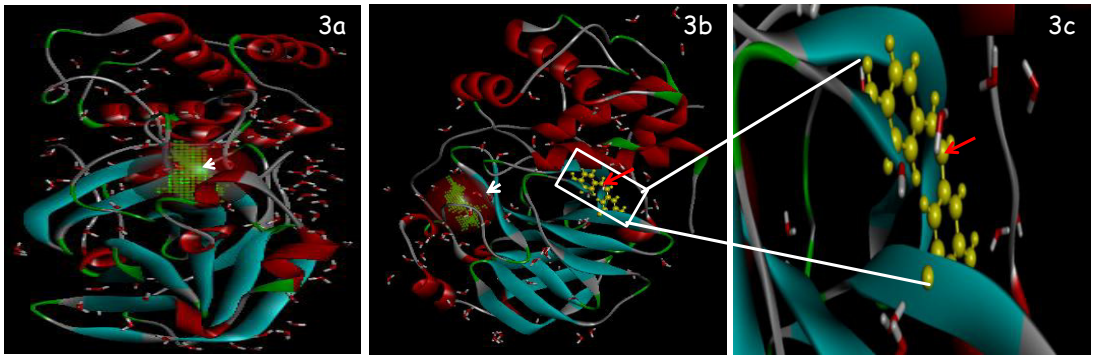

Fig. 3: The active site of the SARS-CoV-2 main protease (M^{pro}) interacts with Resveratrol. 3a: Free form of M^{pro}. 3b: M^{pro} associated with the ligand, resveratrol. 3c: Magnified image showing the association of the resveratrol with the M^{pro}. (The white coloured arrow and the red coloured arrow indicate the active site of the M^{pro} and binding of resveratrol respectively).

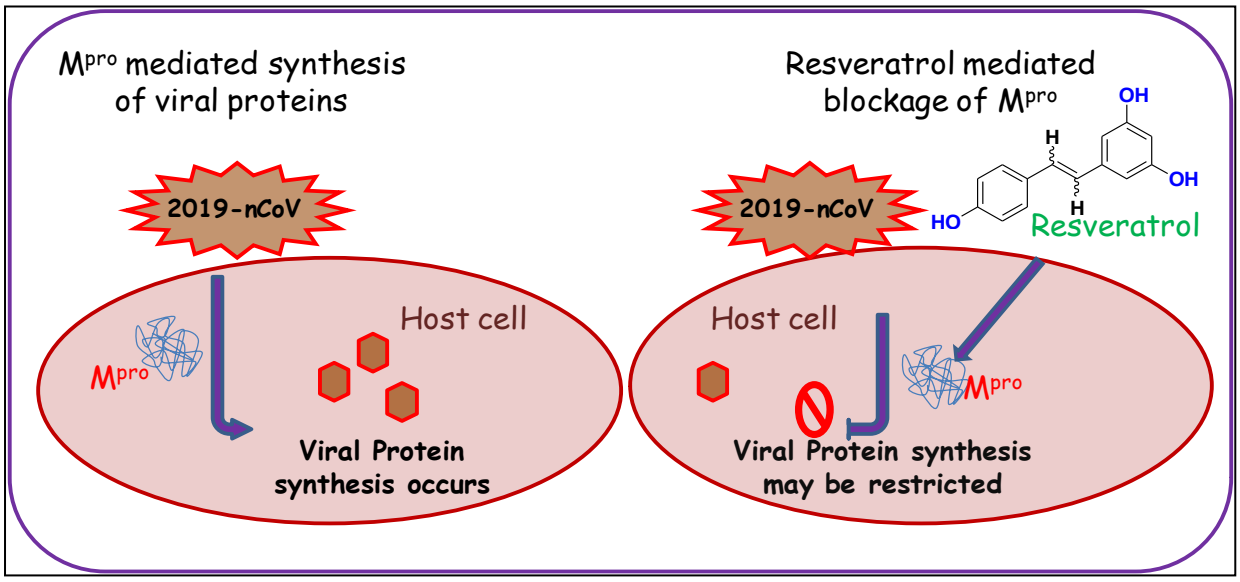

Fig. 4: Resveratrol, a phytochemical may inhibit COVID-19 Mpro and thus restrict the synthesis of viral proteins

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

The Challenge of Producing Methane from Gas Hydrate

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Received: 02 Mar 2020 Revised: 04 Apr 2020 Accepted: 06 May 2020

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ABSTRACT

A large amount of gas hydrates has been explored by many countries and it is widespread. Govt. and private research agencies are helping them for more research and development. But the main challenge is production and exploitation of methane in a safely, methodically and economically. But the major concern is dissociation mechanism of gas hydrates in different environment. As production is concerned, depressurization is a main method for separation of methane from gas hydrates in hydrocarbon sector. Rock properties like permeability play a significant role in enhancing gas production. Form of tight and low permeability directly proportional to the low gas volume. The successful permeability of hydrate-bearing sediments ultimately depends on both porosity and saturation of the hydrate. Compaction of sediments and loads from outside increase during depressurization. Analysis of hydrate gas production is due to the volume changes in formation in response to earth stress.

Keywords: Clathrate, Depressurization, Geo-mechanics, Heterogeneity, In situ stress, Gas Hydrate

INTRODUCTION

Gas hydrates are solids (mostly methane) consisting of water and natural gas. This was first discovered in the oil industry when Solid hydrate was used to block the transmission lines of natural gas in the 1930s. Vasil'ev et al (1970) first acknowledged that gas could form gas hydrates. Naturally occurring methane hydrate was found in Siberian gas reservoirs (Polar Regions of Northern Russia – Makogen et al, 1971, 1981) The first recorded recovery of sediment gas hydrate was found in 1972 using a pressure-core barrel at Arco-Exxon wildcat well on Alaska's northern slope.

First direct observation of gas hydrate was possible by describing gas hydrate crystals found in near surface sediments of the Black sea (1974).

Structure and Formation of Gas Hydrates

Natural gas molecules are entrapped in ice at lower temperature and form an ice like solid structure. (Kvenvolden 1993; Sloan 2000). These are called as gas hydrates and metastable complex in nature. Basically gas hydrates are clathrates,(Sloan, 1990). It is a chemical complex that is formed when one type of molecule completely encloses another type of molecule in a lattice.(Kvenvolden and McMenamin, 1980). Typically speaking, gas hydrates are non-stochiometric crystalline solids composed of hydrocarbon gases trapped within the cavities of a rigid water molecules "cage-like" lattice. These compounds contain clusters (two or more) of gas trapping polyhedra formed by hydrogen-bonded water molecules arranged pentagonally and hexagonally (Figure-2). Van der Waals interactions between the trapped "guest" molecule and the surrounding water cage walls stabilize and help the individual polyhedra forming the hydrate lattice and limit the guest molecule's translational motion. (The Buffet, 2000).

There are typically three types of hydrate structures based on the geometries of their constituent water cages: cubic structures I and II and hexagonal structure H. Each crystalline structure includes geometrically distinct water cages with different size cavities that usually only handle one guest molecule ranging from 0.40-0.90 nm in diameter. (Sloan, 2003) Structure I(sI) hydrates are the most frequently found natural hydrate structure that encompasses small molecules of diameter (0.40-0.55 nm) such as methane or ethane gas.(Sloan, 2003) Structures II (sII) and H (sH) hydrates tolerate larger guest molecules, usually propane or iso-butane for sII or methane gas and nexohexane or cycloheptane for sH varieties, but are less prevalent in nature.The unit cell for sI hydrates consists of 46 water molecules arranged in two small dodecaedral cages (each with twelve pentagonal faces) and six large tetradecaedral cages (each with two hexagonal and twelve pentagonal faces) (see Figure-3). (Sloan et. Al, 2008) The ideal molar guest to water ratio for sI hydrate is 1:5.75, assuming full occupancy.

Here, water molecules bonded with hydrogen form a cage-like structure in which the mobile gas molecules are absorbed or bound. Makogon, et al. [1997] stated in particular that 164.6 m3 of methane was produced from one cubic meter of gas hydrate. Mathematically 924 ft3 of methane is equal to one barrel of gas hydrate and is about six times more gas than the gas found in an unimpeded gas-filled pore system [Selley, 1998, pg, 25]. Volumetrically, 20 percent of the gas is believed to be in gas hydrates and 80 percent remaining to be consumed by vapor. Basically Gas hydrates reserves can be found throughout the world [Selley, 1998; Makogon, et al., 1997]. Their occurrence is on land in sub-Arctic sediments and in offshore on seabeds.(e.g. Kvenvolden 1993; Holbrook et al. 1996; Sloan 1997; Ginsburg and Soloviev 1998; Collett et al. 1999; Collett and Ladd 2000; Milkov and Sassen 2001; Collett 2002) Here water depth is 600ft to 1500ft and water is near freezing. Sea floor temperature varies from 39°F in the Gulf of Mexico and 30°F in some sections of the North Sea for formation of gas hydrates, according to Makogon, et al. [1997], over 700 trillion m3 in explored reserves of methane in the hydrate state exist. Difficulties in cost-effective production have hampered development of the resource.

Challenges in Gas recovery

It is almost a challenging issue to produce methane from methane hydrate. Methane is embedded in a solid form and going to deposit in deep marine and arctic environments. Indeed it is a challenging issues as tectno-stratigraphic is concerned. Studies from geological, geochemical and field studies it was found that methane hydrate could also occur in multi structural setup and different reservoir settings. As petroleum system is concerned first primary reservoir controls like (a) concentration and form of the methane hydrate occurrence, (b) physical properties of host rock (e.g., thickness, porosity,permeability, thermal properties, *in situ* stress, and strength), (c) physical properties of overlying and underlying sediments, (d) pressure and temperature environment, (e) non-uniform conditions such as geologic heterogeneity or possible communication with open faults or fractures, and (f) presence of free gas and/or

free water zones above, below, or within the methane hydrate occurrence. It is important to know the geochemical properties of methane hydrate bearing sediments for exploration and production activities in a safe manner. Its inherent quality is to predict changes in these properties during methane hydrate dissociation and after also. As far as the ecosystem is concerned, there are many problems with the development of gas hydrates, such as the processing of extracted water, possible effects of marine deposits on the seafloor or subsurface, and encounters with permafrost in the case of Arctic deposits.

METHODS FOR EXTRACTION OF GAS HYDRATES

Gas hydrates are one of the unconventional types of energy resources that is increasingly considered as potential energy resource. As per as production is concerned methane extraction from gas hydrates typically based upon conventional hydrocarbon completion and production methods. These three primary methane hydrate production technologies are (1) Depressurization, (2) Thermal stimulation, and (3) Chemical stimulation. But each one has some draw backs. The main objective is to manipulate the *in situ* stability characteristics of the methane hydrate and induce in-place break down to flow free gas and associated hydrate-bound pore water. For each of these methods it has been discussed in more detail below. If it will consider Indian exploration and production history to worldwide, production testing of methane hydrate is very limited. Makogon (1981) indicated that the Messoyakha natural gas field in northern Siberia may have been caught up in methane hydrate and that dissociation of methane hydrate might characterize the development history of this field as the the pressure of the free-gas reservoir with time. Although this interpretation was posed by (Collett and Ginsburg, 1998), and the inadequacy of field evidence to validate the initial in situ conditions or the accurate response to output significantly restricts any modern assessment of engineering. The only other full-scale study of methane hydrate production to be undertaken was in the Mackenzie Delta, Mallik field.In 2002, a consortium of five countries conducted a thermal stimulation test at Mallik, including participation by DOE (Dallimore and Collett, 2005). In 2007 a Canadian-Japanese research program also conducted full-scale depressurization experiments at the site.

Depressurization Technique

The depressurization technique is one of the efficient ways of production techniques adapted by many researchers. It is seen as one of the most cost-effective and practical method of production (Max et al., 2006). Here the in situ pressure of the liquids in the porous rocks in contact with the methane hydrate reservoir is mainly reduced. This process can also be used by changing the pressure regime of the methane hydrate reservoir itself, or by reducing the pressure of the overlying or underlying sedimentary rocks in contact with the methane hydrate reservoir and transferring this change of pressure to the reservoir. The effectiveness of this method is greatly affected by the way the methane hydrate exists (i.e., distributed within sediment or in large form) and the abundance and interconnectivity of the liquid porous water that helps to transmit the decreased in pressure.

Thermal Stimulation

In the thermal stimulation method the initial idea is to raise the in situ temperature of the methane hydrate reservoir above the level of stability of the pressure-temperature. The only full-scale thermal stimulation test at Mallik site was performed in 2002. During this process, hot brine was circulated over a 13-meter perforated test period, depending primarily on heat conduction to dissociate methane hydrate into the formation (see Dallimore and Collett, 2005). During the 124-hour thermal test approximately 500 m3 of gas was recovered.This low volume of gas recovery proposes that this technique's rather limited potential as a primary method of methane hydrate production. Nevertheless, the combination of depressurization with modest thermal stimulation can offer the opportunity to both improve the production of reservoirs and overcome flow assurance problems in the production tubes. One analytical challenge here is to understand the endothermic shift in the dissociation of methane hydrate and the effect this shift

has on the temperature of reformation and the water and gas generated. One category of technique often used to characterize conventional (and even unconventional) hydrocarbon reservoirs is based on pressure testing (or pressure transient testing and analysis). These kinds of techniques are complementary to other characterization techniques because (1) they fill a gap between the small-scale characterization based on cores and logs and large-scale characterization based on geophysical measurement and (2) they provide a measure of flow capacity (e.g., Hancock et al., 2005). Rectification of such techniques for methane hydrate reservoirs can also be beneficial.

Chemical Stimulation

Chemical stimulation is one of the main techniques for maximizing methane hydrates during development. The primary and valid development method for the chemical stimulation of methane hydrate here was to change the conditions of in situ methane hydrate equilibrium by injecting hydrate inhibitors such as salts or evaporites and alcohols; these inhibitors act to minimize methane hydrate stability and induce dissociation. This unique technique has been carried out for decades to deal with methane hydrate obstacle in pipelines. But one demerit is that as regards output it was not used as an option for the long-term. Some restrictive issues include possible operational difficulties in integrating the inhibitor into the process, the considerable cost of the system, and environmental concerns related to disposal after development of the used chemicals.

Novel Production Methods

Now-a-days new techniques applied for extraction of methane from methane hydrates. Out of these few novel concepts have applied to extract methane from methane hydrate. It was also distributed around the world, with various scientific journals and technological patents being given. Although the most recent of these is a modification of a technique of chemical stimulation involving the injection into a methane hydrate reservoir of other gas species such as Co2, effectively sequestrating Co2 and releasing methane at the same time. This concept is based on laboratory observations and thermodynamic considerations (Graue et al., 2006; McGrail et al., 2007; Stevens et al., 2008), which suggest that when Co_2 is brought into contact with methane hydrate it will exchange with methane in the hydrate structure. Perhaps the laboratory and modeling studies are encouraging, the challenge of scaling this technique from the laboratory to field testing has yet to be undertaken.

Other development optimisation techniques patented include inducing in situ combustion of methane hydrate; combustion will heat the formation (Pay zone) and stimulate dissociation of methane hydrate (Collett, 2002; Max et al., 2006).In situ combustion was followed in order to stimulate the output from tar sands; but this principle was not seriously considered for the output of methane hydrate. Other methods, such as seafloor strip mining, were also addressed as a potential solution to extracting methane from near-seafloor hydrate deposits.So there is no problem with all novel production methods, where practical experience is limited and new techniques are being evaluated; the environmental impacts of development and sustainability will require careful consideration.

RESERVOIR SIMULATION MODELING

Simulators are widely used to study the performance of the reservoir and to determine ways to improve the ultimate recovery of hydrocarbons from the reservoir. Models for reservoir simulation are computer models that engineers routinely use to simulate output from a hydrocarbon field over long time scales. In the petroleum industry, they are useful instruments to assess the effectiveness of different production management strategies and methods to stimulate or improve production and, on the other hand, to understand the environmental implications of production.While there is extensive worldwide expertise in the use of reservoir simulators for traditional oil and gas reservoirs, the use of these devices for methane hydrate applications has only recently been considered. The compliance of a verified methane hydrate simulation model would enable prediction of methane production rates

and formation responses from different production strategies (e.g., depressurization, thermal stimulation, chemical inhibitor injection) for either Arctic or marine hydrate reservoirs. The assimilation of modeling and field studies like reservoir rock and fluid properties are essential to efficiently determine various production optimization approach and responses. So reservoir models can forecast both the production rates and responses, as well as in interpreting the experimental observations from the field tests and data. These numerical models assimilate equations accounting for heat transfer, fluid flow, and kinetic mechanisms that govern methane production from hydrate reservoirs.

Since the progress made across history matches the currently available short-term production field datasets (Moridis et al., 2009), there is a shortage of long-term production field data to validate the simulations. Nonetheless, several attempts have been made to compare each model using the same petro-physical parameters and data from some short-term reservoir studies to conduct a series of simulations. This code comparison effort, as described by Wilder et al. (2008), determined that all simulators were able to capture basic heat and mass transfer as well as the overall process of hydrate dissociation. They predicted different front hydrate positions in some parts of the reservoir when ice formation was expected. All simulators showed a rise in the output of methane and water when free pore water is present. The authenticity of the reservoir simulation predictions depend upon (a) knowledge of the parameters and relationships that describe quantitatively the physical processes and thermo-physical properties of all the components of the system under investigation (these physical properties need to be obtained from laboratory experiments and/or from field tests either by direct measurement or through history matching) and (b) availability of field data for the validation of the numerical models (Moridis et al., 2008). Reservoir simulation models need to be carefully validated and tested with long-term production field data. The geomechanicalmodeling is still in the early stages of development, and experimental and field data will also be critical to validate the geomechanical predictions.

Recently, further application of these simulations has been the development of economic models to estimate the commercial viability of methane hydrate output on simulated methane hydrate reservoirs. While very preliminary, these models are the first economic studies to be carried out to estimate the price of natural gas that could lead to commercially feasible methane hydrate gas output (Hancock, 2008; Walsh et al., 2009).Such economic models result in a range of gas prices for methane hydrate energy production which is within the price range traditionally seen in North America.

PROBLEMS ASSOCIATED WITH GAS HYDRATES

Presence of gas hydrates can make field operations more complicated. The presence of hydrates on the ocean floor, for example, will affect drilling operations in deep water. The simultaneous flow of natural gas and water through tubing and pipelines can contribute to the creation of gas hydrates that can hinder or fully block fluid flow across pipeline networks. Heating the gas or treating the gas-water system with chemical inhibitors that prevent hydrates from forming, but increases operational costs, Gas hydrates are usually considered a problem for oil and gas field operations, but their potential commercial value as a renewable energy resource is changing the perception of industry.The potential as a gas resource is due to the relatively large volume of gas contained in the gas hydrate complex.

Indian scenario

With no big gas reserve findings it is important to look for alternative resources such as gas hydrates. Vast continental margins with substantial sediment thickness and organic content provide favorable conditions for gas hydrates to occur in the deep waters adjacent to the Indian continent. In Indian deep offshore gas hydrates mainly occur beyond a bathymetry of 650-700m. So it occurs mainly in continental slope regions. With favorable temperature and pressure condition gas hydrates start forming right from the seafloor down in the shallow sediments. Usually shallow sediments in deep oceans contain lot of gas and water. So gas hydrates form in the pore

spaces of the shallow sediments. As mentioned earlier gas hydrates are highly unstable compounds and can exist only under certain temperature and pressure conditions. ***Caution***: Gas hydrates bear the risk of natural hazards associated with stability of the sea floor, leakage of methane into the ocean and atmosphere, and disrupted gas hydrates during drilling pose a safety problem.***Research***: Development of a field model is quite necessary before the installation of a full scale setup in the sea bed.

CONCLUSION

No one in the world has commercially exploited gas from gas hydrate. But the fact is that why nobody has been able to exploit gas hydrates or rather the problems in exploitation and some concepts which may sound a bit weird or wild today.Unconventional energy like gas hydrates have been the prime focus to fill up the energy demands. But for producing methane has its own challenges. A few would perhaps be:

- Absence of representative deepwater gas hydrates field anywhere in the world.
- Gas production rate (Gas in the production testing of Mallik well in Canada's permafrost area have yielded very low production rate and could not sustain more than 7 days of production using thermal and depressurization methods)
- Managing Water production rate (High amount of water is expected to be produced along with the dissociation of hydrates)
- Sand control since the hydrate reservoirs exist at very shallow depth below sea bed (200-400 mbsf) the sands here would not be consolidated due to absence of overburden pressure.
- Reservoir subsidence and other environmental hazards like tsunami generation and greenhouse gas release.
- Deep water and nature of gas hydrate deposits.
- Behavior of dissociated gas from gas hydrates.
- Difference in the nature of oil/gas and gas hydrate deposits.

Only the sustained dedication of our scientists and the establishment of a proper gas hydrate research and development center in India can resolve these difficulties and challenges. Scientists and researchers must work in tandem with one another to hydrate the global gas communities. Until gas hydrates can be considered a viable choice for affordable supply of natural gas, many critical techno-economic, environmental and extraction problems need to be resolved.

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Figure 1: A pictorial representation of Gas hydrate specimen.Source, *courtesy* of the NGHP-1, India

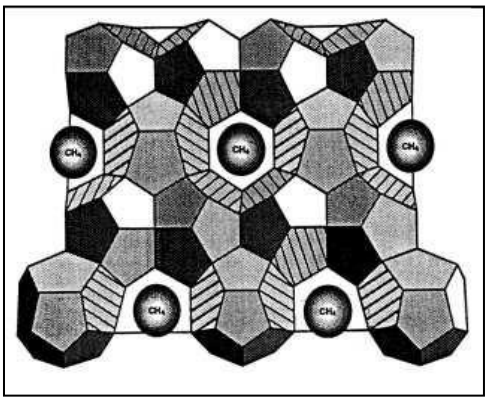

Figure2: Water molecules are arranged in a rigid frame work of cages, Source, *courtesy* Krason and Ciesnik1985

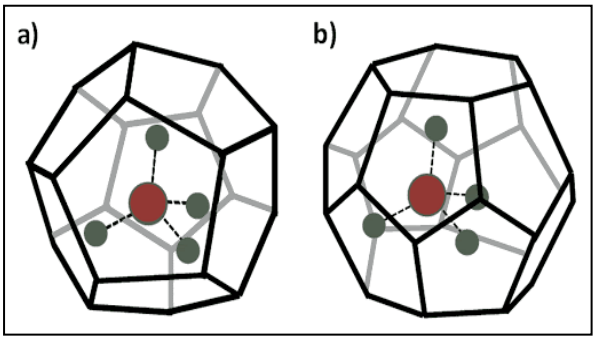

Figure3: Methane hydrate structure: the rigid cages are composed of hydrogen-bonded water molecules, and each cage contains a methane molecule. Source, *courtesy of the Mount Elbert gas hydrate stratigraphic test well project*

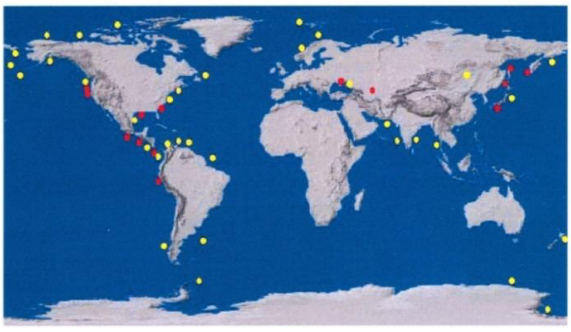

Figure4: Location of gas hydrates in oceanic sediments along continental margins and in polar continental settings. Source: *Loreson TD and Kvenvoloden KA: A global inventory of natural gas hydrates occuarance, USGS, http://walrus. wr.usgs.gov/ globalhydrate/index.html*

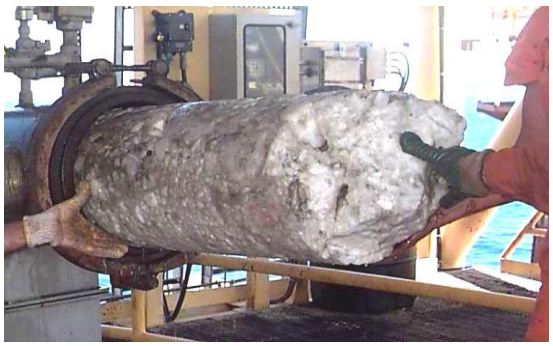

Figure 5: Specimen of methane hydrate extraction, source: courtesy of the NGHP-1, India

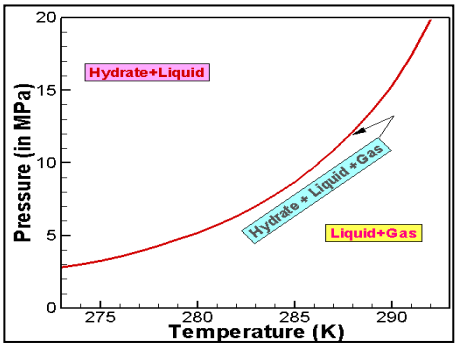

Figure: 6 Phase equilibrium diagram, *source* R.C. Selly

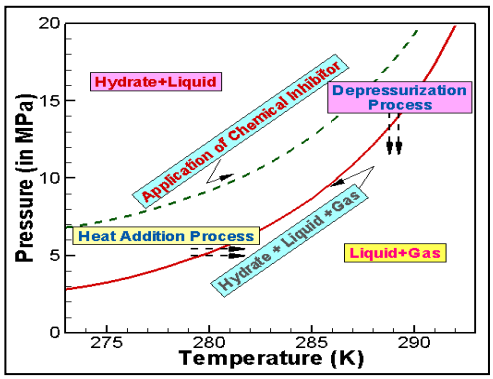

Figure: 7 Decomposition of hydrates by depressurization, thermal, and chemical techniques e.g., Hunt, 197; *source* R.C. Selly

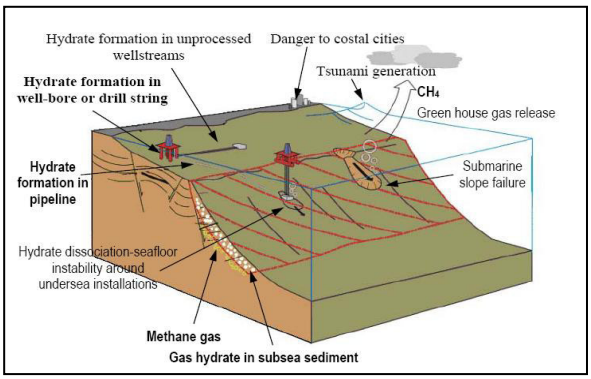

Figure: 8 Various issues related to extraction of gas hydrate

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Gene Expression in Eukaryotes: At the Crossroads of Molecular Surveillance

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Received: 10 Feb 2020 Revised: 13 Mar 2020 Accepted: 16 Apr 2020

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ABSTRACT

A web of events which are inter-related and highly regulated determines the fate of gene expression. Essentially, these vital events are broadly categorized into two types, namely, post-transcriptional and post-translational events. Primarily, processing mechanisms of pre-mRNA including polyadenylation, capping, splicing and modifications of RNA through changes in chromatin [Small interfering RNAs (siRNA), long non-coding RNAs, micro RNAs (miRNA)] are included under post- transcriptional events. Whereas, protein modifications including sumoylation, ubiquitination, phosphorylation are some of the events which occurs post-translation. Both post-transcriptional and post-translational events are considered to be constitutive and also are aggravated by endogenous and exogenous factors. Particularly, in plants, regulation of gene expression is yet to be fully understood. These molecular events ensure proper occurrence of the factor(s) required which would ultimately modulate several downstream cellular processes. This review primarily focuses on some of the key post-transcriptional and post-translational events which are significant in deciding the fate of eukaryotic gene expression.

Keywords: Replication, messenger RNA, post-transcriptional modifications, protein modifications.

INTRODUCTION

Exterior framework of an organism typically referred to as the phenotype, is generally determined by the functional proteins, though their sequence is encoded in DNA. The genetic expression is regarded as one of the elemental process which plays a vital function in the changeover of the complex genome to a substantial life. Since genetic expression process is a strongly regulated event, so any sort of mis-regulation may escort to distorted physical life which includes a variety of genetic diseases. Till date, it is pretty well recognized that the genetic expression is

synchronized at a variety of levels and these miscellaneous mechanisms are well included as a food web. The regulatory mechanisms controlling gene expression is primarily divided into two main types (1) post-transcriptional mechanism and (2) post-translational mechanism. Additionally, upstream of these two events, DNA is mostly synchronized at the transcriptional level prior to entering into the transcription event. Quality control of RNA at the post-transcriptional level is a very important concern for all organisms to ensure precise gene expression, both qualitatively and quantitatively [1]. Transcriptional process has been expansively premeditated as compared to that of post-transcriptional and post-translational events for the reason that of scientific aspects. It is apparent that transcription is one of the essential and instinctively imperative steps within the multistep processes involved in regulation of gene expression and also the scientific methods to decode transcriptional regulation are very well recognized. Post-transcriptional regulation mechanisms engage diverse events such as messenger RNA processing which includes 5′ end capping, polyadenylation and intron splicing. Export as well as localization of messenger RNA, messenger RNA decay, and translation of messenger RNA are also included (Fig. 1). Regardless of this array of regulatory mechanisms, lone thing in general is that they eventually control where and when a messenger RNA is translated to protein. As a result, translation and its regulation are extremely fundamental to post-transcriptional regulation of gene expression. The regulatory mechanisms controlling post-translational events are known as post-translational modification (PTM), essentially which refers to several types of covalent and enzymatic modifications of proteins occurring following to their synthesis.

Post-synthesis of proteins through ribosomes, they endure PTM in order to shape the mature and functional protein product. PTMs may arise on the side chains of amino acid or else at the N- or C- terminal of proteins [2]. Phosphorylation is quite a regular mechanism for modulating the enzymatic activity and is also most common PTM [3]. Countless eukaryotic proteins harbour carbohydrates attached through a process called as glycosylation, which mostly induces stabilization and protein folding, thus allowing the newly synthesized proteins to perform regulatory functions. Alliance of lipids commonly recognized as lipidation, chiefly aim a specific protein or a part of it which is adhered into the cellular membrane. Additional forms of PTMs comprises of cleavage, as in the case of synthesizing a mature form of protein by processing a given pro-peptide. Occurrence of disulfide bonds, formed due to cysteine residues, also is referred to as a type of PTM [4]. PTMs also occur as a result of oxidative stress [5]. Protein aggregates formed post protein degradation may be referred to as carbonylation which primarily targets the newly synthesized protein. Modifications in unambiguous amino acids can thus be applied as biomarkers signifying oxidative dent [6].

RNA processing and export

Prior to the transfer of mRNA from nucleus to the cytoplasm for getting accessible to the translational apparatus, it needs to experience a sequence of dispensation steps: firstly, at the 5' end, the messenger RNA bears a cap like structure, then, splicing event initiates leading to the splicing out of introns harbouring in the pre-messenger RNA, finally a specific 3' end of the mRNA is synthesized, usually referred to as polyadenylation. Every event advance co-transcriptional and influences each other [7]. Initially, m7G cap is added at the 5' end of the budding mRNA and occurs following synthesis of 20-30 nucleotides. This event primarily is a complex process and occurs in a three-step process. At first, the guanosine mono phosphate (GMP) domain from the GTP is supplemented into the foremost nucleotide present in the pre-mRNA and then, GMP is methylated specifically at location N7. For the stability of mRNA and translation process, the m7G cap is imperative. Inside the nucleus, the m7G cap gets associated along with the cap binding complex (CBC). CBC contains two subunits and after being moved into the cytoplasm, it forms a complex with the translation initiation factor 4E (eIF4E), which is essentially an indispensable tread in the initiation process of translation. Since the coding sequences (exons) of a large amount messenger RNAs incase of the higher eukaryotes are broken up by the presence of introns, thus these group of introns needs to be chopped off of the pre-messenger RNA to create functional messenger RNA. Consensus and conserved sequences are required for splicing of the mRNA, which primarily marks the exon-intron limits, and the spliceosome, commonly referred to as the catalytic complex carries out the enzymatic reactions to eliminate the introns and ultimately ligate the flanking exons. Five small ribonucleoprotein particles (snRNP U1, snRNP U2, snRNP U4, snRNP U5 and snRNP U6) make up

the spliceosome unit. Usually, snRNPs are made up of a small nuclear RNA, commonly referred to as snRNA, and associated proteins, many of which are accessory proteins. Preferably, almost hundred of proteins are believed to be engaged as factors, primarily involved in splicing event [8]. Splicing reaction undergoes catalysis process and this event is reliant on RNA-RNA, protein-protein and RNA-protein interactions. Moreover, the unconventional use of exons, referred to as alternative splicing can also add to the formation of protein diversity [9] by allowing a single gene to fabricate manifold isoforms. The majority of messenger RNAs also bear a definite structure, a poly (A) tail at the 3' end. In higher eukaryotes, mRNAs coding for histone proteins lack poly (A) tail, but this is absent in yeast [10]. Polyadenylation at the 3′ end occurs in two steps: firstly, the newly synthesized messenger RNA is cleaved at the site mostly where the polyadenylation is destined to initiate, and then processed for poly (A) creation. In resemblance to the splicing, the polyadenylation protein complex is required for poly (A) tail configuration and also explicit sequence-elements above the pre-messenger RNA. In case of mammalian cells, the position of cleavage mostly lies flanked by a hexamer motif (AAUAAA) along with a GU-rich downstream element, DSE. This hexamer is essentially associated by the cleavage and polyadenylation specificity factor (CPSF).

The downstream elements associate with the cleavage stimulatory factor: cleavage factor I and cleavage factor II are also obligatory. While both the poly(A) polymerase (PAP) and the cleavage and polyadenylation specificity factor are mandatory for cleavage of the pre-mRNA and poly(A) addition respectively, the cleavage stimulatory factor (CstF) is also indispensable for the endonucleolytic cleavage to occur, and CstF together with the CPSF are indulged for recruiting CF I and also the CF II. The synthesis of poly (A) tail occurs in the same way both in the case of yeast and mammalian cells. The protein factors concerned largely bear orthologous components, but also explicit accessory machinery that are specifically found in any one of the species. Additionally, in case of yeast cells, the AAUAAA hexamer pattern is replaced by an erratic A-rich element and instead 3 polyadenylation complexes are present. Cleavage Polyadenylation Factor (CPF), which bears numerous factors which are homologous to CPSF and also the cleavage factor IA (CF IA), poly(A) polymerase and cleavage factor IB (CF IB). The rising poly (A) tail is associated by the poly (A)-binding protein (PAPB). The PABP is mainly thought to persuade the ultimate length of the poly (A) tail, positively by invigorating the processivity of poly (A) polymerase, and negatively by associating with the poly (A) nuclease (PAN) [11]. Moreover, PABPs are concerned with nuclear export and are also imperative for the launch of translation. The poly (A) tail is critical for quite a lot of superfluous mechanisms regulating post-transcriptional events, occurring in the cellular cytoplasm.

The translational state can also be standardized via cytoplasmic polyadenylases and steadiness of a range of target messenger RNAs by manipulating the length of the poly (A) tails. The preeminent illustration is most likely that of translational regulation of the maternal messenger RNAs in case of oocytes of *Xenopus*, stockpile in a translationally subdued state with extremely petite poly (A) tails, which become polyadenylated when activated and as a outcome of which, translated messenger RNA undergoes decay by several exonucleolytic events which is usually preceded by a reduction of the 3′end poly (A) tail [12]. In recent times, poly (A) tails deadenylation has also been exposed to ensue in micro RNA-mediated regulation [13, 14]. The very last component in the expedition from the nucleus (space of transcription process) into the cytoplasm, is the nuclear export of the mature messenger RNA. Export occurs through the nuclear pore complex and happens in the perspective of messenger ribonuleoprotein complexes (mRNPs). Messenger ribonuleoprotein complexes embrace messenger RNA and several associated RNA-binding proteins, which associate to the messenger RNA during the progressing steps [15]. Separately from the aforesaid, Cap binding complex or poly (A) binding proteins, like RNA-binding proteins consist of SR (serine/arginine rich) and hnRNP (heterogeneous nuclear RNP) proteins, the exon junction complex (EJC), which are a group of proteins encumbered onto the messenger RNA, mainly at the upstream region of exon-exon junctions, as a end result of the pre-messenger RNA splicing. These protein components are imperative for the organization of the mRNP complex with the nuclear pore complex and the shuttling from nucleus into the cytoplasm, and a few of them settle, allied with the messenger RNA as it is moved out, whereas others are constrained within the nucleus. Moreover, nuclear export is an central step in quality control, as damaged or un-processed messenger RNAs are not only ineffective, but also potentially detrimental, if incase gets translated within the cytoplasm. Only physiological messenger RNAs are transferred into

the cytoplasm from their site of synthesis and particularly this surveillance step is very closely united to RNA processing and the composition of mRNP. Yet again, it needs to be highlighted that, regardless of the introduction of messenger RNA transcription and other downstream chronological events occurring in the cell are well integrated among each other and are not independent in temporal and spatial perspective [7, 15].

Significance of translational regulation

A varied number of reasonable benefits do occur since the translational regulation is perfectly fitted. Most importantly, the translational regulation happens as an immediate retort without the requirement of undergoing the several processes involved in regulating gene expression such as transcription, processing of messenger RNA or even export of messenger RNA. Moreover, the regulatory mechanism of translation is a reversible in nature since it involves quite a lot of reversible protein structure alterations like, the phosphorylation of several initiation factors. Control of translational machinery is very much inevitable, particularly in the systems where transcriptional regulation is not promising like in the case of reticulocytes, they lack nucleus, RNA viruses or oocytes. Most importantly, the translational regulation is primarily, spatial control of gene expression inside the cell [16]. The significance of dedicated translational regulation is realized especially for localized protein assembly within neurons or else throughout the development process, since transcriptional regulation is limited to the cellular nucleus. For regulating gene expression, translational regulation is a superior alternative owing to its flexible nature. There are numerous molecular targets for regulation of translational process, which ultimately affects the efficiency of translational event for numerous or a few messenger RNAs. Most remarkably, for fine tuning of gene expression cells regulate the translational machinery, as there are several number of genes such as GADD45α or TNF-α which are regulated at the transcriptional and translational level.

Effectors for regulation of translation: Initiation factors, messenger RNA (mRNA) and the ribosome

Translational control is well regulated at a comprehensive level as well as in a messenger RNA specific manner [17]. Primarily, large-scale regulation affects the effectiveness of translation machinery of many messenger RNAs through a common switch-on and switch-off of translation process. The translation of a subset of mRNA is affected by mRNA-specific regulation. Mainly, translational regulation allows or forbids the union of the messenger RNA with that of the translational apparatus. A fundamental target in several regulatory mechanisms is the cap binding protein, eIF4E which binds to many inhibitory proteins, resulting in the unavailability of the messenger RNA. Comprehensive regulation of translation is universally mediated through such modifications, especially of the translation initiation factors. An additional target for translational regulation is the messenger RNA itself. The cis-regulatory elements associate with trans-acting factors (Fig. 2).

The cis-regulatory elements present on the messenger RNA could be present anywhere along the messenger RNA, but typically for the translational regulatory factors, these vital elements are present in the 5' UTR or 3' UTR. Translational regulatory events mediated by messenger RNA, occurs mostly by numerous regulatory proteins, which primarily bind to the cis-regulatory elements of a given messenger RNA. The ribosome itself may also become one of the targets of translational regulation. Quite a lot of its protein constituents undergo post-translational modifications. An exemplar is the phosphorylation process of ribosomal protein S6 mediated by ribosomal S6. It has been reported that the phosphorylation of ribosomal protein S6 fallout in an augment in translation initiation. Ribosomal proteins also undergo a post-translational modification, ubiquitination and methylation. Many studies points towards the heterogeneity of ribosomes; the cell is able to construct a range of different kinds of ribosomes [18], which essentially differs in terms of paralogue composition and post-translational modifications, and many a times dedicated ribosomes could also play a role in the translational regulation of specific subsets of messenger RNAs.

Novel components in translational control: Processing bodies and micro RNAs

Recently, two novel ways to direct messenger RNA turnover at the post-transcriptional level have gripped an immense deal of consideration. The discovery of processing bodies localized in the cytoplasm of a cell, which were originally considered as foci inside the cell with a high concentration of enzymes meant for messenger RNA decay [19], has been a significant outreach in the scientific field. The added detection is that of small RNAs, which may amend the permanence and translation of targeted messenger RNAs [20]. Processing bodies are most likely a site of messenger RNA decay (Fig. 3). Processing bodies were first characterized by several groups using several scientific techniques such as microscopy, as factors involved for the perishing of messenger RNA decay and other factors like LSM, XRN1, DCP1 and DCP2 accumulate in the foci [19]. The messenger RNA decay in case of eukaryotes is mainly controlled in diverse ways mostly by exonucleolytic or endonuleolytic pathways. Degradation occurring through exonucleolytic pathway is typically initiated by deadenylation of the poly (A) tail of the messenger RNA. Then the transcripts will be tarnished from their 5′ ends mostly by the exonuclease such as XRN1, subsequent elimination of the 5' cap, known as decapping. On the other hand, the exosome complex can debase transcripts from their 3′ ends prior to decapping. Factors involved in the nonsense-mediated decay process, which are responsible for the hasty dilapidation of messenger RNAs with a untimely stop codon are also found in mammalian processing bodies [21]. Nonsense-mediated messenger RNA decay (NMD), which is possibly the best-known translation-dependent regulatory mechanism specifically in eukaryotes, selectively destroys messenger RNAs as a means of post-transcriptional gene control [22]. Control can be for the purpose of ensuring the quality of gene expression.The relation between the processing bodies and messenger RNA turnover rate is still captivating. The precise mechanism how messenger RNAs shuttle into the processing bodies and become repressed from translation is not yet clearly deciphered [23].

Small RNAs are mostly riboregulators that have significant roles in most of the eukaryotes. They inhibit the gene expression by acting either on DNA to direct sequence abolition and chromatin remodeling, or on the RNA to direct cleavage and eventually regulate the translation expression [24]. Micro RNAs (miRNAs) and short interfering RNAs (siRNAs) are the two categories of small RNA molecules that appeared as regulating factors of messenger RNA stability and translation. Both the miRNAs and siRNAs are short RNAs ranging around 20-27 nucleotides and are differentiated based on their biogenesis. miRNAs are principally derived from longer precursors that mostly comprise of a ~75 nucleotide improperly based hairpin segment. siRNAs are of comparable length but are derivative of absolutely complementary RNA precursors. During RNA interference (RNAi), siRNAs which are exogenously introduced target messenger RNAs for cleavage in an endonucleolytic manner [25]. In case of animal cells, a large amount miRNAs are only partly complementary to their target messenger RNAs and the down-regulation of translational product of the target is typically greater than the down-regulation of its messenger RNA profusion, which suggests regulation at the stage of translation.

Post-translational modifications of proteins

Post-translational modifications (PTMs) of proteins largely involve covalent alterations that occur subsequent to the translation process. The newly synthesized nascent proteins are eventually exposed to a string of specific enzyme-catalyzed alterations located on their backbones or side chains. Two extensive types of protein post-translational modifications occur. The first type includes every enzyme-catalyzed addition of a few kinds of chemical groups, typically an electrophilic part of a substrate, towards the side chain residue of a protein. This modified side chain is generally electron rich and act as a nucleophile in the transfer process. The second type of PTMs is covalent cleavage of the peptide backbones in proteins. It occurs either by protease action or less commonly mediated by autocatalytic cleavage. A lot of diversifications can be seen in the side chains of amino acids [26].

Covalent modification of proteins

Fundamentally, there are five most frequent types of covalent additions occurring to proteins. They are acylation, phosphorylation, alkylation, oxidation and glycosylation, which are generally catalyzed by dedicated post-translational modification enzymes. Thus, the protein products obtained in this mode result into making up subsets of the complete proteome of an organism commonly referred to as the acyl proteome, the phosphoproteome, the alkyl proteome, the oxidized proteome and the glycoproteome. Most remarkably, every sub proteomes add to extensive diversity [26].

Post-translational modification: reversible and irreversible

Because of the cellular requirement of a meticulous covalent modification occurring in a protein, reversibility and irreversibility of the specific protein modification is critical. The epitome of reversible modification is mainly the protein phosphorylation, reliable with its advancement to the foremost role in protein-based signaling in eukaryotes. All PTMs apart from alkylation have committed enzymes. Mostly, large enzyme families mediate the amputation of several covalent modifications. The enzymes which are involved in acylation, reverse phosphorylation and glycosylation are mostly specific hydrolases, while cleavage of disulfide bonds is mediated by reductases [26].

CONCLUSION

The self-fidelity and unswerving post-transcriptional and post-translational mechanisms make sure of a safe and sound pathway for the genetic makeup, primarily the DNA of an organism and carries out the critical changeover of the DNA into a functional protein which eventually results into an healthy physiological environment within the cell. This inter-relationship and interdependence prevailing among different molecular events similar to a spider's web provides the foundation for a fault free "Central Dogma" of molecular life. A number of events occurring within a cell irrespective of the nature of product to be formed, whether RNA or protein, surveillance mechanisms ensures the fidelity. These molecular events are essentially very critical for maintaining the homeostasis within the cell. For instance, maximum number of immune related genes, both in plants and animals, are tightly regulated by the quality control mechanism, NMD. NMD ensures that during normal and healthy conditions (pathogen unchallenged condition); these immune genes do not synthesize their protein counterparts. This regulation critically saves a lot of energy for the organism by not synthesizing unwanted protein factors. Whereas, the same NMD process shuts off when the organism is challenged with any sort of pathogen and allows the expression of protein factors responsible for defense mechanism. In a nutshell, understanding and deciphering the role of different post-transcriptional and post-translational events would be critical for future benefits.

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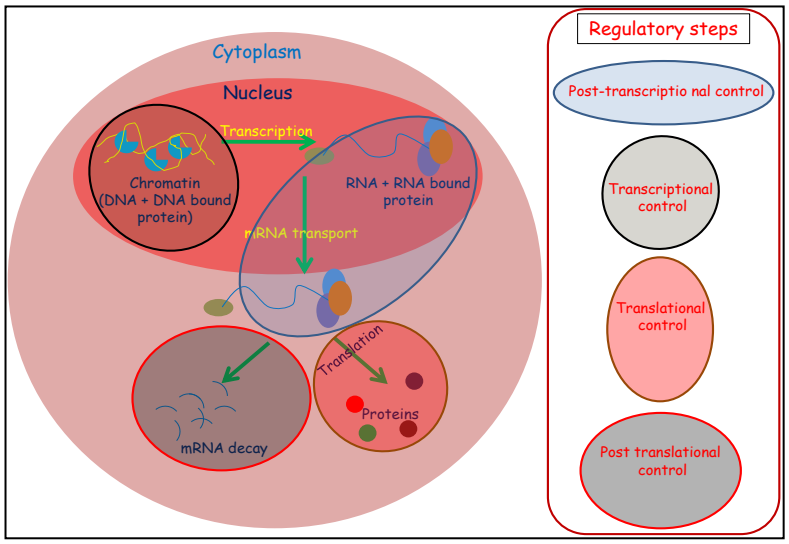

Fig. 1 Multiple layers of regulatory events meant for gene expression

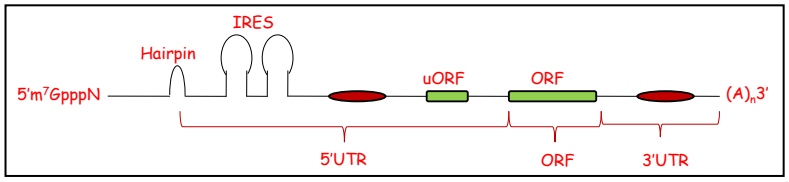

Fig. 2 Cis-acting sequences influence translation initiation of specific messenger RNAs

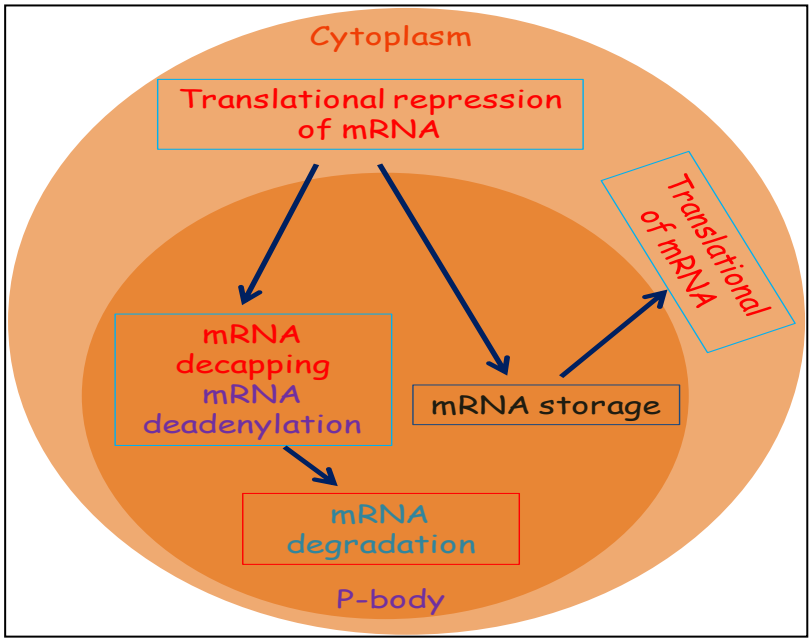

Fig. 3 Processing bodies (P-bodies): The site for mRNA decay

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Antimicrobial Study of an Autochthonous Plant *Achyranthes aspera* Linn.

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Received: 12 Feb 2020 Revised: 14 Mar 2020 Accepted: 16 Apr 2020

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ABSTRACT

The present investigation aims to evaluate the antimicrobial properties of *Achyranthes aspera* leaves extracts using two different solvents: chloroform (nonpolar) and methanol (polar) against human pathogenic microorganisms. The leaves of *Achyranthes aspera* (Family Amaranthaceae) were exhaustively extracted by using Soxhlet apparatus in different solvents like chloroform and methanol. The two extracts were subjected to antimicrobial screening. Extracts of *Achyranthes aspera* from solvents (methanol and chloroform) were evaluated against bacterial strains such as *Escherichia coli* (MTCC723), *Staphylococcus aureus* (MTCC902) and fungi like *Candida albicans* (MTCC4748) using well diffusion method.The methanolic extract of dried leaf of *Achyranthes aspera* L. shows antimicrobial activity against *Staphylococcus aureus* and *Candida albicans* with zone 8mm and 7mm respectively. The chloroform extraction shows antimicrobial activity against *S. aureus, E.coli* and *C. Albicans* with zone 7mm, 9mm, and 11mm respectively. Hence it can be used as drug to inhibit these pathogens. The present study indicates the potential usefulness of *Achyranthes aspera* aerial parts in the treatment of various pathogenic diseases like leprosy, asthma, snake bite, malaria, fever, cough, pneumonia and urinary tract as mentioned in the Ayurvedic literature.The leaves extracts of *Achyranthes aspera* showed significant antimicrobial activity due to the presence of some bioactive compounds in them.

Keywords: *Achyranthes aspera, Escherichia coli, Staphylococcus aureus, Candida albicans,* Phytochemicals, chloroform, methanol.

INTRODUCTION

Nature is the best source of medicinal agents for thousands of years and modern drugs have also been obtained from the natural source [1]. According to WHO report 70-80% of the world population rely on non-conventional medicine that mainly from herbal sources. Medicinal plants contain various active compounds. Secondary metabolites like terpenoids, quinones, flavonoids, tannins, resins and saponins etc. are bioactive compound which can be exploited for drug designing. Secondary metabolites play a vital role in plant disease defense mechanisms. These compounds

are protecting the plants from microorganism, insects and other natural pests. In the recent past, the plant herbal health products have been used tremendously in developed and developing countries and they have gained wide acceptance [14].Ayurveda is one of the oldest medication system of disease prevention in the world and is called as Maharshi ayurveda [2]. In Ayurveda different parts of the plant is used to treat leprosy, asthma, snake bite, malaria, fever, cough, pneumonia etc.[2]. Herbal medicines are developed due to their wide biological activity, less cost and high safety of margin than synthetic drug [7]. An antimicrobial is an agent which is used to kill microorganisms and stop its growth. Antimicrobial medicine is grouped on the basis of microbes primarily acting against, bacteria and fungi. For example an antibacterial is used against bacterial growth [8]. Antibiotics have more effect to destroy bacteria but it may have side effects, traditional medicines are used to reduce side effects [13].

Achyranthes aspera is a perennial herb belonging to family Amaranthaceae which is used for medicinal purpose [3]. It is also known as "Prickly chaff flower" in English, and "Onga", "Latjeera" and "Apamarg" in local language [4]. It is an annual,, erect herb and commonly found as weed all over the india [5]. This plant is used for treatment of cough, renal dropsy, fever, asthma, snake bites [3].*Achyranthes aspera* is used as herbal medicine in Bangladesh [6]. It has hypoglycemic activity, analgesic and antipyretic activity, anti-oxidant activity, anti-tumor activity, cardiac stimulant activity, anti-asthmatic,diuretic,anti-helminthic, antiviral, antibacterial, antifungal, anti-plasmodial, hepatoprotective, nephroprotective, wound healing, anxiolytic, and antidepressant activities [11].It was reported that the chloroform and ethanol root extracts of the *A. aspera* have anti-implantation and abortifacient activity [12]. The present investigation is to find out antibacterial and antifungal activity of leaf extract of plant against selected bacterial and fungal pathogens. For this study human disease caused pathogens were taken..

MATERIALS AND METHODOLOGY

Collectionof Plant materials

The fresh, healthy plant leaves of *Achyranthes aspera* was collected from road side of Centurion University, Ramachandrapur, Jatni, Bhubaneswar. The plant is identified taxonomically and authenticated in the Department of Botany, Centurion University, Bhubaneswar.

Preparation of leaves for Extraction

The leaves were washed thoroughly 3-4 times in tap water and finally rinsed with distilled water and shade dried for about 15-20 days [15]. Periodically the moisture levels of the leaves were observed. Once it is completely dried, using a mechanical grinder the leaves were powdered. The ground samples were stored in a sterile glass container for future process.

Plant extract preparation

Plant leaf extraction was done by using a polar solvent methanol (CH3OH) and a non-polar solvent chloroform (CHCl3). Plant extraction was prepared by using Soxhlet apparatus [9.] After preparation of extract it was stored in a sample bottle and kept in refrigerator for use.

Test organism

In this investigation human disease caused pathogen were taken. Two bacterial strain i.e gram negative bacteria *Escherichia coli* (MTCC723), gram positive bacteria *Staphylococcus aureus* (MTCC902) and a fungal strain *Candida albicans* (MTCC4748). The cultures of bacteria and fungi were sub cultured on Nutrient Agar (NA) and Potato Dextrose Broth (PDB) slant respectively and stored at refrigerator at 4℃ until required for study.

***In-vitro* Antimicrobial activity study**

The antimicrobial activity study is done by using agar well diffusion method [10]. In this method for bacteria culture Mueller hinton agar media was used and for fungal culture Sabouraud dextrose agar was used. The media was autoclaved and poured in sterilized petri dishes. The microorganisms swabbed on the agar surface. Hole was made by using cork borer and the floor was sealed with nutrient agar .Crude sample was loaded in particular concentration i.e. 50µl, 60µl, 70µl, 80µl, 90µl and 100µl.[1] Then the microbial culture was allowed to incubate. The bacterial culture was incubated for 37 0C for 24hours and fungal at 28 0C for 72hours .After incubation period the zone of inhibition was measured and calculated..

RESULTS AND DISCUSSION

In the present investigation antimicrobial activity of plant extract from methanol and chloroform was evaluated against gram positive bacteria *Staphylococcus aureus*, gram negative bacteria *Escherichia coli* and fungal species *Candida albicans*.[6].Chloroform extract of leaf of plant *Achyranthes aspera* L. was found to inhibit the growth of *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* in zone diameters were 7mm, 9mm and 11mm respectively. Methanol extract of leaf of plant *Achyranthes aspera* L. does not show zone of inhibition against*Escherichia coli* and found to inhibit the growth of *Staphylococcus aureus* and *Candida albicans* in zone diameters were 8mm and 7mm respectively. This shows that the methanol extract have no effect on the growth of *Escherichia coli* where as indicates a remarkable result in case of *Staphylococcus aureus* and *Candida albicans*.

CONCLUSION

The selection of this plant is due to its traditional use to treat skin and urinary tract infection. *E. coli* is responsible for causing urinary tract infection in human so also *S. aureus* may cause respiratory tract infection and *Candida albicans*. causes skin infection. Our results against this human disease caused pathogen helps in preparation of herbal drug. From the current study we can draw the conclusion that plant *Achyranthes aspera* shows antimicrobial properties which inhibit growth of bacteria and fungi. This antimicrobial study by using plant indicates that folklore claim is very true and the plant can be effectively used as medicine at par with synthetic medicine to reduce growth of microorganisms. This plant can be well used in the drug designing process and will pave a new way for herbal drug.

ACKNOWLEDGEMENT

The authors thank Centurion University of Technology and Management, Bhubaneswar, Odisha, India for providing laboratory and equipment facilities to carry out the research work.

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Table - 1 (Methanol Extract)

Table - 2 (Chloroform Extract)

SL.NO	Test Organism	Activity	Zone of Inhibition Diameter
01	*Escherichia coli*	Negative	--
02	*Staphylococcus aureus*	Positive	8mm
03	*Candida albicans*	Positive	7mm

SL.NO	Test Organism	Activity	Zone of Inhibition Diameter
01	*Escherichia coli*	Positive	9mm
02	*Staphylococcus aureus*	Positive	7mm
03	*Candida albicans*	Positive	11mm

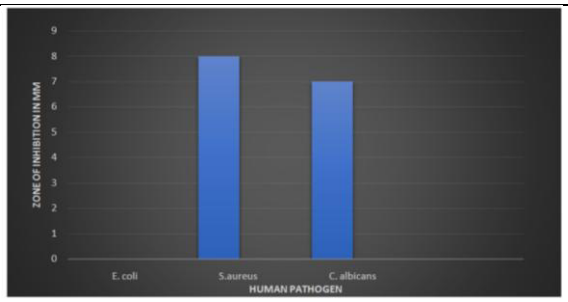

Fig. 1. (Methanol Extract)

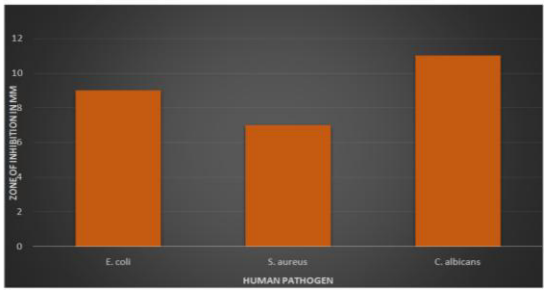

Fig. 2 . (Chloroform Extract)

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Studies on Comparative Analysis of the Morphometric and Meristic Characters of Family *Lutjanidae* at Southern Coast of Gopalpur, Ganjam, Odisha

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Received: 14 Feb 2020 Revised: 16 Mar 2020 Accepted: 18 Apr 2020

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ABSTRACT

Due to identification problem that exist between fishes of different species, hence the need to compare the Morphometric and meristic character of family Lutjanidae. The species diversity of Lutjanus and their comparative analysis from the Southern Coast of Gopalpur was studied during Jan 2020 to March 2020. The sample were collected from two landing centre. A station I (lat 19.3272° N and long 84.9766° E) Arjyapalli fish centre and station II(lat 19.2547° N and long 84.9059° E) Gopalpur fish landing centre. Sample and Specimen were collected regularly on a seasonal basis. The five species observed were *Lutjanus jhoni, Lutjanus indicus, Lutjanus lutjanus, Lutjanus fulvus and Lutjanus fulviflamma.* A *total* of 30 samples of family Lutjanidae were caught from the two sampling station located in the Southern Coast of Gopalpur. Morphometric measurements and meristic characters were determined on the specimens to ascertain the possibility of morphological and meristic diversity among them. The Morphometric measurement i.e eye diameter(ED), head length(HL),Standard length(SL),body depth(BD), total length (TL) and pectoral fin length (PCL) for *L. jhoni* varied from 1.5-1.7cm, 6.8-7.5 cm, 18.5-20.5cm, 6.5-8cm, 21.5-24cm, 5.2-6cm respectively. Eye diameter(ED), head length(HL), Standard length(SL), depth(BD), total length (TL) and pectoral fin length (PCL) for *L. indicus* varied from 1.7-2cm, 7.5-10cm, 21-26cm, 8-8.9cm, 25-30cm, 5.5-6.5cm respectively. Eye diameter(ED), head length(HL), Standard length(SL), body depth(BD), total length (TL) and pectoral fin length (PCL) for *L. fulvus* varied from 1.8-2cm, 7.8-9.5cm, 19.2-23.5cm, 8.3-9.7cm, 24.5-27cm, 6.5-7cm respectively. Eye diameter(ED), head length (HL), standard length(SL), body depth(BD), total length (TL) and pectoral fin length(PCL) for *L. lutjanus* varied from 2cm.,7.5cm, 20cm, 7.2cm, 23.5cm, 5.8cm respectively. Eye diameter(ED), head length(HL), standard

length (SL), body depth (BD) and pectoral fin length (PCL) for *L. fulviflamma* respectively. The meristic characters i.e. dorsal fin spines, dorsal fin rays, anal fin spines, anal fin rays count are 10,14,3,8 of six species are similar. But the meristic trait like scale count for *L. jhoni, L. indicus, L. lutjanus, L. fulvus, L. fulviflamma* varied from 56-60, 51-58, 54-58, 56-62, 52-56 respectively. The populations of the species showed variations in morphometric measurements and similarities in meristic counts. This variation may be related to the geographical ecological activities of the diversity.

Keywords: Lutjanidae, Morphometric and meristic characters, diversity, comparative analysis of five species.

INTRODUCTION

A fish is a poikilothermic (cold-blooded) water dwelling vertebrate with gills and includes 4000 species in world and nearly 2600 species in India. Fisheries sector have been gaining important globally due to their role in national economy, Foreign exchange earnings and employment generation. It also provides nutrients food and cheap protein not only to the fisher folk but also the rapidly growing population (Eyo, 2010). The fishes of the family Lutjanidae are commonly known as Snappers. These are important marine food fishes of high commercial value occurring in the tropical Indo-Pacific region. Although there is report of some species causing fish poisoning (ciguatera), they have high demand in international market. They are also of great demand in aquarium trade for many of them have attractive body colour pattern (Olatunde, 1989). Snapper are usually demersal species, found near the bottom in tropical and subtropical seas throughout the world from shallow water to depths of about 550 meters. Several species are found in coral reef areas, but some also occur in estuaries, rakish water bodies, mangroves and even hyper saline lagoons, with several deep-water and only a few freshwater species (Allen, 1985).

Allen (1985) has studied some biological aspect of this species. In fish, identification may be determined based on two factors which are morphometric and meristic characters. Mostly the morphometric means of determining the growth rate of the fish is carried out by measuring some parts of the physiological structure of the fishes, while meristic is determined by performing some numerical counts on the fish in order to determine the species and class of the fish. morphometric and meristic analysis are part of important rigorous tools used to differentiate closely related species of organism having huge similarity indices of various parameters. Morphometric characters are not only essential to the understanding of the taxonomy but also the health of a species as well as its reproduction in an environment (Wainwright and Richard, 1995).

In Lutjanidae, the common adult length is usually 25cm but may extend to 100cm.The fish lutjanidae is estimated to have 10 spines, 14 dorsal rays, 3 anal spines and 8-9 anal soft rays, which is a determinant features that distinguish from other similar fishes especially the so called popular lady fish (Allen, 1985).Hence, it is aimed to investigating the comparative analysis of the Morphometric and meristic characters of lutjanidae from Southern Coast of Gopalpur.

MATERIALS AND METHODS

Study site

The sample were collected from two landing centre. A station I latitude 19.3272° N and longitude 84.9766° E at Arjyapalli fish centre and station II latitude 19.2547° N and longitude 84.9059° E at Gopalpur fish landing centre of Southern Coast Gopalpur.

Collection and analysis of specimen

A total of 30 samples of family Lutjanidae were caught from the two sampling station located in the Southern Coast of Gopalpur. These were purchased randomly from artisanal fishermen during the period of Jan 2020 to March 2020 and the sample is collected on a seasonal basis. The five species were observed *Lutjanus jhoni, Lutjanus indicus, Lutjanus lutjanus, Lutjanus fulvus, and Lutjanus fulviflamma.* The sexes are not differentiated in this study. A tag number was given to each fish specimen while location and date of collection were noted.

Morphometric measurement (axial growth)

Morphometric measurement as length which quantify axial growth of parts of the fish samples(fresh) were carried out with the aid of measuring board, ruler, divider and tailor tape.
The following morphometric characters as length were measured and recorded to the nearest centimetres.

Total length (TL)

Length of the fish measured from the tip of snout to the posterior extremely of the caudal fin (Paulin, 1988).

Standard length (SL)

Measured from tip of the snout to end of body that is, the base of caudal fin, where the fin reach the hypurals (Bagenaln, 1978; Paulin, 1988).

Head length (HL)

The longest measurement from anterior edge of the lip to the most popular part of the bony opercular edge (Paulin, 1988).

Eye diameter (ED)

Length of orbit, the greatest distance between the free orbital rims.

Pectoral fin length (PCL)

Distance between the origin and posterior tip of the left pectoral fin.

Meristic measurement of characters

Meristic measurement takes cognisance of the counting of number of certain parts of the fish into consideration. For counting of meristic trait compound microscope is required.

Dorsal spines and rays

The spines are hardened and single shafted, located at the anterior part of the dorsal fin. The dorsal rays are soft and flexible, located the posterior part of the dorsal fin. Both are designated by a common letter 'D' (Standing for Dorsal Fin) with the Dorsal Spines (DS) counted and recorded in Roman numerals, while the Dorsal Rays (DR) were counted and recorded in Arabic numerals with two rays that have common root being counted as are (Futch and Brugal, 1976; Begenal,1978).

Anal spines and rays

The spines are also hardened and single-shafted, located at the anterior part of the anal fin while the rays are soft flexible and located at the posterior part. Both are designated by a common letter 'A' (standing for anal fin) with the anal species (AS) counted and recorded in Arabic numerals.

Scale count

This represent the number of dorsal scales in the lateral line scales counts were made according to the procedure described by Begenal (1978).The Lutjanidae have lateral line in single part. Counting was made to the end of upper lateral line.

Statistical analysis

Statistical data analysis was done by using Microsoft EXCEL.

RESULTS AND DISCUSSION

The morphometric measurement in *L. jhoni*, eye diameter (ED) varied from 1.5-1.7cm;head length(HL) varied from 6.8-7.5cm; standard length (SL) varied from 18.5-20.5cm; body depth(BD) varied from 6.5-8cm; total length varied from 21.5-24cm and pectoral fin length(PCL).The mean value of *L. jhoni* for each morphometric trait i.e. Eye diameter (ED), head length (HL), standard length (SL), body depth (BD), total length (TL) and pectoral fin length (PCL) is 1.6cm,7.15cm,19.5cm,7.25cm, 22.75cm, 5.6cm respectively.

The morphometric measurement in *L.indicus*, eye diameter (ED) varied from 1.7-2cm;head length(HL) varied from 7.5-10cm; standard length(SL) varied from 21-26cm; body depth(BD) varied from 8-8.9cm; total length varied from 25-30cm and pectoral fin length(PCL).The mean value of *L.indicus* for each morphometric trait i.e Eye diameter(ED), head length (HL), standard length (SL), body depth (BD), total length(TL) and pectoral fin length (PCL) is 1.85cm, 8.75cm, 23.5cm, 8.45cm, 27.5cm, 6cm respectively.

The morphometric measurement in *L. fulvus*, eye diameter (ED) varied from 1.8-2cm;head length(HL) varied from 7.8-9.5cm; standard length (SL) varied from 19.2-23.5cm; body depth(BD) varied from 8.3-9.7cm; total length varied from 24.5-27cm and pectoral fin length(PCL).The mean value of *L. fulvus* for each morphometric trait i.e. Eye diameter(ED), head length (HL), standard length (SL), body depth (BD), total length(TL) and pectoral fin length (PCL) is 1.9cm, 8.65cm, 21.35cm, 9cm, 25.75cm, 6.75cm respectively.

The morphometric measurement in *L. fulviflamma* eye diameter (ED) varied from 2-2cm; head length (HL) varied from 7.5-7.5cm; standard length (SL) varied from 20-20cm; body depth (BD) varied from 7.2-7.2cm; total length varied from 23.5-23.5cm and pectoral fin length (PCL). The mean value of *L. fulviflamma* for each morphometric trait i.e. Eye diameter(ED), head length (HL), standard length (SL), body depth (BD), total length (TL) and pectoral fin length (PCL) is 2cm, 7.5cm, 20cm, 7.2cm, 23.5cm, and 5.8cm respectively.

The morphometric measurement in *L. lutjanus*, eye diameter (ED) varied from 1.2-1.7cm; head length(HL) varied from 5-6.3cm; standard length(SL) varied from 14.5-16.4cm; body depth(BD) varied from 5-6cm; total length varied from 16-19.7cm and pectoral fin length(PCL).The mean value of *L. jhoni* for each morphometric trait i.e. Eye diameter(ED), head length (HL), standard length (SL), body depth (BD), total length(TL) and pectoral fin length (PCL) is 1.45cm, 5.65cm,15.45cm, 5.5cm,17.85cm, 4.25cm respectively.

The meristic characters i.e. dorsal fin spines, dorsal fin rays, anal fin spines, anal fin rays count are 10,14,3,8 of six species are similar. But the meristic trait like scale count for *L. jhoni, L. indicus, L. lutjanus, L. fulvus, L. fulviflamma* varied from 56-60, 51-58, 54-58, 56-62, 52-56 respectively. The mean value of *L. jhoni, L .indicus, L. fulvus, L. fulviflamma* and *L. lutjanus* for each meristic trait like dorsal fin spine, dosal fin rays, anal fin spine and anal fin rays is 10, 14, 3 and 8 respectively. The mean value of *L .jhoni, L. indicus, L. lutjanus, L. fulvus,* and *L. fulviflamma* for meristic trait of scale number is 58, 54.5, 56, 59, and 54 respectively.

CONCLUSION

The present study highlighted that little variation in morphological characterisation and no variation occur in meristic count of family lutjanidae among five species. As evident from the above notable discussion on morphological meristic and shape may result in separation and differentiation of stock. These characteristics may be more applicable for studying short-term, environmental induced disparities, and the findings can be effectively used for improved fisheries management.

ACKNOWLEDGEMENT

The authors are acknowledged to Dr Anil Mohapatra, Scientist-D and OC and his Researchers' team, EBRC, Gopalpur on Sea, Gopalpur, Ganjam for their help and cooperation.

COFLICT OF INTREST

There is no conflict of interest among the authors.

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Table 1. Mean±SD value for morphometric trait variation among five species of lutjanidae

Morphometric trait	Mean±SD of *L. jhoni*	Mean±SD of *L. indicus*	Mean±SD of *L. fulvus*	Mean±SD of *L. fulviflamma*	Mean±SD of *L. lutjanus*
ED	1.6±0.923	1.85±0.534	1.9±0.718	2±1.154	1.45±0.4585
HL	7.15±4.128	8.75±2.525	8.65±3.269	7.5±4.330	5.65±1.786
SL	19.5±11.258	23.5±6.783	21.35±8.069	20±11.547	15.45±4.885
BD	7.25±4.185	8.45±2.439	9±3.401	7.2±4.156	5.5±1.739
TL	22.75±13.134	27.5±7.938	25.75±9.732	23.5±13.567	17.85±5.644
PCL	5.6±3.233	6±1.732	6.75±2.551	5.8±3.348	4.25±1.343

Table 2: Mean±SD value for meristic trait variation among five species of lutjanidae

Meristic Characters	Mean±SD of *L. jhoni*	Mean±SD of *L. indicus*	Mean±SD of *L. fulvus*	Mean±SD of *L. fulviflamma*	Mean±SD of *L. lutjanus*
DS	10 ± 5.773	10 ± 2.886	10 ± 3.779	10 ± 5.773	10 ± 3.162
DR	14 ± 8.082	14 ± 4.041	14 ± 5.291	14 ± 8.082	14 ± 4.427
AS	3 ± 1.732	3 ± 0.866	3 ± 1.133	3 ± 1.732	3 ± 0.948
AR	8 ± 4.618	8 ± 2.309	8 ± 3.023	8 ± 4.618	8 ± 2.529
Scale number	58 ± 33.486	54.5 ± 15.732	59 ± 22.299	54 ± 31.176	56 ± 17.708

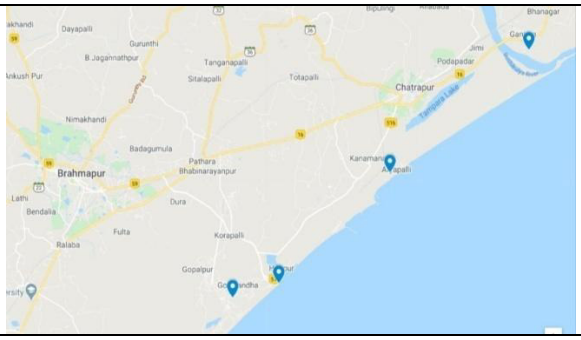

Fig.1. Study site of fish catch

Fig.2. *Lutjanus. Jhoni* specimen

Fig.3. *Lutjanus indicus* specimen

Fig.4. *Lutjanus fulvus* specimen

Fig.5. *Lutjanus fulviflamma* specimen

Fig.6. *Lutjanus lutjanus* specimen

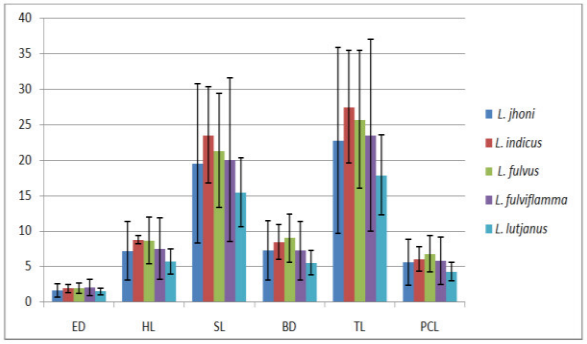

Fig. 7. Graph showing Mean±SD value for morphometric trait variation among five species of lutjanidae

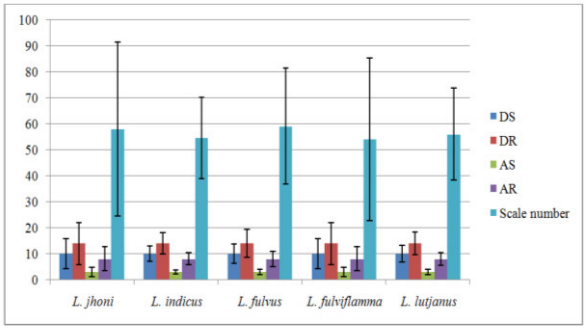

Fig.8. Graph showing Mean±SD value for meristic trait variation among five species of lutjanidae

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Time Series Analysis of 2019-nCoV Cases in INDIA using Seasonal Autoregressive Integrated Moving Average

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Received: 02 Mar 2020 Revised: 04 Apr 2020 Accepted: 06 May 2020

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ABSTRACT

A novel coronavirus (2019-nCoV) is identified in people with acute respiratory disease. Around 210 countries are affected with 2019-nCoV and billions of people are forced into lockdown.The present study aims to explore the distributive feature of 2019-nCoV and construct a Seasonal Autoregressive Integrated Moving Average (SARIMA) model to predict the future trend of spread of 2019-nCoV in India. Statistical data was collected from 01st March 2020 to 30th April 2020 on a daily basis. Standard operating procedure was followed to analyze the data. Minitab was used for statistical analysis. 50 days data were used to train the model and 11 days data were used to testthe model. A short term forecast was executed and analyzed. The SARIMA model works best when linear tendencies in the data are eliminated showing consistent pattern over time with a minimum amount of outliers.

Keywords: Time series analysis; 2019-nCoV; Future trend; SARIMA; Prediction.

INTRODUCTION

Respiratory diseases are rampant and causing mortality across the globe. Precise diagnosis and treatment of respiratory diseases at molecular level is needed [1]. These respiratory diseases are infectious and non - infectious. Numerous studies have been established the patterns of infectious diseases such as SARS–CoV, MERS–CoV [2], Pneumonia [3] and Bronchiolitis [4]. Most recently a new virus, Coronavirus (2019-nCoV) has emerged in China and declared pandemic by World Health Organization (WHO) [5]. 2019-nCoVspread across the globe through carriers (air or human to human) and to-date poses a severe threat to mankind.Almost 210 countries across the globe are suffering from this pandemic disease.The spread of virus is escalating, despite the containment and stringent measures taken by authorities. The incubation period for this 2019-nCoV differs from person to person. However, Centers for Disease Control and Prevention (CDC) claims that the incubation period is between 2-14 days of exposure [6]. Liu et al reported that transmissibility of 2019-nCoV is very high than other viruses but the mortality

rate is low as compared with other viruses [7]. The situation report 1 published by WHO dated: 20January 2020 states that, 282 people were infected by 2019-nCoV from four countries such as China, Thailand, Japan and Republic of Korea [8] of which casualties were 6 in number. The situation report 66 published by WHO dated: 26 March 2020 states that, the number of confirmed cases across the globe was 4, 62,684.In India, the first case of 2019-nCoV was reported on 30 January 2020. The statistics about India reported by WHOas per situation update report -8 revealed that, 360 people were confirmed as infected with 2019-nCoV and 7 causalities as on 22 March, 2020 [9].As per the statistics published in [10], the number of 2019-nCoV confirmed cases as on 01stFebruary 2020 was one but by 30th April 2020, the number of active cases escalated to 25807 and the number of deceased was 1154.The number of people affected by 2019-nCoV is increasingalarmingly inspite of stringent containement of people. This intrigued authors to analyze the time dependent data and make an attempt to anticipate the statistical forecast.

Time series data analysis is a scientific method of analyzing data in such a way to extract meaningful inferences out of statistics and also permits to predict the future values based on the observed values of the past and present. Various methodologies have been used to analyze the time dependent data and the same are very well documented [11-16]. Methodology of best fit of a model is a more accurate approach in time series analysis [17]. In the present study, Seasonal Autoregressive Integrated Moving Average (SARIMA) modelwas constructed to analyze the dynamics of statistical data collected i.e. number of people affected by 2019-nCoVand execute a short term forecast of the same such that preventive measures can be taken as effectively as possible.Minitab was used for selection of apprpriate SARIMA model,for statistical analysis and forecast.

MATERIALS AND METHODS

Source of data

The data used for this study on prevalence of 2019-nCoV were collected on a daily basis from 01st February 2020 to 09th April 2020 from the official website of WHO (https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports), Ministry of Health and family Welfare, Government of India (https://www.mygov.in/covid-19) and https://www.covid19india.org/.Data from 01stMarch 2020 to 15th April 2020 were used to train the SARIMA model.Data collected from 16thApril 2020 to 30th April 2020 were used to testthe model and forecast was done for a period of ten days i.e. from 1st May 2020 up to 10thMay 2020.

Seasonal Autoregressive Integrated Moving Average (SARIMA) model

A Seasonal Autoregressive Integrated Moving Average (SARIMA) model is one step ahead of Autoregressive Integrated Moving Average model where the seasonal effects or trends are taken into consideration in the time series data [18]. SARIMA models mostly depend on seasonal lagsand also differences to fit the data. The general equation form of SARIMA model is SARIMA$(p,d,q)(P,D,Q)_x$. Where p,d,q stands for order of autoregressive, degree of differencing and order of moving average model respectively. P,D andQ are seasonal auto regressive, seasonal differencing and seasonal moving average terms respectively and x is the length of the period. The time series data was subjected to stationarity test and if any non-stationarity found,should be made stationary by differencing. In the present study, Minitab was used to check the data for stationarity. The plot of time series data before stationarity is presented in Figure 1(a). As the data was not stationary, the differencing of data was done using Box-Cox transformation and the same is presented in Figure 1(b). Kwiatkowski-Phillips-Schmidt-Shin (KPSS) test [19] shall also be used to check the stationarity. The non-seasonal and seasonal difference values were estimated. The autocorrelation function (ACF) and partial autocorrelation function (PACF) were depicted for the series data to confirm the parameters of auto regression and moving average, the same are presented in Figure 1(c) and Figure 1(d) respectively. Based on the ACF and PACF plots, the appropriate SARIMA mode should be selected. The determination of each parameter i.e. p, q, P and Q can be done by conventional method using maximum likelihood

estimation [20]. To reduce the ambiguity in selection, the auto ARIMA function in R 3.4.2 software was used. The same was verified with the values of Akaike Information Criterion (AIC) orBayesian Information Criterion (BIC) for standard SARIMA models. SARIMA(1,1,0)(0,1,2)$_7$was chosen from among various models with the AIC value of 683.29 which was minimum from among various models. The goodness of models fit was checked and Ljung-Box test [21] was performed to validate the estimated results for 'White Noise' check and found False. Forecasting for the time series was applied and also the reverse forecast was executed of the original data and verified.

RESULTS AND DISCUSSION

The adequacy of the SARIMA model was checked using residual plots and presented in Figure 2. It can be seen from the normal probability of residuals plot, Figure 2(a) that the residuals lie very close to the normality line. The plot of residuals against the fitted values exhibits no strong pattern, Figure 2(b). The histogram of residuals confirms the normality assumption holds good, Figure 2(c). The residuals plotted against time, Figure 2(d) shows that they are structure less and exhibits constant variance [22] subsequently; the assumption was satisfied for the model. Modified Box-Pierce (Ljung-Box) Chi-Square statistic was done to support the non-significant p values [16, 23] such that the model shall be deemed valid (p-value > 0.05 level of significance) and the results are presented in Table 1. In the present study, time series data from 1st March 2020 to 20th April 2020 were used for training and data collected from 21stApril 2020 to 30th April 2020 were used for testing of the model and forecast was done for a period of ten days i.e. from 1st May 2020 up to 10thMay 2020. The plot of forecast is presented in Figure 3.

CONCLUSION

The present study demonstrates the trend of 2019-nCoVcases in India. Data of number of people affected were collected and the database was prepared in MS Excel. Minitab was used for statistical analysis of data. The data of ten days were used to test the model and the results of estimations were compared with actual values. Short term forecast of 2019-nCoV cases in the near future was done using Minitab.This model shall be used to predict the values of ongoing pandemic, 2019-nCoV. The authors would propose to maintain social distancing and self-hygiene to break the chain of spreading of 2019-nCoV.The authors also propose to extend this model to incorporate number of people in incubation period. As such there is no specific criterion established about period of incubation.

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Table 1: Modified Box-Pierce (Ljung-Box) Chi-Square statistic values for SARIMA(1,1,0)(0,1,2)

SARIMA(1,1,0)(0,1,2)			
Lag	12	24	36
Chi-Square	34.9	37.3	38.6
DF	8	20	32
P-Value	0.00	0.011	0.197

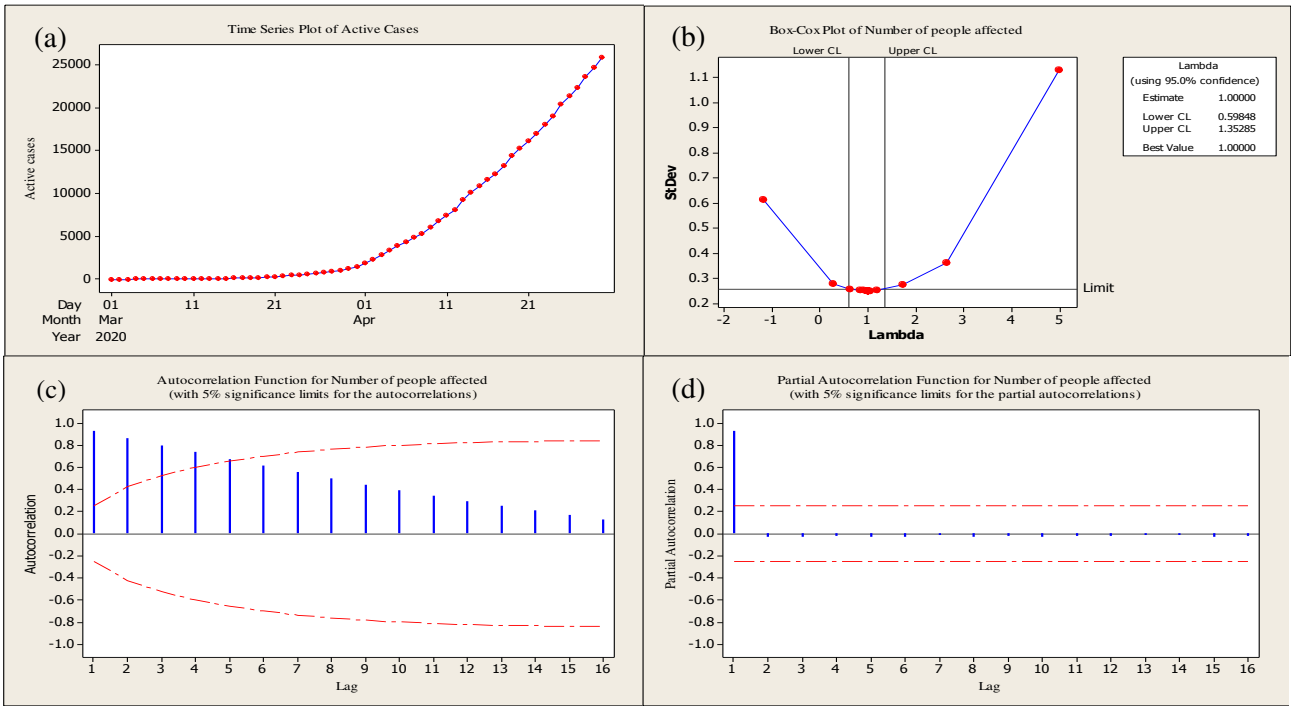

Fig. 1(a) Time series plot of number of people affected from 01-03-2020to 30-04-2020, 1(b) Box-Cox plot of number of people affected, 1(c) Autocorelation function for number of people affected and1(d) Partial autocorrelation function of residuals for number of people affected.

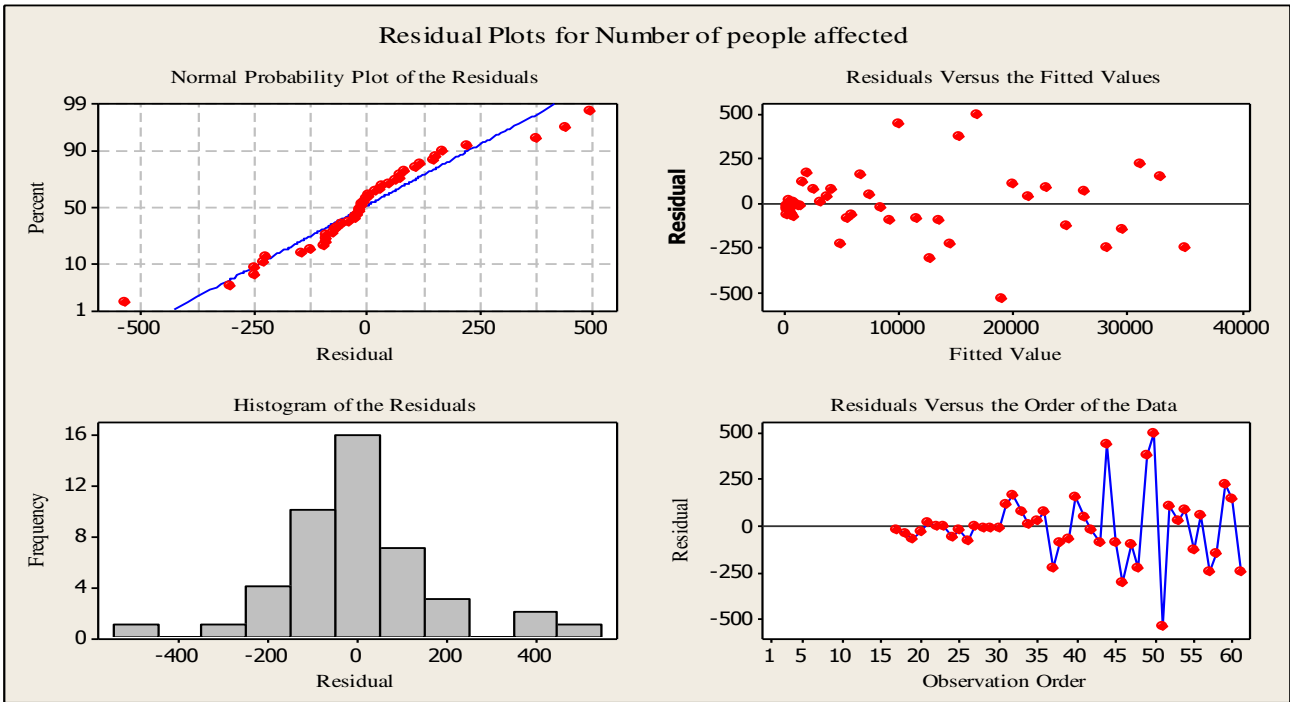

Fig 2. The residual plots for number of people affected

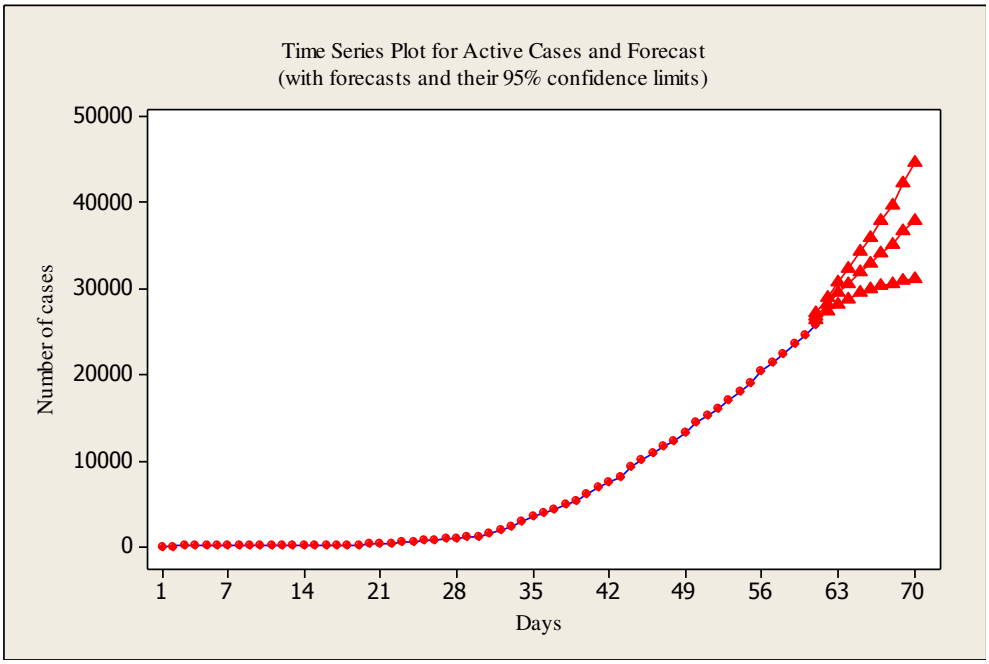

Fig 3. Time series of number of peopple affected upto 30th April 2020 and forecast up to 10thMay 2020

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Studies on Morphometric and Meristic Count of Species *Mystus vittatus*

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Received: 14 Feb 2020 Revised: 16 Mar 2020 Accepted: 18 Apr 2020

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ABSTRACT

The aim of the studies is to describe the morphometric and meristic characteristics of the fresh water fish species *Mystus vittatus* (Bloch,1794) in Nuagoan, Bhubaneswar, Odisha.Atotal10 specimen ranging from total length (9.9±12.3) that use to studies morphometric and meristic characteristics. There were 15 morphometric characters are measured. These morphometric characters were Total length (TL) (9.9±12.3)cm, Standard length (SL)= (8.1±9.6)cm, Forked length (FL) = (8.1±9.6) cm, Head length (HL) = (1.5±2.2) cm, Snout length (SnL) = (0.6±0.75)cm,Eye diameter(ED)=(0.5±0.6)cm, Pre-dorsal length(PDL)= (2.6±3.3), Pectoral fin length (PPL)=(1.5±1.9)cm, Pre-pelvic length (PPvL)=(3.5±4.1)cm, Pre-anal length (PAL) = (4.9±6.3)cm, Height of dorsal fin (HOD)=(1.6±2.5), Anal fin length (AL)= (0.6±1.7)cm, Body depth (BD) = (2.7±3.7), Caudal depth (CD)=(0.8±1.0), Caudal length (CL)= (2.1±2.9)cm. In these morphometric characters there statistical characters such as Arithmetic mean, Standard deviation, Correlation of co-efficient were measured .The Correlation of the all morphometric traits compared to Total length were TL/SL (0.964683) cm, TL/FL (0.975351)cm, TL/HL(0.637695)cm, TL/SnL(0.05417)cm, TL/ED(0.2246934)cm, TL/PDL(0.67222)cm, TL/PPL (-0.3019833)cm, TL/PPvL(0.311706)cm, TL/PAL(-0.18059)cm, TL/HOD (0.09384)cm, TL/AL (0.52552)cm, TL/BD (0.5235969)cm, TL/CD (0.810168)cm, TL/CL (0.821535)cm. Itindicates that the growth of *Mystus vittatus* in one part of the body is directly related to the other part of the body Pre-anal length (PAL) and Pre-pelvic length(PPL) show the negative correlationship with total lengthas compared to other morphometric traits.Maxillary barbel present from head to middle of the body. Teeth arranged uninterrupted semilunar band across the palate. Specimen had short anal fin, adipose fin small,dorsal spine weak, finely serrated on its inneredge. 4pairs of maxillary barbell present, lateral line is clearly visible from head to tail. Specimen was generally grey-silvery to shining golden. A narrow dusky spotis present on the shoulder.The fin glass with dark tips, steel black steep present side of the body. Above all the morphometric characteristics the species were identified as *Mystus vittatus* the fresh water fish.

Keywords; Morphometric traits, Meristic traits, Species identification

INTRODUCTION

The term "fish" is a group of poikilothermic (cold-blooded) aquatic vertebrates under the Phylum: Chordate that takes breathes with gills (Nelson, 2006). Catfishes are large group of fishes of the order: Siluri formes (Green wood *et al.*, 1966), there are present thirty one families in this order. Majority of catfish habitat are fresh waters, while two families are in marine. These fishes have naked that connect to the innerearand the swim bladder called the Weberian apparatus. A nadiposefin is present in between the dorsal fin and tail. A flap of fatty tissue is covered by skin. Mouth is bordered by dentaries and premaxillaries , that generally supplied with viliform teeth. The maxillaries are toothless, and base for maxillary barbels. Spinous rays are present in Pectoral and dorsal fins, these are the defense organs. Barbels help in locating food that surround the mouth are sensory in the function (Burgess, 1989). Bagridae family (Regan,1911)is a large family of Siluri formes which are fresh water cat fishes distributed in Ethiopean, Oriental and Palaearctic regions (Jayaram, 1955). The body of this family have elongatd and head is depressed or may or may not be granule, may be covered by thick or thin skin, the snout is blunty pointed rounded or spatulate. There are three to four pairs of barbels : maxillary pair are very long, then as alpair and two pairs of mandibular barbels. Mouth is transverse, crescent with jaws Teeth are arranged in bands and are usually viliform.

MATERIALS AND METHODS

Fish samples (n=10) were collected from Nuagaon near the Daya River. Morphometric measurements were measured by the help of divider, forcep, thread and measuring scale. Then the samples were preserved in a jar with formalin solution. Then the data were taken for statistical analysis (mean, standard deviation, co-relation) by the software Microsoft Excel to determine the relationship between the species.

RESULTS

SPECIES IDENTIFICATION

Adipose dorsal fin short, commencing after an interspace from the rayed dorsal fin, occipital process reaching basal bone/dorsal fin. Adipose dorsal fin base was shorter than anal fin base, median longitudinal groove on head, short/long as 1/2 fontanelles, not reaching base of occipital process.Body was with one or two longitudinal colour bands on the either side of lateral line.Body had with 3/4 longitudinal colour band above and below the lateral line.A dark shoulder spot no spot present at the base of caudal fin (Jayram, 1981). Maxillary barbels extend to between origin of ventral and middle of anal. Nasal barbels reach pre opercula, outer mandibular barbels extend to origin of pectoral fin ; first dorsal spine short, closely opposed to the base of second spine, body is elongated , upper surface of head is rough with peduncle.

COLOUR AND PIGMENTATION

Upper surface of the body and head dull grey, 4 horizontal band along flanks formed by melanophores an other band along the middor saline from napetocaudal base; black blotch behindoper cleand above the pectoral. Due to this characters the species are identified as *Mystus vittatus*

MERISTIC CHARACTERS COUNT

Meristic count sare countable characters. Example of meristic counts are then umber off in rays, number of lateral line scales. Meristic characters are use to measuring intra specific variation among the species.

SPECIES DESCRIPTION

Mystus vittatus (Bloch, 1794) Synonyms:
Silurus vittatus(Bloch 1794), *Aoria vittatus*(Bloch 1794*),*
Bagrus vittatus(Bloch 1794*), Macrones vittatus*(Bloch 1794),
Mystus vittatus vittatus (Bloch 1974)

Body is elongated, body with horizontal band present above or below the lateral line. Upper surface of head is rough with tubercles. Maxillary barbell reach to nearly middle of the body, median longitudinal groove reaching midway behind the hind border of the eye. Total length of the specimen that I observed 9.9-12.3 cm, head length is 8.1-9.6 cm, snout length from – 0.75cm, eye diameter from 0.6 –0.75 cm. Maxillary barbel present from head to middle of the body. Teeth arranged un interrupted semilunar band across the palate. Anal fin short, adipose fin small. Dorsal spine weak, finely serrated on its inner edge 4 pairs of maxillary barbel present, lateral line is clearly visible from head to tail.

COLOUR
Specimen generally grey-silvery to shining golden. A narrow dusky spot is present on the shoulder. The fin glass with dark tips, steel black steep present side of the body.

DISCUSSION

Sabbir *et al.,* (2017) studied on hepatosomatic index(HSI), aliment osomatic index(ASI), gastrosomatic index(GaSI), condition factor, relative length, food and their habitat of Nona Tengra(*Mystusgulio*)fromKhulnaRegionof West Bengal. They concluded that the sample were heterogeneous, mean of hepatosomatic (0.41- 0.06) and alimentosomatic index (0.53-0.083) indicate greater liver activity. They feed Planktons, crustacean, rotifera and shrimps so it identified as a carnivorous species. Brraich and Akhter (2015) studied on morphometric and meristic count of *Garra gotyla gotyla* from Ranjit Sagar wetland. They concluded that two characters were environmentally controlled and in eighteen charactersten characters were high value of co-relation show moderate co-relation. Lalwani *et al.,* (2019) analysis of morphometric and meristic characteristics of *Mystus bleekeri* from the Narmada River. They concluded that the co-relation between the total length and other parameter was found highest fork length and lowest pelvic length, that proportional was increasing the growth and increasing the total length. Aryani *et al.,* (2017) studied on morphometric characters of Asian catfish from Riau Province of Indonesia.

They concluded that more favourable morphometric characteristics of *H. swyckll* were found in Kampar Kanan River than Koto Panjang Reservoir and Kampar Uri River. Ahmad *et al.,* (2012) studied on morphometric relationship and condition factors of two freshwater Barbs *Puntius sophone* and *Puntius tictof*rom Padma River Bangladesh. They studies on the length-length, length-weight relationship for detecting their value, they concluded that data helpful for fishery management in Bangladesh and also all over the world. Chakkarvorty *et al.,* (2016) studied morphometric and meristic count of three *Mystus* species from Chandubi Beel Kamrup of Assam. They concluded that the total length of *M. bleekeri* was longest 97.48mm and*M. vittatus* was 83.9mm and their barbell length was 74.6% of total length.

That is varied geographical, climatic and nutritive factor. Rahman *et al.,*(2019) Studied on morphometric and meristic characteristics of Banded Gourami *Trichogaster fasciata* (Bloch and Schneider, 1801) from the Bangladesh. They concluded that *T. fasciata* was dorsal D: XV-XVII/ 10-14. Pectoral PC: 9-10 Pelvic and anal XV-XVIII/15-19 and caudal C: 18-20. Gupta *et al.,* (2018) studied on morphometric differences among the 5 species of subfamily Barbinae from Ganga River They analysis 20 morphometric and 10 meristic counts through traditional morphometric and truss network systems. The variation are same in both methods, geometrically these species are different. To identify the

correct analysis Traditional and Truss methods gives the correct classification of these species. F.A*et al.,* (2014) comparative studied on morphometric and meristic characters of family Lutjanidae, species *L. goreensis* (Valenciennes, 1830) and *Lagennes*(Blecker, 1863) from the Lekki lagoon and Badagry creek of Lagos state Nigeria. They observed there was no taxonomical variation among the species *L.goreensis* and *L.agennes* they were diverse genetically. Claytor and MacCrimmon (1985) studied on meristics and morphometric identity of Baltic stocks of Atlantic salmon (*Salmo salar*). Ward (2001) Studied on morphometric evaluation of whitefish complex in Bear Lake, Utah. Morphometric characteristics quantified the result from otolith aging and morphological analysis. In laboratory there is only one taxonomical group of *P. spilonotus* and *P. abyssicola* are distinguish from each other by using the measurement methods.

CONCLUSION

The present study highlighted that the variation in morphological characterisation is primary steps for the stock structure analysis of the species *Mystus vittatus*. The correlation between various morphometric traits was found to be positive except Pre-anallength (PAL) and Pre-pelvic length (PPL). In study the morphometric traits the maxillary barbel present from head to middle of the body. Teeth arranged uninterrupted semilunar band across the palate. Anal fin short, adipose fin small, dorsal spine weak, finely serrated on its inner edge, 4 pairs of maxillary barbel present, lateral line is clearly visible from head to tail. Specimens were generally grey-silvery to shining golden. A narrow dusky spot is present on the shoulder.The fin glass with dark tips, steel black steep present side of the body.

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Table.1. Meristic Characters Count

ACRONYMS	MORPHOMETRIC TRAITS	DESCRIPTION
TL	Total length	Measurementofbodylengthfromthe tip of largest jaw to largest part of caudalfin
SL	Standard length	Length from the snout to origin of caudal fin
FL	Forked length	Length from the snout to the point of bifurcation caudal fin
HL	Head length	Length from snout to the posterior most part of operculum
SnL	Snout length	Length from snout to anterior most margin of eye orbit
ED	Eye diameter	Maximum length of eye orbit from one margin to other
PDL	Predorsal length	Length from the snout to origin of dorsal fin
PPL	Pectoral pre-fin length	Length from snout to origin of pectoral fin
PPvL	Pre pelvic length	Length from snout to origin of pelvic fin
PAL	Pre anal length	Length from snout to origin of anal fin
HOD	Height of dorsal fin	Height of dorsal fin from base of origin of last anal fin ray
AL	Anal fin length	Length from origin of 1st anal fin ray to origin of last anal fin ray
BD	Body depth	Maximum vertical length of body(deepest part of body)
CD	Caudal depth	Minimum vertical length of the body (depth of caudal peduncle)
CL	Coudal length	Total length to standard length

Table 2. Species Description

Specimen	1	2	3	4	5	6	7	8	9	10
Total length	12.3	11.4	11.5	10.3	9.9	10.3	12.2	10.3	11	10.8
Standard length	9.6	8.8	9.2	8.4	8.1	8.3	9.2	8.2	8.6	8.6
Forked length	9.6	8.9	9.2	8.5	8.1	8.5	9.6	8.2	8.7	8.8
Head length	2.2	2.0	2.1	2.0	1.7	1.6	1.9	1.7	1.7	1.5
Snout length	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.75	0.7	0.6
Eye-diameter	0.6	0.6	0.5	0.6	0.5	0.6	0.6	0.6	0.55	0.5
Pre-dorsal length	3.3	3.1	3.0	2.8	2.9	2.6	3.1	2.9	3.0	3.3
Pre-pectoral fin length	1.9	1.6	1.4	1.6	1.7	1.9	1.5	1.9	1.7	1.9
Pre-pelvic length	4.1	3.8	3.7	3.7	3.5	3.9	3.6	3.8	3.5	3.8
Pre-anal length	6.3	5.6	5.4	5.6	5.5	6.2	4.9	5.8	5.5	6.0
Heightof dorsalfin	2.5	1.9	1.7	1.9	2.5	1.6	1.9	1.9	2.1	2.0
Analfin length	1.7	0.7	0.6	0.62	0.65	0.7	0.7	0.8	0.8	0.8
Body depth	3.2	3.7	3.0	3.3	3.3	2.7	4.0	2.8	3.3	3.1
Caudal depth	1.0	0.8	0.9	0.8	0.8	0.8	0.9	0.8	0.9	0.8
Caudal length	2.8	2.7	2.6	2.1	2.5	2.2	2.9	2.3	2.5	2.2

Table 3. Morphometric traits

MORPHOMETRIC TRAITS	MEAN IN CM.	STANDARD DEVIATION
TL	11	0.8334
SL	8.7	0.494413
FL	8.81	0.525885
HL	1.84	0.2319
SnL	0.695	0.36893
ED	0.565	0.047434
PDL	3	0.216025
PPL	1.71	0.185293
PPvL	3.74	0.183787
PAL	5.68	0.413118
SHOD	2	0.298142
AL	0.807	0.322216
BD	3.24	0.389301
CD	0.85	0.070711
CL	2.48	0.274064

Fig.1- Sample specimens of *Mystus vittatus*

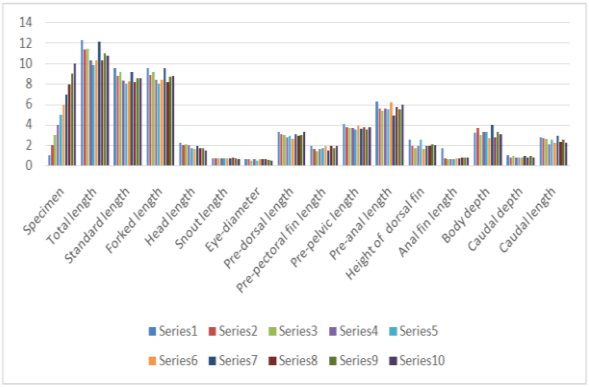

Fig.2- Graph showing all morphometric traits

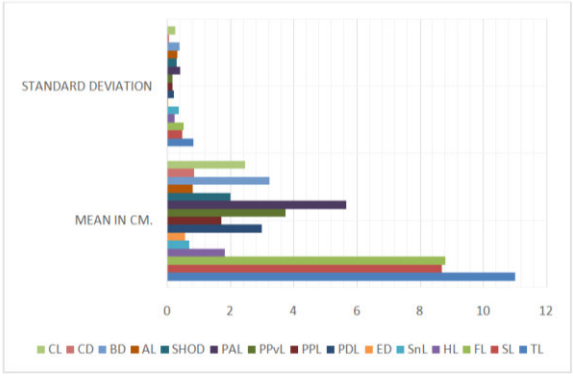

Fig.3-Graph showing the Arithmetic mean and Standard deviation of all morphometric traits

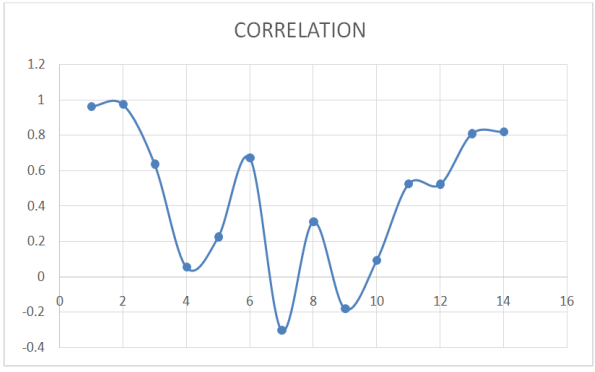

Fig.4- Graph showing Correlation between total lengths to all morphometric length.

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Investigations on the Detection of Disease on Leaves using Machine Learning Techniques

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Received: 02 Mar 2020 Revised: 04 Apr 2020 Accepted: 06 May 2020

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ABSTRACT

An agricultural area plays a vital role in the economy of nation. Agricultural output is very vital in several developing countries. Increase in population and growth in the life expectation is pressurizing the agricultural area to come out with new types of high yielding crops. The diseases in the plants are common, early detection and controlling the disease which increases the yield of a crop. Development of technology in the field of computer science can be useful to detect these diseases early. Image processing and classification methods can be applied to recognize the plant disease in the early stage. The features are passed on to the classifiers to classify the diseases. This research work has been framed to classify and distinguish the leaf disease based on its features. The proposed researchwork (AANN) implements the classification techniques, such as Advanced Artificial Neural Network (AANN), Support Vector Machine (SVM) and Naive Bayes classifiers to analyze and predict the disease based on the sample leaf images.

Keywords: Disease detection; accuracyAdvanced Artificial Neural Network; Support Vector Machine; Naive Bayes.

INTRODUCTION

Agricultural disease identification is one of the predominant areas of research. Identification of leaf diseases at an early stage will help the farmers to reduce pesticide usage. In recent days lot of research work happened on image processing, which are used to solve the problems in different agriculture applications like plant disease identification

[1-2]. Disease of plant can be identified through leaf by using various image processing and machine learning techniques [3-4]. For any image based framework image pre-processing is a basic step, which is a technique to enhance the image quality. The images can be captured from various means like cameras or sensors, space probes etc. In order to ensure the success of the subsequent steps the accuracy of this technique needs to be significantly high, hence dissemination and development of new technology is ankey factor in defining the future of farming. Manual classification methods are being used largely by the farmers, which will be done with the expert's advice on identifying the leaf diseases on basis of its geometric parameters. Even though, an accurate identification of leaf diseases is very important while classifying the leaf diseases and leaf type. This research work proposes a method that processes the captured digital image of leaves and extract the relevant features. To identify the types of diseases, the features like texture, shape and color of the leaf were used. Image processing techniques are applied to extract various features from leaves and to classify them based on its features. The collected features are then used for disease classification using AANN, SVM and Naive base classifiers.

LITERATURE SURVEY

Agriculture is the mother of all nations. Research in agriculture area is aimed towards increase the quality and quantity of the product at less expenditure with more profit. The quality of the agricultural product may be despoiled due to plant diseases. These sicknesses are produced by pathogens viz.., fungi, bacteria and viruses. Therefore, to detect and categorize the plant disease in early stage is a significant task. Farmers need constant watching of experts which might be excessively expensive and time consuming. Depending on the applications, many systems have been planned to solve or at least to decrease the problems, by making use of image processing and some automatic classification tools [5, 6].

SuhailiKutty et al. [7] planned the process to classify Anthracnose and Downey Mildew, watermelon leaf viruses. For this region of attention need to be identified from infected leaf sample based on RGB color component. Then to decrease noise and for segmentation median filter is used. And for group, neural network pattern recognition toolbox is used. Proposed method reached 75.9% of accurateness based on its RGB mean color component. The goal of SanjeevSannaki et al. [8] is to identify the disease using image processing and artificial intelligence methods on images of grape plant leaf. They categorize mainly two diseases, downy mildew and powdery mildew of grape leaf. Masking is used to remove training to improve accuracy. For preservinginformation of precious portion of leaf, Anisotropic Diffusion is used. Segmentation is carried out by k-means clustering method. After segmentation, feature extraction take place by controlling Gray Level Co-occurrence Matrix. And lastly classification is done using Feed Forward Back Propagation Network classifier. They have used only Hue feature which gives more perfect outcome.

Akhtar et al. [9] have used the support vector machine method for the classification and detection of rose leaf viruses as black spot and anthracnose. Authors have used the threshold method for segmentation and Ostu's algorithm was used to define the threshold values. In this approach, features of DWT, DCT and texture created eleven haralick features are extracted which are more used with SVM approach and shows effective accuracy value. S. Dubey and R. Jalal [10] travelled the concept of finding and classification of apple fruit diseases, namely, scab, apple rot and apple blotch. For that, segmentation is done using K-means clustering technique. Then features are extracted from the segmented image. For classification Multiclass Support Vector Machine (SVM) is used. UsamaMokhtar et al. [11] described technique of Tomato leaves diseases detection and diseases are: Powdery mildew and early blight. Image preprocessing involved several techniques such as softness, remove noise, image resizing, image isolation and background removing for image enrichment. Gabor wavelet transformation is useful in feature extraction for feature vectors also in classification. Cauchy Kernel, Laplacian Kernel and Invmult Kernel are applied in SVM for output decision and preparation for virus identification.

Sachin Khirade and A. B. Patil [12] discussed nearly the main steps of image processing to identify disease in plant and classify it. It includes steps like image acquisition, image preprocessing, image segmentation, feature extraction and classification. For segmentation, approaches like, otsu's method, converting RGB image into HIS model and k-means clustering are there. Among all, k-means clustering technique gives accurate result. After that, feature elimination is carried out like, color, texture, morphology, edges etc. Among this, morphology feature extraction gives better result. After feature extraction, classification is done using classification methods like Artificial Neural Network and Back Propagation Neural Network.

Bhog and Pawar [13] have combined the concept of neural network for the classification of cotton leaf disease examination. For segmentation, K-means clustering has been used. Different cotton leaf sicknesses are like Red spot, white spot, Yellow spot, Alternaria and Cercospora happening the Leaf. For testing, MATLAB toolbox has been used. The credit accuracy for K-Mean Clustering method using Euclidean distance is 89.56% and the execution time for K-Means Clustering techniqueby Euclidean distance is 436.95 second. Ms. Kiran R. Gavhale et al. [14] presented number of image processing techniques to extract diseased part of leaf. For Pre-processing, Image boost is done using DCT domain and color space change is done. After that segmentation take place using k-means clustering technique. Feature extraction is done using GLCM Matrix. For group of canker and anthracnose disease of citrus leaf, SVM with radial basis kernel then polynomial kernel is used.

PROPOSED METHODOLOGY

The main objective of this studypaper is to improve a leaf disease identification and classification system using its color, texture and shape features, which classifies the type of disease as well as the portion of the leaf affected by the disease using AANN, SVM and NB classifiers. Classification is used to identify the class of the new observation using training data whose class label is known. It is performed by using three different machine learning techniques. For classification Advanced Artificial Neural Network, Support vector machine and Naive Bayes classifiers are used.

Advanced Artificial Neural Network (AANN)

AANN is a mathematical model used for pattern recognition. This concept is derived from biological human neurons system. In this each node behave like neuron. There are different type of AANN is there based on its network structure. In that Back propagation neural (BPN) network is one and which is widely used. In BPN error is back propagated from output layer to input layer for correction. It can handle noisy data effectively and well suited for complex problems. AANNs are trained using large collections of images. From these large collections, AANNs can learn many of the feature representations for the whole of the collection of images. As an easier way to perform the classification without shedding time and effort, they are trained prior as an extractor of the features. The following are the steps involved:

Algorithm: AANN Classifier

Step 1: Load feature vector of the leaf.
Step 2: Calculate the predictor variables in each class.
Step 3: Initialize weights to all neuron and evaluate the termination criteria.
Step 4: Compute Gradients Vector v.
Step 5: Estimate the conjugate direction between the hidden layers.
Step 6: Recomputed the optimal output weights and update output layer.

Support Vector Machine (SVM)

Support vector machines can have good prediction speed and memory usage with few support vectors. Best hyper-plane is found which separates one class of data points from the data points of other class. The hyper-plane which has the maximum margin between the support vectors of two classes is known as the best hyper plane. The slab containing maximal width which is parallel to the hyper plane is referred to as the margin. The classifier will assign the label to the photograph and it specifies which category it belongs to, from where the classifier is predefined primarily based upon the feature. This classification is used for each study and the trying out phase. SVM makes use of the method referred to as the kernel. To classify the images using SVM is obtained by taking into account the training feature vector comparing with the testing feature vector to conclude target class, as

$P = \{(x,) \mid xi \in Rn, i \in \{-1, 1\}\} t1 = 1)$ ….. Equ (1)

The SVM is used for the classification of the disease which are classified by using the selected features of the texture and color.

Algorithm

Step 1: Load feature vector of the leaf.
Step 2: Calculate the predictor variables in each class.
Step 3: Optimize a_i and a_j to select closest Attribute pair.
Step 4: Compute the weight vector of dimension lengths.
Step 5: Calculate the ranking criteria for all i.
Step 6: Find the feature with smallest ranking standard.
Step 7: Apprise feature ranked list.
Step 8: Finally classify the feature with smallest ranking criterion.

Naive Bayes (NB) Classifier

Based on Bayes theorem classification is performed using probabilistic analysis, which is a statistical classifier. A computational model in plant identification system of digital images of plants utilizing biometric features such as shape and vein patterns via hidden naïve Bays classifier. Naïve Bayesian classification is used to find the probability of input leaf image fits to the each cluster. Input leaf image goes to the cluster which has the maximum probability. Naïve Bayesian classifier is based on the Bayes theorem is given as follows:

$$P\left(\frac{Ci}{X}\right) = \frac{P\left(\frac{X}{C}\right)P(C)}{P(X)} \quad \text{…..Equ(2)}$$

Where, X: feature vector of the given leaf, C: Leaf clusters, i: Number of Clusters.

Algorithm

Step 1: Load feature vector of the leaf.
Step 2: Compute the predictor variables in each class.
Step 3: Determine the probability of f_i using the gauss density equation in each class.
Step 4: Till the probability of all predictor variables (f_1, f_2, f_3,…..f_n) has been calculated.
Step 5: Compute the likelihood for each class.
Step 6: Get the greatest likelihood.

Step 7: Update likelihood feature.
Step 8: Finally classify the feature with greatest likelihood feature.

Performance evaluation

The classifiers will produce the, disease type as well as the portion of disease affected area in terms of percentage. The performance evaluation of the obtained result is done based on the values of accuracy.

Accuracy

It is the ratio of reflection predicted correctly to the total number of explanations. It is calculated as,

$$Accuracy = \frac{Correctly\ Predicted\ Observation}{Total\ Number\ of\ Observations} \quad \text{..... Equ (3)}$$

Percentage of disease affected to the leaf is calculated using Equation (4). This will give the severity of the disease. This information will help the farmers to decide or select amount of pesticide to be used.

$$Percentage\ of\ disease = \frac{Pixels\ in\ disease\ portion}{Total\ pixels\ in\ leaf\ area} \quad \text{..... Equ (4)}$$

RESULT AND DISCUSSION

The Figures presents a comparison of the best results achieved by each method. It appears that much better results in classification were obtained using AANN than SVM and Naive Bayes. It also seems that AANNs are more resistant to insufficient data amount, because even for small set of datasets results were satisfactory. That cannot be said about SVM and Naïve Bayes, which gives less accuracy as compared to AANN in datasets.

Accuracy

Accuracy is the proximity of measure results to the true value; precision, the repeatability, or reproducibility of the measurement. In the fields of science, engineering and statistics, the exactness of a measurement system is the degree of closeness of measurements of a portion to that quantity's true value. Accuracy of AANN is always better than SVM and Naive Bayes in classification of integer datasets. Comparison of Accuracy using AANN,SVMand Naive Bayes is presented in figure 1.

CONCLUSION

AANN perform best in classification when talk about accuracy. CPU time is divided into two times: training time, testing time. Experimental results indicate that SVM performed significantly better than AANN and Naïve Bayes when talk about training time but in testing AANN performs better. In the Neural Network, number of hidden nodes is already decided but support vector machine generates number of support vectors by the model produced by training process itself. For the comparison of Neural Network and Support Vector Machine we used equal number of hidden nodes in Neural Network and number of Support Vectors in Support Vector Machine. The proposed methodology gives the 99% accuracy. At last we analyses that overall AANN is better than SVM and Naïve Bayes.

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Table 1. Accuracy comparison of three classifiers

Disease Class	AANN Classifier(Proposed)	SVM Classifier	Naïve Bayes Classifier
Cercospora Leaf Spot	99%	89%	80%
Bacterial Blight	98%	85%	66%
Powdery Mildew	99%	91%	91%
Rust	100%	89%	97%
Average	99.00%	88.50%	83.50%

Table 2. Error rate comparison of three classifiers

Disease Class	AANN Classifier(Proposed)	SVM Classifier	Naïve Bayes Classifier
Cercospora Leaf Spot	2%	17%	20%
Bacterial Blight	4%	51%	34%
Powdery Mildew	2%	9%	9%
Rust	0%	11%	3%
Average	0.80%	19.80%	15.40%

Figure 1: Original image of the leaf affected by Bacterial Blight disease

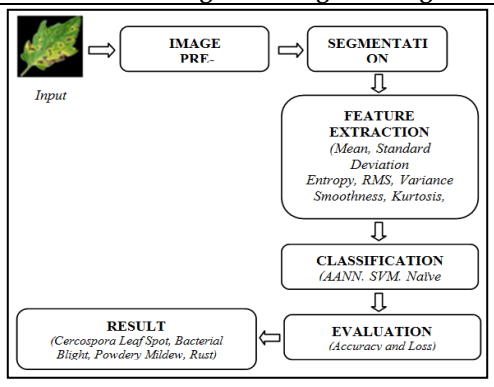

Figure 2: Overall Architecture of the proposed Methodology

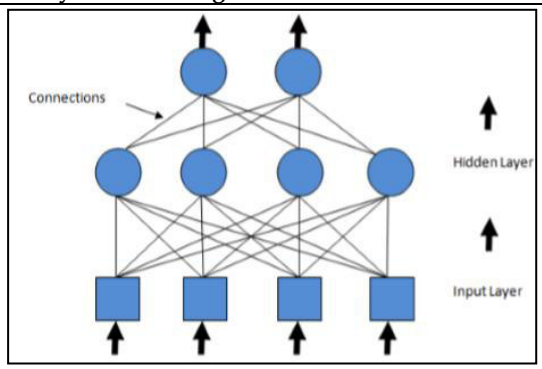

Figure 3: Network diagram of AANN classification problem

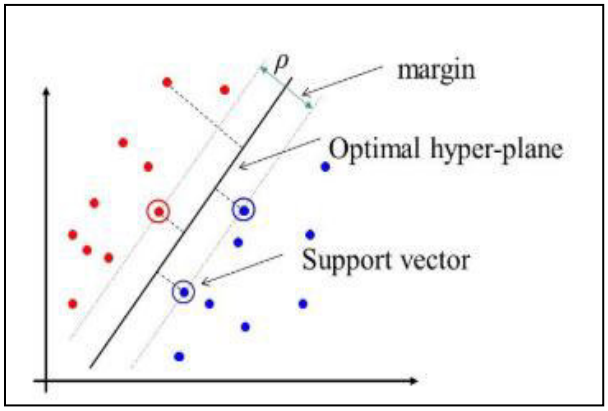

Figure 4: Optimal Hyper Plane separating Support Vectors in SVM

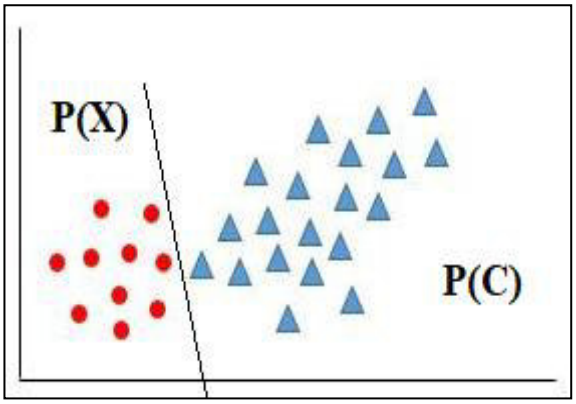

Figure 5: Cluster framing based on Probability using Naïve Bayes

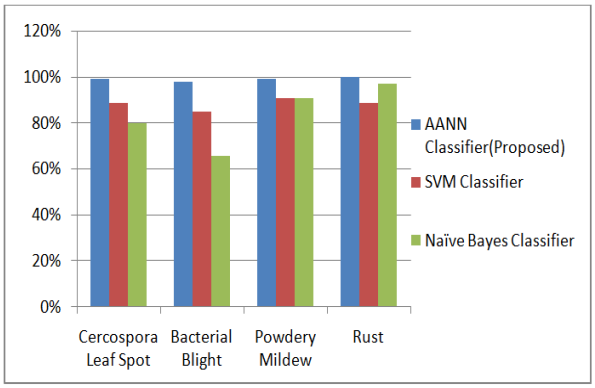

Figure 6: Accuracy Comparison based on AANN, SVM and Naïve Bayes Classifier

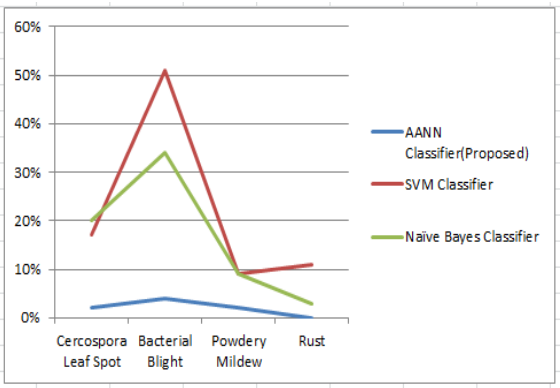

Figure 7: Error Rate Comparison based on AANN, SVM and Naïve Bayes Classifier

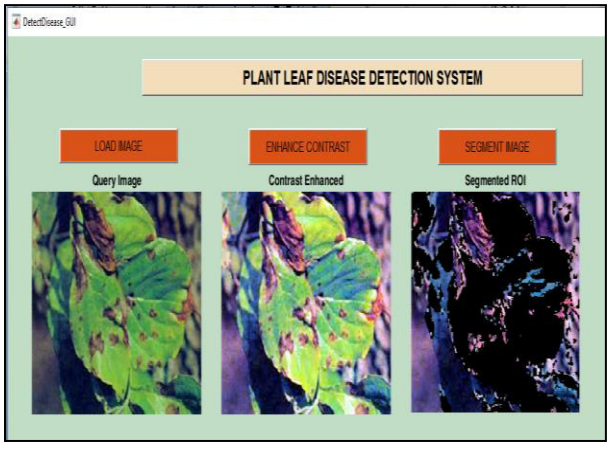

Figure 8: Image Pre-processing using Color Transformations and Masking Green Pixels

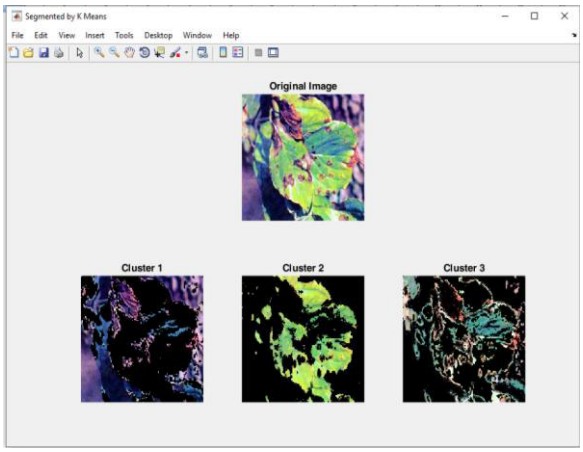

Figure 9: Segmentation of Region of Interest by k means clustering algorithm

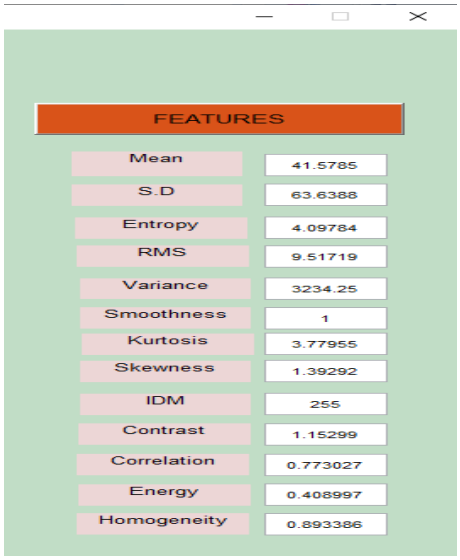

Figure 10: Important features measures extracted from the segmented Leaf Image

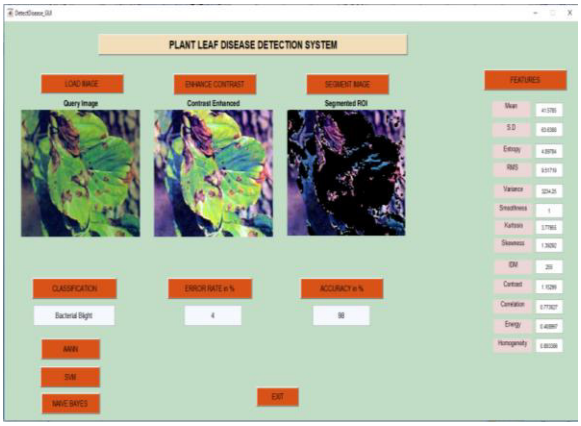

Figure 11: Framework for Plant Leaf Disease Detection System using Machine Learning Techniques

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Studies on Morphology of Some Grasshopper in CUTM Campus, Bhubaneswar

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Received: 14 Feb 2020 Revised: 16 Mar 2020 Accepted: 18 Apr 2020

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ABSTRACT

Grasshoppers are generally found in cultivated crops, grasslands woodlands and deserts semiarid regions of the world which belong to the order Orthoptera of phylum Arthropoda. Grasshoppers are the crucial pest, which eat green leaves, so it is known as herbivorous insect. Sometimes, these serious pests destroy crops over wide areas. Like all insects, the body of grasshoppers is divided into three main regions: head, thorax, and abdomen. Grasshoppers are enriched with source of protein, carbohydrate, fiber, fat, so it have been eaten in Southern Mexico and Central America. For the study on identification and morphological characterization, 30 individuals were collected from CUTM campus District Khordha, Odisha, India. These samples were identified with the help of standard taxonomic keys and different morphological characteristics slightly different pattern recorded. Then these samples were preserved in 70% formalin solution for further study. The present study record shows that the collected species were distributed evenly. Due to the detailed identification features and patterns of morphological variation four types of species were recorded. Those species includes *Spathosternum prasiniferum prasiniferum, Oxyahylahyla, Acridaexaltata, Diabolocatantops pinguise*. The present study provides the important information on the morphological variation in different species of Grasshopper from the CUTM campus of khordha, Odisha, which will be useful in acquiring a better understanding of the economic importance of this pest and planning more appropriate and effective control measures in future.

Keywords: Grasshopper, Identification, morphological variations.

INTRODUCTION

Grasshoppers are herbivorous insects of the suborder caclifera in the order orthopteran. Acrididae includes more than 8000 species of grasshoppers and locusts distributed worldwide (S Ramani, PrashanthMohanraj, Yeshwanth HM 2019). They are found in almost all types of habitat including the tropics, temperate grassland, deserts and mountains region. Each species has unique characteristics when it comes to geographic location, anatomy and trends in behavior. Grasshopper and crickets are similar insects both being of the order orthopteran, but they are different and actually, are in different scientific suborders. Grasshoppers are plant eaters. Due to their relatively short horned antennae, Acrididae are sometimes referred to as the short-horned grasshoppers. However, other families that are sometimes thought of as grasshoppers include the Tetrigidae or pygmy grasshoppers, the Eumastacidae or monkey grasshoppers, the Tanaoceridae or desert long horned grasshoppers, the Tettigoniidae or long horned grasshoppers or katydids; the Gryllacrididae, or wingless long horned grasshoppers. Blatchley (1920) and Helfer(1972) .Other than the acridid, only the tettigoniids are known to people as katydids.

The system of classifying grasshoppers by earlier workers was mainly based on easily recognizable externally visible character. (Slifer& King, 1936; Slifer,1939;1940;1943; Dirsh 1975a,band Meinodas et al.,1982) showed the taxonomic significance of spermathecal in Acridoidea. They are typically ground dwelling insects with powerful hind legs which enable them to escape from predators. They are mostly solitary and residential species often abundant as individuals but which may occasionally migrate. It is a cold blooded animal. Some grasshopper species can change colour and behavior and form swarms at high population densities and under certain environmental conditions. Whenpredators comes, many species were brilliantly changed their coloured, while jumping and (if adult) launching themselves into the air, usually flying for only a short distance.

Grasshopper is relatively a large insect reaching up to 8 cm in length. Body is narrow, elongated, cylindrical and bilaterally symmetrical (Rastogi and Kishore, 1997). Body of grasshopper is yellowish or brownish with different colour spots and markings. The AK plant grasshopper (*Poecilocerus pictus*) (Fabricius,1775) is blue and yellow in colour. The body is covered by exoskeleton having chitin. Each body segment is made up of separate hard exoskeletal plates, called as sclerites. Body of grasshopper is segmented. Grasshopper is divided into 3 region such as (1-head, 6-fused segments, 2-thorax, 3-segments, 3-abdomen, 11-segments).Grasshopper also have six jointed foot, 2 pairs of wings, and 2 antenna. The mantis have 5 eyes (2- compound eye, and 3-ocelli).Metamorphosis has 3 stages such as egg, nymph and adult. Head is at end of the grasshopper's body and is the location of the brain, the two compound eyes, the mouth parts, and the points of attachment of its two antennae. Eyes are made up of hexagonal lenses. The jaws are located near the tip of the head, by the palps the jaws crush the food. Palps are long, segmented mouth parts that grasp the food. It is the middle region of body attached to head and abdomen by flexible joints. The three segments of thorax are prothorax, mesothorax and metathorax.

In grasshopper, each of the mesothorax and metathorax bears a pairs of wings. Each wing is supported by numerous longitudinal cuticular thickenings called nervures or veins. Abdomen is slender, cylindrical, and consisting of 11 segments. Each segment typically has a dorsal tergum and a ventral sternum, there being no pleura. 8 pairs of small spiracles are present. Male grasshopper is smaller than female in size. In female grasshopper, abdomen is more tapering or pointed than in male. Most of the internal space or body cavity is not a true coelom but a haemocoel filled with haemolymph or blood. Muscles of grasshopper are striated type. They are soft, delicate but strong. In abdomen they are arranged segmentally for respiratory and reproductive movements. The alimentary canal consists of the three principal parts: foregut, midgut and hindgut. Foregut and midgut are lined with cuticle. They move from plant to plant by walking, hopping and flying in order to feed upon their leaves. Circulatory or blood vascular system is of open or lacunar type. Blood is confined to vessel (heart and aorta) during circulation. The principal excretory organs are a number of minute, slender, blind and thread like Malpighian tubules, lying coiled about in

haemocoel. Removal of dry excretory wastes is characteristic of small land animals that have only a limited water supply. Sexes are separate.

MATERIALS AND METHODS

Study area

Centurion University of Technology and Management is the first multisector, private state university from Odisha, India. With its main campus at Paralakhemundi in the Gajapati district and our campus located at Jatni, on the fringes of Bhubaneswar. It was accorded the status of a university in the year 2010. It spread over 4o acres land in the foothill of Barunei hills, near Jatni town, the campus is adjacent to National Institute of technology.

Collection and Preservation of Sample

Grasshopper were collected by hand and by sweeping using an aerial insect net. The net was used for catching insects individually or by sweeping over crops. The insects caught were transferred to a bottle that containing cotton soaked in ethyl acetate, to kill the specimen. Once killed, the specimen was removed from the bottle to prevent morphological changes, specifically colour change.

Identification

Specimens were first relaxed (right wings were stretched putting a piece of paper on it, and pinned by inserting a pin on the posterior right on thorax) on a stretching board and left to dry for 72 hours. The specimens were later identified up to species level on the basis of external morphological characters with the help of binocular microscope and keys in available literature.

Morphometry

This study was done (in mm) with the help of Vernier calipers, thread, scale, divider, slide, microscope. Total body length, head, thorax, abdomen, wing, fore limb, hind limb, femur, tibia, antennae, pronotum were measured.

Storage

Pinned specimens were kept in storage boxes and cabinets, with naphthalene 70% ethyl alcohol in plastic vials.

RESULTS AND DISCUSSION

SPECIES-1

The specimen are green in colour.Body small sized and cylindrical in shape. Head is convex.Antenna is 21 segmented.A black band is running from behind eyes to pronotum along lateral carinae. Tegmina light brown or green. Fastigium of vertex wide, slightly depressed, apex parabolic. 10-11 spines are present.Mesosternal lobes are separate.Dorsum flattened with three transverse sulci. Males are smaller than female. Due to the above characteristics the specimen is *Spathosternum prasiniferum prasiniferum* (Walker, 1871).

SPECIES-2

Body colouration is green. A brown band start from each eye along superior margin of lateral lobe continuing uptoepisternum. Head is smooth. Eyes are oval in shape. Antennae is filliform and slightly shorter than head and

pronotum together. Fastigium is horizontal. Apex is rounded, smooth, and gradually merging with frontal ridge. Prosternal process is subconical, anteroposteriorly compressed. Hind tibia with eight dorso-external spines and dorso-internal spines. Four transverse sulci are present.Due to the above characteristics the specimen is *Oxya hyla hyla (Serville, 1831).*

SPECIES-3

The specimen are green in colour.Wings are infuscated. Head is long, conical, sloping upwards. Fastigium of vertex long extending well beyond eyes, wider than distance between eyes, slightly concave. Apex roundly trunicate at extremity. Fastigialfoveolae absent. Antennae is ensiform. Pronotum compressed, hind margin acutely angular. Prozona shorter than metazona.Due to the above characteristics the specimen is *Acrida exaltata (Walker, 1859)*.The biological studied is done by Bhowmik and Halder.

SPECIES-4

The colour of the specimen is brown in colour. Head is obtusely conical. Fastigium of vertex wide, trapezoidal. Frontal ridge is flat. Fastigialfoveolae is lacking. Pronotum is finely tectiform. Lateral carinae is absent. Mesosternal interspace open, moderately wide, lobes rounded, slightly wider than long. Median carina obtusely present. Due to the above characteristics the specimen is *Diabolocatantop spinguise.*

DISCUSSION

Grasshoppers have chewing mouthparts and are commonly thought of as foliage feeders, but they also feed on flowers, fruits, seed heads, stems, and essentialy all aerial plant parts, causing considerable damage to agricultural crops, pastures, and forests (Joshi et al. 1999).Grasshoppers are widely distributed in Odisha. Grasses have poor nutrition as compared to other crops (Tscharntke and Greiler, 1995) hence grasshoppers tend to move on crops or other mixture of plants for their nutritional requirements for better growth and reproduction (Behmer and Joern 1993). The impressive growth of Indian agriculture has helped the country achieve food security at national level. From ANOVA it was observed that in all the four species, the degree of freedom (df) between the group is 5 and within the group is 36. The value of p between the groups is 0.9925 in *Spathosternum prasiniferum prasiniferum*, 0.9856 in *Oxya hyla hyla,* 0.8792 in *Acrida exaltata,* and 0.9795 in *Diabolocatantops pinguise*. Similarly the value of F between the groups is 0.09486 in *Spathosternum prasiniferum prasiniferum,* 0.1258 in *Oxya hyla hyla,* 0.3499 in *Acrida exaltata* and 0.1776 in *Diabolocatantops pinguise.*

CONCLUSION

This experimental study record as describe about the four different types of the species and their morphological characterstics. Due to the chewing type of mouthparts grasshopper that tear away plant tissue commonly thought of as feed on flowers, fruits, foliage and stem. The paddy fields are heavily infested by grasshopper.

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Table 1.Calculated length, mean and standard deviation of *Spathosternum prasiniferum prasiniferum*

Measurement (mm)	Male			Female			Male		Female	
							Mean	±SD	Mean	±SD
Body length	15.56	15.80	16.19	18.58	19.22	19.72	15.85	±0.25	19.17	±0.46
Head	2.73	3.15	3.68	3.48	3.96	4.24	3.19	±0.38	3.89	±0.31
Thorax	3.16	3.57	3.98	3.68	4.12	4.56	3.55	±0.33	4.12	±0.35
Abdomen	5.56	5.98	6.24	5.78	6.12	6.34	5.92	±0.28	6.08	±0.23
Femur	8.46	9.28	9.64	9.48	9.76	10.74	9.12	±0.49	9.99	±0.54
Tibia	7.56	7.78	8.24	8.38	8.96	9.52	7.86	±0.28	8.95	±0.46
Antennae	4.26	4.56	4.87	3.14	3.44	3.88	4.56	±0.24	3.48	±0.30

Table 2: Test for equal means (ANOVA) of *Spathosternum prasiniferum prasiniferum*

	Sum of the square	Df	Mean square	F	P(same)
Between groups	11.9629	5	2.39259	0.09486	0.9925
Within groups	907.984	36	25.2218		Permutation p(n=99999)
Total	919.947	41			0.9927

Table 3.calculated length, mean and standard deviation of *Oxya hyla hyla*

Measurement (mm)	Male			Female			Male		Female	
							Mean	±SD	Mean	±SD
Body length	21.93	22.56	23.17	21.89	22.56	25.22	22.55	±0.50	23.22	±1.43
Head	5.15	5.39	5.92	6.17	6.54	6.79	5.48	±0.32	6.5	±0.25
Thorax	6.17	6.53	6.66	7.14	7.44	7.98	6.45	±0.20	7.52	±0.34
Abdomen	10.45	10.62	11.13	11.67	11.86	12.15	10.73	±0.28	11.89	±0.19
Femur	15.77	16.43	17.54	18.97	19.35	20.78	16.58	±0.73	19.7	±0.77
Tibia	14.92	16.79	17.11	16.98	18.34	19.56	16.27	±0.96	18.29	±1.05
Antennae	7.96	8.11	8.15	6.50	7.35	7.97	8.07	±0.08	7.28	±0.59

Table 4: Test for equal means (ANOVA) of *Oxya hyla hyla*

	Sum of the square	Df	Mean square	F	P(same)
Between groups	27.4862	5	5.49724	0.1258	0.9856
Within groups	1572.79	36	43.6886		Permutation p(n=99999)
Total	1600.28	41			0.9854

Table 5.calculated length, mean and standard deviation of *Acrida exaltata*

Measurement (mm)	Male			Female			Male		Female	
							Mean	±SD	Mean	±SD
Body length	41.54	43.33	46.89	61.32	64.35	67.57	43.92	±3.85	64.41	±4.42
Head	10.42	10.88	11.24	12,62	13.56	14.28	10.84	±0.58	13.48	±1.17
Thorax	11.78	12.24	12.56	13.44	13.82	14.56	12.19	±0.55	13.94	±0.63
Abdomen	19.54	20.34	20.78	20.44	20.73	21.95	20.22	±0.88	21.04	±1.13
Femur	25.66	27.44	30.78	35.12	37.78	38.56	27.96	±3.67	37.15	±2.55
Tibia	24.86	27.34	29.64	34.52	35.38	37.74	27.28	±3.38	35.78	±2.36
Antennae	11.97	12.33	12.98	11.64	11.82	12.28	12.42	±0.72	11.91	±0.46

Table 6: Test for equal means (ANOVA) of*Acrida exaltata*

	Sum of the square	Df	Mean square	F	P(same)
Between groups	444.861	5	88.9722	0.3499	0.8792
Within groups	9165.99	36	254.611		Permutation p(n=99999)
Total	9610.85	41			0.8866

Table 7.calculated length, mean and standard deviation of *Diabolocatantops pinguise*

Measurement (mm)	Male			Female			Male		Female	
							Mean	±SD	Mean	±SD
Body length	29.11	31.56	32.55	37.88	38.63	39.67	31.07	±2.50	38.72	±1.27
Head	5.96	6.55	7.17	6.45	7.61	8.12	6.56	±0.61	7.39	±1.21
Thorax	7.23	7.56	7.99	8.20	8.31	8.59	7.59	±0.53	8.36	±0.28
Abdomen	12,57	13.48	14.43	13.45	14.81	15.87	13.49	±1.31	14.71	±1.71
Femur	16.77	17.85	18.05	18.96	20.34	21.56	17.55	±0.97	20.28	±1.83
Tibia	15.36	16.52	17.64	17.38	17.72	18.99	16.50	±1.61	18.03	±1.20
Antennae	7.68	7.33	8.97	8.72	8.97	9.12	7.99	±1.22	8.93	±0.63

Table 8: Test for equal means (ANOVA) of *Diabolocatantops pinguise*

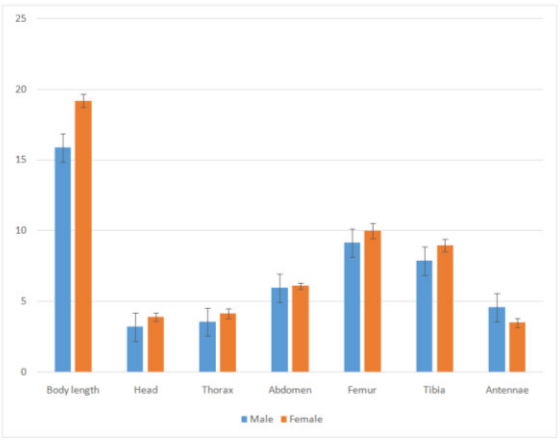
	Sum of the square	Df	Mean square	F	P(same)
Between groups	71.5897	5	14.3179	0.1476	0.9795
Within groups	3492.52	36	97.0145		Permutation p(n=99999)
Total	3564.11	41			0.9805

Figure 1. ***Spathosternum prasiniferum prasiniferum***

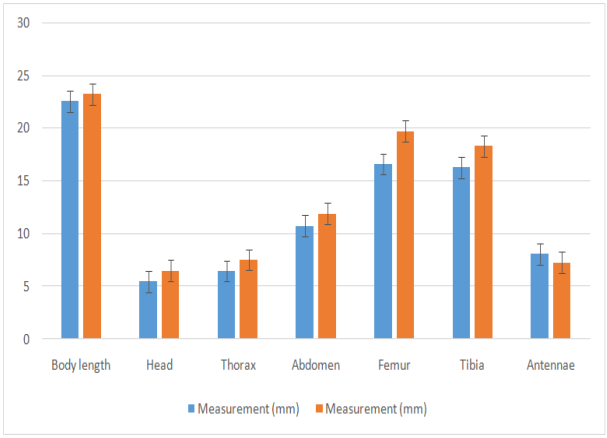
Figure 2.*Oxya hyla hyla*

Figure 3. ***Acrida exaltata***

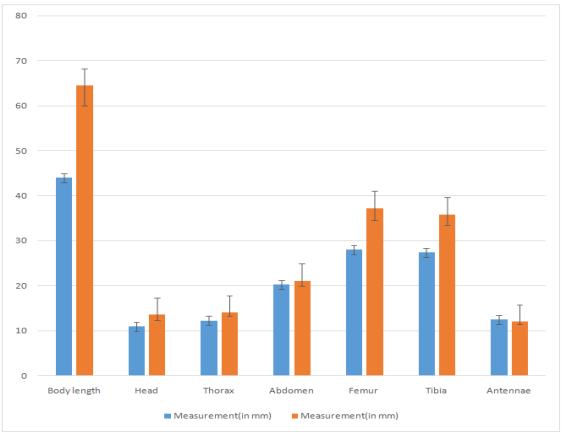
Figure 4.*Diabolocatantops pinguise*



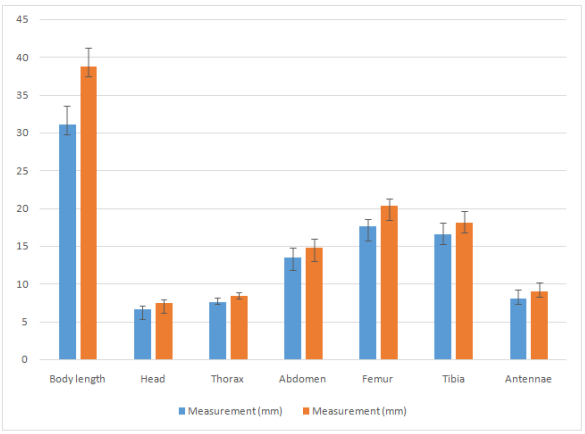
Graph.1. *Spathosternum prasiniferum prasiniferum*



Graph.2.*Oxya hyla hyla*



Graph.2. *Acrida exaltata*



Graph.3. *Diabolocatantops pinguise*

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

SHORT COMMUNICATION ARTICLE

A Comrade As a Guardian Angel Battling for the life of a Bezzie in O'Henry's Short Story 'The Last Leaf'

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Received: 04 Mar 2020 Revised: 06 Apr 2020 Accepted: 08 May 2020

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ABSTRACT

The short story is a different genre in the field of Literature. He focuses on the main plot with all the delicious ingredients of pleasure, thrill, and excitement to quench the thirst of craving of the readers in the vast pool of American Literature. O' Henry is a great American short story writer who has handled the tool of short story in an artistic way and most of the issues that had been dealt with is relevant in the scenario of contemporary world. 'The Last Leaf' is O Henry's master piece in which he has conveyed a powerful message of humanitarianism and friendship that could save lives. This is really the need of the hour to make the world a better place to live in. He is very unique in entwining surprise shocks, terrible twists in all his short stories which make the readers thrilled and peppy when they travel through all the aspects of the story. The story depicts the struggles for survival, pain of poverty, loss of laughter and longing for lights of success of the lower class people in American society. This paper aims to focus on 'A Comrade As A Guardian Angel Battling For The Life Of A Bezzie In O'henry's Novel, 'The Last Leaf''

Key words: excitement, contemporary, humanitarianism, entwining, peppy, Comrade, Bezzie.

A short story is a brief work of prose fiction, and most of the component elements, the types, and the various techniques of the novel are applicable to the short story as well. The plot form may be comic, tragic, romantic or satiric; the story is presented to us from one of the many points of views; and it may be written in the mode of fantasy, realism or naturalism. The influence of the short story has been international. Its popularity has grown and spread to and from England, France, Russia and America. As

a youth, Kipling achieved world-wide success with tales from India. The French author, Gue de Maupassant, had a whole troop of followers to every country. The Russian, Chekhov, came to have considerable influence upon short story writers between 1900 and 1920, while the Americans, Ernest Hemingway and William Saroyan have been widely imitated more recently. The modern short story writers like Poe and O' Henry have contributed a lot to modern English literature with a variety of their short stories. The short story consists of various elements. The elements are plot, character, setting, point of view, theme and style.

O' Henry is a minor classic who occupies a permanent, unique spot in American literature. It has to be recognized that at the core of O' Henry's being lays an element of surprise and wonder. The most obvious technical manifestation of O' Henry's delight in the unexpected is, of course, in his famous surprise endings; for scarcely a single story among his three hundred fails to meet his specifications for a conclusion other than the one the reader is apparently being prepare for O' Henry's stories are liberally sprinkled with asides in which he addresses the reader in familiar tone. Coincidence figures largely in his stories, and they often have a surprise twist, or "Snapper", as O' Henry called it. In addition, he usually made his contrived stories illustrate some more or less serious theme.

O' Henry's stories have a variety of settings, but most of them are laid in either New York or Texas. His characters include shop girls and millionaires, policemen and burglars, cowboys and tramps, confidence men and southern gentlemen and other assorted types. He is usually that of extensively talkative tale teller with slangs and usage of trig words for humorous effect. He usually used stock story formulas and he had a gift for devising ingenious variations on them. O' Henry's pervasive tendency to lay bare the construction of a story and subject the plot to periodic play, the "Unexpectedness" of his ending acquires special meaning. Unexpectedness of ending is the most striking and consistently commented on feature of his stories, the unexpectedness, moreover, being almost invariably of the "Happy Ending" variety. For O' Henry this quality of the unexpected constitutes the very heart of the construction and bears a perfectly special character.

'The Last Leaf" takes place in Greenwhich village, a bohemian neighborhood in New York city, sometime in the early 20th century. Greenwich Village is a haven for poor artists and bohemian young people and O' Henry depicts a vibrant creative community. The story centers on Sue and Johnsy, two young artists who met in a restaurant, discovered their shared tastes in art and decided to live and work together. The author characterizes pneumonia as 'a strange male visitor' to the neighborhood perhaps helps to further establish men as an antagonistic presence in the lives of these two unusual women. The author adorns the story by featuring the characteristics beautifully. The description of the characters brings the persons in real in front of the readers to visualize clearly. All the incidents are painted with real and touching flavors with an essence of emotions which readers could sense it and the impact it creates holds the hearts of the readers for a long period of time.

The author introduces pneumonia as a male visitor. In the ancient days male chauvinism was predominant and the wings of women's freedom, joy are under the complete control of men. The deeds, orders of men cause the negative impact of ripples to the women. The troubles and problems faced by women because of men are endless. The pain, sorrow that the women underwent can be rightly compared to the pain during death. Therefore, the author represents pneumonia as a male visitor.

The doctor's contemptuous response to Sue's suggestion that Johnsy might be depressed over frustrated artistic ambition, demonstrations preventing attitudes about gender at that time. The doctor assertively objects that artistic ambitions are not her concern and be confidently tells that romantic troubles frustrate her.

Sue tells the doctor with conviction that no man has any role with her frustration and it is her artistic ambition that she is engrossed with. This firm conviction of Sue exhibit their true friendship and displays very vividly that there are no secrets between them. Sue plans to do everything possible to help Johnsy. She cooks, cleans and cares for her friend in her illness, while also act as the primary breadwinner in their household. The author clearly sketches the true love between them. Sue's single determination is to save the life of Johnsy. Sue is obsessed with the thought of saving her life. She works very hard by working in a magazine by selling the painting which would help her make prepare some good food for Johnsy. Despite all the efforts, however Johnsy is increasingly cold to Sue, turning away from friendship as she psychologically prepares herself for death.

Johnsy strongly believes that her death is imminent and the hope of living is nil. Therefore she is at the state of despair, she loses interest in life, unwilling to communicate, no zeal to look at people. So the great weapon 'silence' takes complete hold of her. The silence wails her agony of dying at the young age, the grief of not achieving her goal. The death is common to everyone if the news of death is known to a person, each and every second she dies of the news of death. Johnsy's desire to go "sailing down" like one of the leaves demonstrates her fading hope and loss of the will to live.

The leaves are fresh, green and dance with spirit for the music of breeze. These leaves can withstand strong wind. But when the leaves turn pale yellow, it is so light and hold of the leaf to the stalk is weak and it falls down very lightly. The life of leaves is rightly compared to the life of human beings. At the young age, the human beings are dynamic, energetic and live with spirits. Sickness, tiredness, weakness become the unwelcoming guests to the old aged people. Therefore the author compares the life of human beings to the leaves that wither. The old yellow pale leaves fall with lightness. Johnsy's heart is heavy with the visitor 'death' whom she believes that is about to visit. The distress of death, the sorrow of living with disease builds a heap of heaviness in her heart. She starts to trust that the death would relieve her from all these heaviness and she would leave the world very light like the old, pale yellow leaves. Sue comes to know that Johnsy strongly believes that when the last leaf of ivy vine tree falls she also would depart from this world.

This pessimistic thought weaves the negative rumination in her and she has the faintest of chance of surviving. When Sue reveals to Behrman, he feels very sorry for the young girl Sue. That night the pessimistic thought of Sue causes a lot of distress to him. The author knits his mastery by revealing the end of the story with surprises and twists. Johnsy keeps counting the leaves from 300 to 10. She could see three or four leaves clinging to the tree. She looks at the tree keenly for falling of those leaves. But Sue forces her to sleep and not to look at the tree anymore. She closes her eyes but she strongly believes that these leaves would definitely fall because of the strong wind. The next day, Johnsy could see the last leaf of the ivy vine that was clinging to the stalk. It was a surprising shock to her. In spite of the strong wind, the sustained leaf builds confidence, hope and life in her. The last leaf that clasps the tree gives her the message or desire of the God that she is blessed with life to prove her artistic talents and death is not

imminent. This optimistic thought energizes her. She develops a desire to live and the great thought of many years ahead to paint the Bay of Naples make her feel excited. The last leaf represents the long life of Johnsy. The denouement of the story is where the surprise twists of the author play an eminent role. The theme of the story is that hope and life are inextricably intertwined. The last leaf rekindles the hope of Johnsy.

At last, the author reveals that the last leaf that clung to the tree is not real and it was actually painted by Behrman. Behrman has faced long, hard life of social alienation, economic struggle and creative disappointment. His desire is to paint a masterpiece in his life time. When Sue reveals to Behrman, he feels very sorry for the young girl Sue. That night the pessimistic thought of Sue causes a lot of distress to him. The significance of his long solemn look at the ivy vine is revealed later-as he had decided then to paint the illusion of a leaf on the wall outside the window of Johnsy. Behrman's painting of the last leaf can be rightly called as masterpiece as it is a selfless act of sacrifice and it had saved the life of Johnsy. The painting was extraordinarily realistic and it exhibits the love and generosity of him for those young girls.

True friendship blossoms when true love, ultimate affection, consistent care and comfortable concern are exchanged one another. They weave a colourful spectrum where they live in the most enjoyable, funny environment and one is the perfect reflection of other reflecting both happiness and sorrows. 'The Last Leaf' is the perfect garland woven by the author because where all the aspects of friendship roses are wreathed into a perfect festoon in which the pleasant essence oozes out as a sweet aroma of friendship to all the readers. A true friend reads the pain of the hiding tears in her friend's eyes behind her smiles on her lips. The true friend Sue struggles a lot to save the life of Johnsy in spite of her indifferent attitude after she is affected by pneumonia.

The author makes the readers travel through all the emotions that had been blended in each and every aspects of the story. The emotions take its height not only in the plot but also laid in beautiful description. The rays of hope and positivity are aimed by the author through the arrows of his plot. God plays the magical tunes in the life of two friends as there was music of true love between their two hearts. This turns turtle the wheel of impossibility to possibility when true, lovable hearts are embraced. An unsuccessful artist in his lifetime gets an opportunity to paint a masterpiece of a simple leaf which brings back life to a girl by painting the scoops of confidence, hope, optimism and energy and desire to live in her.

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

REVIEW ARTICLE

Fish Composition in Ten Reservoirs of Karnataka: A Review

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Received: 04 Mar 2020 Revised: 06 Apr 2020 Accepted: 08 May 2020

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ABSTRACT

Ichthyofaunal composition in the 10 reservoirs of Karnataka is reviewed based on published literature. In this review, a total of 23 fish families and 10 orders are recorded from 10 reservoirs. Among the families cyprinidae is dominant in Linganamakki reservoir with 31 species followed by Karanja (29 species) and Tunga reservoir (23 species). Bagridae is dominant in Karanja reservoir (19 species) preceded by Linganamakki reservoir (6 species). With respect to orders cypriniformes and siluriformes were dominant at Karanja reservoir followed by Tunga reservoir. The reservoirs were found to be rich in fish fauna, most of the fishes were found to be indigenous as well as exotics. Their abundance and composition influenced by the pre-impoundment situation. In the course of environmental changes and human intervention, however, a few fish species appeared to be vanished, while others flourished in the new habitat. Some exotic fishes got introduced into these reservoirs during the course of stock enhancement programme.

Keywords: Fish fauna, Reservoirs, Indigenous fishes, Exotic fishes, Karnataka

INTRODUCTION

Reservoirs are viewed as the developing assets in India with huge fish yield potential and are intended to help the fishing exercises. These water bodies are mind complex frameworks that show wide scope of ecological co operations (Panikkar and Khan, 2008). Habitat space changeability answerable for supply efficiency might be ascribed to climatic, morphometric and hydro-edaphic factors. Wetlands are natural surroundings that are in part submerged by water and incorporate living spaces like bogs, swamps, lakes including lakes, reservoirs. They work as ecotones, the advances between various living spaces and have attributes of both aquatic and terrestrial biological systems. These environments bolster differing greenery and are exceptionally beneficial biological systems much the

same as the tropical rainforest in terrestrial biological systems (Ramachandra et al, 2005). Fisheries segment assumes a transcendent job as far as nourishment esteem as well as produces income and work to general society. Definitely, the nation needs to rely vigorously upon the inland catch fishery assets, among which the reservoirs establish the pillar. Anyway Sinha (2001) observed low fish yield from the reservoirs. The sustainability of fish assorted variety and its plenitude depends on the nature of water existing in that region. Stocking of monetarily suitable local types of fishes in the stores may change over the supplies increasingly profitable and is significant measure from aqua-biotic preservation perspective. Assume if the reservoirs are overseen under such conditions, these frameworks will be progressively profitable as far as biomass and fish too. Later their production retreats. Such conditions are known as aquatic deserts. So as to maintain a strategic distance from such circumstance these water bodies must be overseen a long time before any progressions happen.

DETAILS OF THE RESERVOIRS

The Anjanapura reservoir lies in semi-malnad area of Shikaripura taluk, Shimoga locale. It was developed over the River Kumudvathi for water system and household reason, yet it is being utilized today for inland fish culture. The catchments territory of repository comprises of evergreen and semi evergreen forest types with deciduous (moist/dry) forest close to Anjanapura supply region and lower basin comprises of scrub jungle. The normal yearly temperature is around 26^{o}C. The zone gets great precipitation from south-west monsoon and the mean yearly rainfall is 699.1 mm. Bhadra reservoir is situated at Chikmagalore locale of Tarikere taluk close to Lakkavalli town, of Karnataka. The supply is arranged at 13^{0} 42$^{|}$ 00$^{||}$ N latitude and 75^{0} 38$^{|}$ 20$^{||}$ E longitude. It is situated at a height of 601 m above Mean ocean level. The Bhadra River emerges from Ganga Moola. This is multipurpose venture for power age just as for water system. The Bhadra basin gets the inflows from the south west monsoon (June-September) and Northeast rainstorm (October-December). The catchment region at site is around 1968 Sq.km. the normal precipitation of that territory is 117 cm to 513 cm. The depth is around 186 feet and all out length is 1445 feet. Reservoir is intended to impound 61.70 TMC of water to flood a territory of 1, 05,570 ha of land in Chikmagalur, Shivamogga, Chitradurga and Bellary region. The water of the reservoir is utilized for drinking, fisheries, irrigation and furthermore for delivering power. The atmosphere of this area is reasonably cool.

The Tunga reservoir is a medium reservoir created due to the construction of dam across the river Tunga which is a major tributary of Tungabhadra River in Shivamogga district of Karnataka. This reservoir is situated at 75°40'20"E longitude and 14°0'24"N latitudes and the total water spread area of the reservoir is about 1600 ha. Fish were sampled monthly at three fish landing centers of the reservoir namely Gajanoor, Sakrebylu and Mandagadde. Gajanoor is located near dam site, Sakrebylu is located at the middle part of the reservoir and Mandagadde is a point where river Tunga joins the reservoir. The Tunga reservoir is a medium reservoir created due to the construction of dam across the river Tunga which is a major tributary of Tungabhadra River in Shivamogga district of Karnataka. This reservoir is situated at 75°40'20"E longitude and 14°0'24"N latitudes and the total water spread area of the reservoir is about 1600 ha. Fish were sampled monthly at three fish landing centers of the reservoir namely Gajanoor, Sakrebylu and Mandagadde. Gajanoor is located near dam site, Sakrebylu is located at the middle part of the reservoir and Mandagadde is a point where river Tunga joins the reservoir.

The Tunga reservoir is a medium one because of the development of dam over the waterway Tunga is a significant tributary of Tungabhadra river in Shivamogga locale of Karnataka. This reservoir is situated at 75°40'20"East longitude and 14°0'24"North latitude and the all out water spread region of the store is around 1600 ha. Gajanoor area is situated near to this dam site, The Karanja reservoir a significant perennial water body of the district and situated at Bhalki taluka of the Bidar region at 17°22′30″ N latitude and 76°59′0″ E longitude. It is made because of the development of dam over the waterway Karanja, a tributary of Manjra River of Godavari framework. It is a medium reservoir having water spread region of 5,673 ha with net water system capability of 1, 62,818 hectares.

Jambadahalla dam is an artificial water body arranged between 75° 45' 0"E longitude and 13°41' 0"N latitude. The complete territory of this water body is 2.186 sq. km and the profundity is around 11-12 meters. The water storage limit is 85.5 billion cubic feet and catchment region is 23.9 square kms. Anthropogenic and household exercises are nearly less here. The water is utilized for agriculture and fishery purposes. Tungabhadra Reservoir is situated at 76° 21' 10" East Latitude and 15° 15' 19" North Longitude, connecting to Mallapur town around 5 kilometers from Hospet town, of Bellary area, Kamataka. It is a multipurpose dam serving water system, power age, flood control, and so on. This is a joint task of recent Hyderabad state and past Madras Presidency when the development was started (The Secret History of Hyderabad State of the Nizam (South India; 1724 – 1948*)* later it turned into a joint venture of Karnataka and Andhra Pradesh after its culmination in 1953.The basic worker for the dam was Venkat Reddy Mulamalla, from Konour town, Mahabubnagar, Hyderabad .

In 1979, Hemavathy dam was constructed over the River Hemavathi in the Hassan district. This stone work dam highlights earthen flanks and a focal spillway. The dam was built above Gorur and downstream from its juncture with the Yagachi River. The dam has a complete height of 58 meters and a length of 4,692 meters. It impounds a 8,502-hectare repository that can amass to 37.1 tmc ft of water. Water from this dam is utilized to flood over 6.5 sections of land of land in the Hassan, Mandya, and Tumkur districts (https://www.karnataka.com/chikmagalur/ stream hemavathi).

Vani VilasaSagara dam is located at Hiriyur taluk, Chitradurga District in Karnataka. It is located around 20 km to Hiriyur and 32 km to Hosadurga, 58 km to Holalkere, 50 km to Huliyar, 60 km to Chitradurga, and 180 km away from Bangalore. Vani Vilasa Sagara was worked by the Mysore Maharajas pre-autonomy over the waterway Vedavathi. Vani Vilasa Sagara is the most seasoned dam in the state. The dam is a flawless bit of design, a building wonder for that time. The dam inundates a huge territory of the Deccan area of Central Karnataka, which is in any case to a great extent a dry land. It waters more than 100 km² of land in Hiriyur and Challakere taluks through right and left bank trenches. Vani Vilasa Sagara dam is likewise the wellspring of household water for water limit is high because of uncommon downpour dam become dry Hiriyur and Chitradurga. The Linganamakki Dam was developed by the Karnataka State Government in 1964. Situated in the Kargal town of Sagara taluk, the dam has a length of 2.74 kilometers (1.70 mi) extending over the Sharavathi waterway. It is situated around 6 km from Jog Falls (Jain, Sharad et al.,2007). The Upper Mullamari reservoir is developed over the river Mullamari which is a minor tributary of river Bheema in Bidar district of Karnataka. It is a perennial reservoir with complete water spread territory of around 277 hectare. The reservoir is arranged in the Northern piece of Karnataka state between (77^0 00' 00" E longitude and 17^0 42' 00" N latitude) presents common place atmosphere of peninsular India with semi dry conditions.

FISH COMPOSITION IN THE RESERVOIRS OF KARNATAKA

Anjanapura reservoir

Fish abundance and richness of Anjanapura reservoir,Karnataka was considered by Basavaraja et al (2014) from November 2005 to October 2006. Their examination has demonstrated that Anjanapura reservoir bolster 25 fish species having a place with 04 orders, 09 families and 18 genera. The order Cypriniformes was prevailing with 14 fish species followed by order Siluriformes with 6 species, and the Perciformes with 4 species and Osteoglossiformes with one species. To the extent biodiversity status (IUCN-1994) is worried, out of 25 species, 11 fish species are sorted into Lower risk Near threatened(LR-nt), 08 Not surveyed, 03 Vulnerable, 01 each in regard of Endangered, Data Deficient, and Lower risk least concern (LR-lc) individually. Occasional water quality portrayed that it was reasonable for fish populace. It very well may be presumed that reservoir upheld rich fish population. It needs appropriate administration and usage of this fish wealth and sustainable steps to monitor and save this fish wellbeing. The significant and cultivable fishes recorded viz., *Notopterus notopterus, Cyprinus carpio, Catla catla, Glossogobius giuiris, Labeo rohita, Cirrhinus mrigala, Sperata seenghala, Sperata oar* and *Channa marulius*. Similarly, this

dam is likewise occupied by the ornamental fishes like *Puntius sp,, P. sarana sabnastus, Chanda nama* and *Rohtee ogilbii*. Some uncommon species include *Mastacembalus armatus, Cirrhinus cirrhosus, Labeo fimbriatus* and *Wallago attu*.

Bhadra Reservoir

Thirumala et al (2011) studied the fish community of the Bhadra reservoir in relation to physico-chemical parameters from June-2004 to May-2005. They recorded a total of 33 fish fauna belongs to Cyprinidae 18 species, Channidae 2 species, Bagridae and Siluridae with 3 species and a species each of Mastacembelidae, Ambassidae, Cichlidae, Claridae, Notopteridae, Cobitidae and Heteropneustidae. All fishes are useful as food fishes except *Ambassis, Puntius and Gambusia*, which are useful as decorative and larvicidal fishes. The species diversity is maximum after monsoon, coinciding with favorable conditions such as enough water and sufficient food resources. The diversity was minimum before monsoon season may be due to the reduction in the water spread area of the reservoir. Based on water quality they reported that this reservoir is moderately oligotrophic in nature.

Tunga reservoir

Kumar Naik et al (2012) investigated on the ichthyofaunal diversity of the Tunga reservoir of Shivamogga district, (Karnataka) India. They recorded 43 species belonging to 13 families and 6 orders were recorded by them. The order Cypriniformes dominant with 27 fish species preceeeded by Siluriformes with 10 species and Perciformes with 3 species respectively. Among fish families, Cyprinidae was found as the leading with 23 species with 53.48% followed by Bagridae & Channidae with 3 fish species with 6.97% each.

Karanja Reservoir

Kumar Naik et al (2013) has made an attempt to assess the ichthyofauna in Karanja reservoir of Karnataka between 17°22′30″ N latitude and 76°59′0″ E longitude. During their study they observed 64 species of fin fishes belonging to 37 genera, 16 families and 5 orders. The order Cypriniformes was dominant with 31 fish species followed by Siluriformes with 20 species, Perciformes with 10, Osteoglossiformes by 2 species and the order Synbranchiformes with one fish species.

Jambadahalla reservoir

Thirumala and Kiran (2017) studied the fish fauna in Jambadahalla dam of Chikmagalur district in Karnataka. A total of 26 fish species belonging to 11 families were recorded with cyprinidae was dominant by 14 species followed by Bagridae and Channidae with 02 species and rest of the families consists of one species each. Among orders, Cypriniformes was dominant by 14 species (53.84%), followed by Siluriformes and Perciformes by means of 05 species (19.23%) and Osteoglossiformes and Cyprinodontiformes with one species each respectively (3.85%). Regarding biodiversity status (IUCN, 1994), out of 26 species, 12 species as lower risk-near threatened- 46.15 %, not assessed 05 species -19.23 % vulnerable 07 species- 26.92%, lower risk least concern and Endangered each with one species by 3.85 % . The diversity of fish fauna in this dam is attributed to the introduction of exotic species and water quality.

Tungabhadra reservoir

Nagabhushana Charantimath (2013) investigated 89 fishes from Tungabhadra reservoir. All together 89 species belonged to 49 genera and 17 families were recorded. Among them 2 species belonged to family Ambassidae, 1 each from Anguillidae and Aplochelidae, 6 from Bagridae, 4 from Balitoridae, 1 from Belonidae, 3 of Channidae, 2 of Cobitidae, 53 from Cyprinidae, 1 from Gobidae, 2 from Mastacembellidae, 1 each from Notopteridae,

Osphronemidae & Pangassidae, 5 from Schilbeidae, 3 from Siluridae and 2 from Sisoridae. lUCN status of fishes showed rare species (R=l), endangered (EN=5), vulnerable (VU=5), near threatened (NT=4), data deficient (DD=4), least concerned (LC=47) and remaining 23 were not listed in the Red Data Book.

Hemavathy reservoir

Hemavathy reservoir is well exploited with 100 licenced units. Besides local fishermens, parties from Chickmagalur district migrate temporarily during the fishing season, making temporary settlements. As compared to Kabini, fishing intensity is of low order during early years of formation, weed fishes like *Oxygasrer* spp. and catfishes dominated the catches. Stocking of catla, rohu and common carp changed the catch composition with predominant occurrence of Indian major carps, exotic carp -*Cyrinus carpio.* along with minor catfishes like O*mpok bimaculatus* and *Mystus cavasius*. Peak fishing was noticed during pre monsoon months. Catla catla appears to grow well as it has been estimated to attain 700 mm with 6.5 kg after 2 years (Ramakrishniah et al.,2000).

Vani Vilas sagar reservoir

It is an old and well exploited reservoir with 85-120 fishing units were engaged during peak season establishing 2 to 3 temporary scttlements along the reservoir During the post-monsoon months 13-16 coraclcs were observed at two centers. The fish catches consisted of Indian major carps, Exotic carps, Indigenous fishes(*Cyprinus carpio. Labeo rohita, Labeo fimbriatus, Notopterus notopterus, Mystus cavasius, Cirrhinus reba, Channa marulius* and *Mastacembelus armatus)*. *Cyprinus carpio* occurred in thc size range 500-900 mm with dominance of 700-800 mm group and belongs to above 3 agc group. *Labeo rohita* occurred in the length of 610-760 mm, perhaps belonging to above 2 age group *Labeo fimbriatus* occur in the length range of 300-460 mm with dominant size of 300-360 mm. probably bclonging to I+ age group. The growth of *Cyprinus carpio* and *Labeo rohita* was found to be good. The aquatic vegetation seem to be favourable for the growth of *Labeo rohita* as it offcrs good substrate for periphyton. Similarly miscellaneous fishes viz., *Notopterus notopterus* , murrels seem to thrive well. Natural recruitment has been observed in *Labeo fimbriatus* as well as in all miscellaneous fishes, but in *Cyprinus carpio* and *Labeo rohita* it appears to be doubtful. Presence of submerged plants offers ideal conditions for the breeding and successful recruitment of *Cyprinus carpio*. But the length composition in the catch did not show any recruitment of this species (Ramakrishniah et al.,2000)

Linganamakki reservoir

Ramakrishniah et al.(2000) reported that 200 coracles are engage in the management of this reservoir. Major fishing season is May to August. *P. kolus* was the dominant species during pre- monsoon and monsoon season. Besides P. kolus is catch included *Catal catla, Labeo rohita, Cirrhinus mrigala, Cyprinus carplo*, *Wallago attu, Ompok bimaculatus, M armatus* and *Channa marulius.*. A number of weed fishes like *P. mahecola. Puntius sp., Danio aequipinnatus. M. cavasius* also recorded by them. *P. kolus* occurred in the length range of 200-390 mm, *C. catla* with 700-850 mm and *Cyprinus carpio* with 230-250 mm. *Oreochromis mossambicus* was recorded after monsoon season. During the year 2000 according to fishermen, *Oreochromis* occurred for the first time. Fish catches are disposed off to the middlemen who proceed them loans (Ramakrishniah et al.,2000).

Ramachandra et al (2012) studied the fish diversity in the Linganamakki reservoir of the upper catchment area and recorded 64 species from 32 genera and 17 families. 51 freshwater fish species have been recorded from lower catchment stretch.The total species collected from these basin alone accounts for 6.88 % of Indian fresh water fish (930 species) and 22.2 % of Western Ghats species (288). Based on the IUCN categories of the fish species, of the 51 species, 35.3 % of 18 species are endemic to Western Ghats, about 43.1 % of 22 species were threatened species and the remaining 19.6 % with 10 species are data deficient. The yearly fish yield of the reservoir was estimated to be 200

tons. Prohibition of over-fishing, migrant fishermen and breeding season fishing can contribute to sustainable fishery in the reservoir. Strict use of restricted mesh size can minimize the death of non-target fishes. Educating local fishermen and activating the inactive cooperative society can pilot to sustainable fishery .

Mullamari Reservoir

Kumar Naik et al (2013) investigated on fishes in Upper Mullamari Reservoir in Bidar district, Karnataka. They observed 32 species of fishes belonging to 26 genera, 14 families and 6 orders. Among them Cypriniformes represented by 17 species followed by Siluriformes (9 species), Perciformes (3 species). Synbranchiformes, Osteoglossiformes and Clupiformes contributed to one species each respectively. Among the families the Cyprinidae formed the major group constituting to 50% followed by Bagridae with 9.37%, Siluridae of 6.25%, and Channidae with 6.25%.The other families like Balitoridae, Claridae, Heteropneustidae, Schilbeidae, Pangasiidae, Ambassidae, Mastacembelidae, Engraulidae, and Notopteridae each contributed to 3.12% of the total fish species.

DISCUSSION

Reservoirs have protected a rich assortment of fish species in spite of the disastrous faunistic changes related with their impoundment, and the ichthyofauna of the reservoirs fundamentally speak to the faunistic decent variety of the parent river framework (Sugunan, 1995). Jhingran (1991) has given a decent record of fish and fisheries of the reservoirs related with the different significant waterway frameworks of India. He has identified the endemic and indigenous fish species harboring the supplies, and saw that in many stores the carps and catfishes add to the fisheries. A few of the animal varieties were endemic to their specific living space. Also, numerous types of carps, especially Indian major carps, common carp and tilapia were transplanted in huge numbers of the reservoirs, contributing considerably to the commercial fisheries of these water-bodies. A few of the presented angles have obscured the indigenous fish fauna and, thus, the assorted variety of fish species in numerous reservoirs has declined extensively over some undefined time frame. The Indian significant carps have, be that as it may, neglected to have an effect on any of the peninsular reservoirs basically in light of their inability to breed in this environment.

CONCLUSION

Being significant reservoir of Karnataka, it harbours assortment of fish species and every species regularly comprises of a few indigenous groups with an divergent hereditary make up. There could be vulnerabilities with every single logical undertaking to screen bounty and profitability of stocks and the hidden causes. Further, there are vulnerabilities concerning environmental change, aquatic system productivity, predation and fishing pressure. Protection of fish fauna expect top most need under changing conditions of progressive natural surroundings debasement. The acceptance of fish stocking plans and its results on the indigenous fish fauna of the reservoirs ought to be read for further management and improvement of fisheries right now. The findings of this review study are relied upon to profit the planning and the management towards economical fishery and protection programmes of reservoir of Karnataka.

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Table 1: Family wise distribution of fish species in few Reservoirs of Karnataka

Families	Anjanapura	Bhadra	Tunga	Karanja	Jambadahalla	Tunga Bhadra	Mullamari	Linganamakki
Cyprinidae	14	18	23	29	14	14	16	31
Bagridae	1	3	3	9	2	1	3	6
Clariidae	1	1	3	2	1	1	1	2
Cichlidae	0	1	2	2	1	1	0	1
Balitoridae	0	0	1	1	0	0	1	8

Siluridae	3	3	2	3	1	1	2	3
Schilbeidae	0	0	1	2	0	1	1	1
Channidae	1	2	3	3	2	4	2	3
Mastacembelidae	1	1	1	1	1	0	1	1
Ambassidae	1	1	0	5	1	0	1	0
Pangasiidae	0	0	0	1	0	0	1	0
Sisoridae	0	0	0	2	0	0	0	1
Heteropneustidae	1	1	1	1	1	1	1	1
Anabantidae	0	0	0	1	0	0	0	0
Notopteridae	1	1	1	2	1	1	1	0
Cobitidae	0	1	0	1	0	0	0	1
Belonidae	0	0	1	0	0	0	0	1
Aplocheilidae	0	0	0	0	0	0	0	1
Chandidae	0	0	0	0	0	0	0	2
Gobiidae	1	0	0	1	1	1	0	1
Belontidae	0	0	0	0	0	0	0	1
Rasboridae	0	0	1	0	0	0	0	0
Poecilidae	0	0	0	0	0	1	0	0
References	Basavaraja et al,2014	Thirumala et al,2011	Kumar Naik et al,2012	Kumar Naik et al,2013	Thirumala & Kiran,2017	Manasi et al,2009	Kumar Naik et al,2013	Ramachandra et al,2012

Table 2: Order wise occurrence of fish species in Reservoirs of Karnataka

Orders	**Anjanapura**	**Bhadra**	**Tunga**	**Karanja**	**Jambadahalla**	**Tunga Bhadra**	**Mullamari**
Cypriniformes	14	18	27	31	14	14	17
Siluriformes	6	7	10	20	5	5	9
Perciformes	4	2	3	10	5	6	3
Synbranchiformes	0	0	1	1	0	0	1
Osteoglassiformes	1	0	1	2	1	1	1
Clupiformes	0	1	0	0	0	0	1
Beloniformes	0	0	1	0	0	0	0
Cyprinodontiformes	0	2	0	0	1	1	0
Channiformes	0	2	0	0	0	0	0
Mastacembaliformes	0	1	0	0	0	0	0
References	Basavaraja et al,2014	Thirumala et al,2011	Kumar Naik et al,2012	Kumar Naik et al,2013	Thirumala & Kiran,2017	Manasi et al,2009	Kumar Naik et al,2013

Table 3: Diversity indices of fish species in Reservoirs of Karnataka

Indices	Anjanapura	Bhadra	Tunga	Karanja	Mullamari
Shannon-Weiner index	2.4-3.0	2.2-4.1	2.47-2.66	2.69-3.089	2.37-2.68
Simpson dominance index	0.08-0.2	-	0.882-0.907	-	-
Simpson diversity index	0.89-0.95	-	-	0.88-0.92	0.86-0.894
Pielous Evenness	0.6-0.9	0.99	0.65-0.70	0.65-0.75	0.75-0.8
Margalef Index	1.48-2.4	-	-	6.62-7.11	-
References	Basavaraja et al,2014	Thirumala et al,2011	Kumar Naik et al,2012	Kumar Naik et al,2013	Kumar Naik et al,2013

Table 4: Biodiversity status (IUCN,1994) of fishes in the Reservoirs of Karnataka

	Anjanapura	Bhadra	Jambadahalla	Tunga Bhadra	Linganamakki
LR-nt	11	11	12	4	21
NA	8	12	5	23	11
VU	3	5	7	5	12
EN	1	3	1	5	9
DD	1	1	-	4	10
LR-lc	1	1	1	47	-
Rare	-	-	-	1	-
CR	-	-	-	-	1
References	Basavaraja et al,2014	Thirumala et al,2011	Thirumala & Kiran,2017	Nagabhushana charantimath, 2013	Ramachandra et al,2012

CR – Critically Endangered, EN – Endangered, VU – Vulnerable, LR – Lower risk,
DD – Data deficient, NA-Not assessed

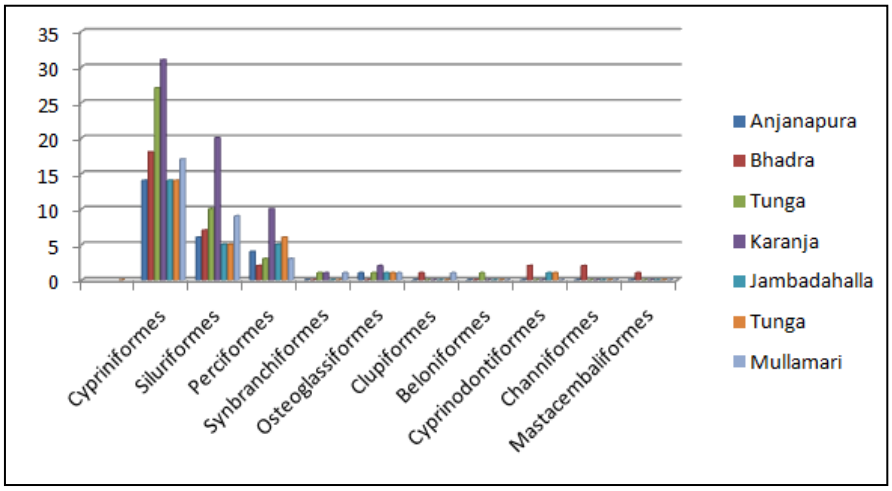

Figure 1: Order wise distribution of fish species in Reservoirs of Karnataka

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Designer Novel Triazole Molecules: for Highly Selective Recognition of Silver Metal Ion

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Received: 13 Feb 2020 Revised: 15 Mar 2020 Accepted: 18 Apr 2020

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ABSTRACT

Metal ions are ubiquitous in nature and are present in many enzymes to carry out various vital biochemical processes in living systems. Therefore, design and synthesis of new receptors for selective recognition metal ion has always attracted considerable interest in the scientific community. We report herein, the design and synthesis of triazole based molecules (E20, E21 and E12) using Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition reaction. We demonstrate the use of triazole moiety in designing molecules endowed with ability for molecular recognition of metal ions. The receptors E20 and E21 having open structure bind to silver ion with high selectivity over other metal ions.

Keywords: Triazole, Cation receptor, Molecular recognition, Cycloaddition reaction

INTRODUCTION

The intermolecular forces, such as hydrogen bonding, metal coordination, hydrophobic forces, van der Waals forces, π-π interactions, and electrostatic interactions, play major roles in governing the function of many biological processes. The control of intermolecular interactions to make a supramolecular assembly is an ongoing research topic in chemical sciences.[1] Some of the major areas of research in supramolecular chemistry are molecular self-assembly,[2] molecular recognition,[3] mechanically-interlocked molecular architectures,[4] and dynamic covalent chemistry.[5] Molecular recognition has attracted considerable interest in the recent years because it is integral in many scientific areas, such as: biology, chemistry, and pharmacology. Molecular recognition of cations, anions, and neutral molecules are important in biology and chemistry. Although rigorous attention has been paid for recognition of anions due to countable numbers, but the recognition of cations has received little attention. Moreover, Cations are everywhere in the living systems and are vital in carrying out many biochemical operations for sustaining life.

Metal ions are ubiquitous in nature and are present in many enzymes to carry out various vital biochemical processes in living systems. Therefore, design and synthesis of highly sensitive and specific artificial sensors for detection of metal ions occupies a central place in the development of molecular devices for diagnostic purposes.[1] Molecular frameworks that are used extensively for designing of receptors are porphyrin, expanded porphyrin, calixarene, and calixpyrrole (Figure 1).[2-3] Porphyrins, which are tetrapyrrolic systems, are the most intensively studied macrocycles. The interest in porphyrin is derived from their applications in catalysis,[4] photodynamic therapy[5] and in organic electronics.[6] The uses and biological relevance of porphyrin inspired the study of other porphyrins analogues, which are contracted, expanded or other porphyrin derivatives.

In the recent years, an increasing attention has been devoted to the synthesis of receptors for recognition of cations. But only fewer scaffold that are known so far such as, porphyrins, aza-crown, and crown ethers etc. Inspired with the interdisciplinary interest in porphyrins and significant role of metal ions in biology, prompted us to synthesize a different class of molecular scaffold with similar complexing or structural similarity. Inspired by the developments in porphyrin chemistry, we have utilized the Cu(I)-catalyzed 1,3-dipolar cycloaddition reaction (Figure 2) to generate a new variety of molecular scaffolds that might behave similarly to porphyrins or calixpyrrole-like systems (Figure 1). The triazole moiety, containing nitrogen atom, could be exploited as a coordinating side for metal ion binding, while remaining part can act as an excellent amide bond mimic, providing hydrogen bond donors in the form of acidic CHs. These structural features in the triazole moiety could be used in design of a new class of molecular scaffolds. The triazole units must be precisely arranged in the scaffold in order to provide appropriate geometry and flexibility with respect to analytes. In light of these advantages of triazole moiety, we have designed and synthesized various molecular scaffold for recognition metal ion with an ideal disposition of the triazole moiety.

RESULTS AND DISCUSSION

The Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition reaction involving a terminal alkyne and azide unit is the simplest method to introduce a triazole moiety in to the molecular scaffolds. From the last few years, this reaction has become central to many investigations owing to its simple handling procedure, high regioselectivity, and affording high yield. Therefore, it has been extensively utilized in for making triazole based molecule for applications in bioconjugation and in supramolecular chemistry.[17] The interdisciplinary interest in porphyrins prompted us to synthesize a different class of macrocyclic molecules with similar complexing or structural similarity. Inspired by the developments in porphyrin chemistry, we have utilized the Cu(I)-catalyzed 1,3-dipolar cycloaddition reaction to generate a new variety of molecular scaffolds that might behave similarly to porphyrins or calixpyrrole-like systems (Scheme 1)

In search of an ideal molecular scaffold for the correct disposition of triazole units for the synthesis of triazole based macrocyclic compound, we embarked on a systematic analysis for the synthesis of various benzenoid anchored triazolophanes containing di-triazole ring similar to porphyrin. To synthesize di-triazole containing macrocycles (E11), in the beginning we treated terminal di-alkyne moiety containing amide linkage (E8) with p-xylene diazide (E10) in presence of Catalytic amount of CuI and acetonitrile (5 volume) as a solvent. The resulted product was observed to be highly insoluble material even in high polar solvent like DMF, DMSO and water at high temperature. The insolubility of amide based triazolophane (E11) is anticipated, owing to its formation of rigid macrocycles which forms strong intermolecular hydrogen bonding among the amide moiety. Therefore, to overcome this insolubility, we turn our attention to synthesize corresponding ester-based macrocycles (E12) which is devoid of strong hydrogen bonding amide moiety in the core structure. Having this intention in mind, we treated ester based di-alkyne (E9) moiety with di-azide (E10) in acetonitrile (5 Volume). Thin layer chromatography result, clearly showed the formation of multiple products. The resulted product was soluble in normal semi-polar solvents like DCM, Chloroform, methanol, ethyl acetate, acetone and acetonitrile. This increase in solubility behavior of E12 reaction mass clearly indicates that the amide back bone triazolephanes (E11) is imparting strong inter molecular hydrogen

bonding within the molecule in the solid state that makes the molecule insoluble. The product formation was further purified by column chromatography. The formation of two singlets at 5.4 and 5.5 ppm in 1H NMR and the absence of peak corresponding to terminal alkyne in 1H NMR clearly indicates that the resulted product is a symmetrical cyclic molecule.[18] The di-triazole cyclophanes product formation was further confirmed by carbon-13 NMR and mass analysis which shows the product ion peak at 431 ($[M+H]^+$).[18] These results clearly support the formation of di-triazole cyclophanes (E12) formation. The overall yield of the di-triazolephanes was found to be 25%. The formation of the low yield is expected due to execution of the reaction in low solvent volume (5 volume) which may lead to the formation tetra-triazole along with polymeric product formation appearing on the bottom of the TLC plate. The binding studies of macrocycle E12 toward metal ions, namely, Pb^{+2}, Hg^{+2}, Zn^{+2}, Cd^{+2}, Cu^{+1}, Ag^+, Rb^+, and Cs^+, were performed by NMR spectroscopic methods. E12 did not bind to any of the above metal ions. It is anticipated due to the inappropriate rigid cavity size of the macrocycle and unfavorable geometrical position of the triazole moiety hindered the binding of the metal ions. To over come this difficulty, we turn our attention to install the triazole moiety in to a flexible scaffold such that it can take the preferable orientation in three dimensions which may allow the binding of metal ion.

Therefore, we envisioned that a flexible system could overcome the problem faced in the above system. In order to have a semi-rigid system we choose 3,3′-dithiobisbenzoic acid for anchoring triazole units. This was chosen due to the rigidity imparted by its aromatic rings and the flexibility of its S-S linkage. Because the macrocycles did not bind, we were persuaded to synthesize acyclic triazole-based derivatives E20 and E21 (Scheme 2), with an expectation that these compounds may show a better metal uptake potential. The 3,3′-dithiobisbenzoic acid E14 converted to corresponding acid chloride E15 by treating with thionyl chloride under reflux for 3 hours. The acid chloride E15 was treated with propargyl amine or alcohol in the presence of triethyl amine as a base to get the dialkynes E16 and E17, respectively. The dialkynes E16 and E17 were further reacted with methyl azido acetate E18 via click reaction to get triazole-based molecules E20 and E21 (Scheme 2). The products were further purified by column chromatography in 2% methanol and chloroform system. The ^1H NMR data clearly reveals that both the products E20 and E21 are symmetrical in nature. The formation of three peaks with integration area 4:4:6 proton ratio in the range of 3 to 6 ppm (Figure 3) and the absence of peak corresponding to terminal alkyne in ^1H NMR (Figure 5) clearly indicates that the resulted products (E20 and E21) formed are symmetrical di-triazole (Figure 5 and 8). The di-triazole formation was further confirmed by carbon-13 NMR (Figure 6 and 9) and mass analysis which shows the product ion peak in the form of ($[M+H]^+$), ($[M+Na]^+$) (Figure 7 and 10). These results clearly support the formation of di-triazole (E20 and E21). All the acyclic di-triazole compounds (E20 and E21), unlike cyclic system, are observed to be highly soluble in solvent like methanol, ethanol, chloroform, acetonitrile, and ethyl acetate. It is expected due to the presence of flexible back bone in to core structure.

We prompted the synthesis of opened di-triazole system from E20 to E21, anticipating that triazole bearing nitrogen atom in conjunction with amide and ester moiety may support for the binding of the metal ions. Moreover, these acyclic triazole molecules may provide enough flexible orientation during binding with metal ion. Therefore, we examined the binding behaviour of triazole-based molecules E20 and E21 with various metal Ions, such as Pb^{+2}, Hg^{+2}, Zn^{+2}, Cd^{+2}, Cu^{+1}, Ag^+, Rb^+ and Cs^+, using ^1H NMR studies. Interestingly, these compounds selectively responded only to Ag^+ ion over all other metal ions. Furthermore, ^1H NMR titration study showed the significant downfield shifting of triazole proton (Figure 4) upon gradual addition of Ag^+ ions into the host solution, indicating the binding of triazole nitrogen with silver ion. Due to binding of the triazole nitrogen with silver ion, the -CH proton of the triazole ring became deshielded. As a result of which, the -CH proton of the triazole ring is shifted downfield upon gradual addition of silver ion. 1H NMR titration data (Figure 4) clearly shows that methylene proton attached with amide and ester group does not show any shifting in NMR signal upon addition of silver ion. This clearly indicates that ester and amide moiety are involved in binding with silver ion (Figure 4). Both the compound has almost same response and selectivity towards silver ion. The binding constant values for the receptors (E20 and E21) were found to be in the range of ~10^4 M^{-1}.

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CONCLUSION

In conclusion, we have demonstrated the synthesis of various macrocyclic compounds with an aim to bind cations. The demonstrated methodology can be adopted for the synthesis of various macrocycles containing many triazole units. The results from the binding studies showed that these macrocycles are not strong binders of cations, indicating that the orientation of triazole nitrogen is a crucial factor. However, the acyclic compounds E20 and E21 were found to be have strong binding affinity than the cyclic triazole molecules, which emphasizing the importance of correct orientation of triazole units for metal ion binding. Moreover, the receptor E20 and E21 were highly selective for silver ion. Therefore, the present methodology can be further utilized for the generation of macrocycles containing metal ion binding units on the linker regions. The conformationally restricted macrocycles, by the correct choice of linkers, may provide macrocycles with high binding affinity similar to porphyrins.

ACKNOWLEDGEMENTS

We thank DST-New Delhi, CSIR-New Delhi, IIT-Delhi and CUTM-BBSR for support.

Experimental Section

General method of preparation of E20 and E21

To an ice cold and homogeneous solution of dialkynes E16 and E17 (2 mmol) in dry acetonitrile was added DIEA (4 mmol), followed by monoazide E18 (4 mmol) under N_2 atmosphere. To the reaction mixture under nitrogen, was added CuI (0.2 mmol) and stirred for 24h at room temperature. The reaction mixture was evaporated and the solid obtained was washed sequentially with aqueous solution of NH_4Cl + NH_4OH (9:1), 2N H_2SO_4, saturated solution of $NaHCO_3$, water, and diethyl ether. The crude product was either purified using column chromatography or crystallized from suitable solvents.

Data of E20: Yield: 85 %, Mp: 134-136 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.82 (s, 6H), 4.72 (d, J = 5.7 Hz, 4H), 5.19 (s, 4H), 7.37 (t, J = 7.8 Hz, 2H), 7.56 (t, J = 7.5 Hz, 2H), 7.64 (t, J = 8.85 Hz, 4H), 7.85 (s, 2H), 8.04 (br s, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 35.5, 50.7, 53.1, 124.2, 126.4, 127.6, 129.6, 132.0, 135.1, 137.5, 145.1, 166.8, 166.9. IR (KBr): 3307, 3128, 3064, 2941, 1644, 1541, 1460, 1429, 1300, 1236, 1165, 1124, 1051 cm^{-1}. HRMS: Calcd for $C_{26}H_{26}N_8O_6S_2Na$ m/z = 633.1314, found m/z = 633.1314.

Data of E21: Yield: 93 %. Mp: 74-80 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 3.80 (s, 6H), 5.20 (s, 4H), 5.47 (s, 4H), 7.36 (t, J = 7.8 Hz, 2H), 7.66 (d, J = 7.5 Hz, 2H), 7.87 (d + s, 4H), 8.1 (s, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 50.7, 53.0, 58.2, 125.4, 128.7, 128.8, 129.3, 130.7, 132.1, 137.3, 143.0, 165.5, 166.6. IR (KBr): 3434, 2956, 1757, 1720, 1493, 1258, 1228, 1120 cm^{-1}. HRMS: Calcd for $C_{26}H_{24}N_6O_8S_2$ Na m/z = 635.0995, found m/z = 635.0998.

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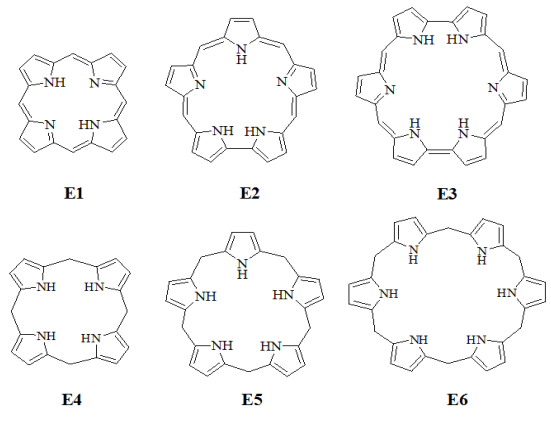


Figure 1: Porphyrins and calixpyrrole receptors E1-E6

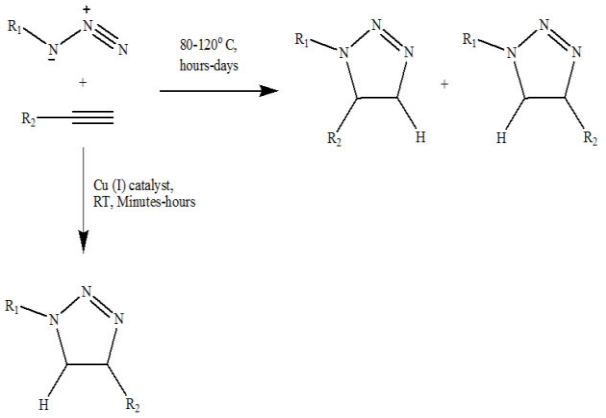


Figure 2: 1,3-dipolar cycloaddition reaction between azide and terminal alkyne

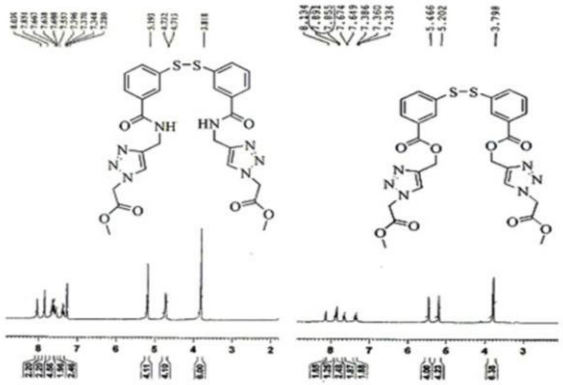


Figure 3: ^{1}H NMR Data of E20 and E21 to compare the range from 3-6 ppm for di-triazole product formation

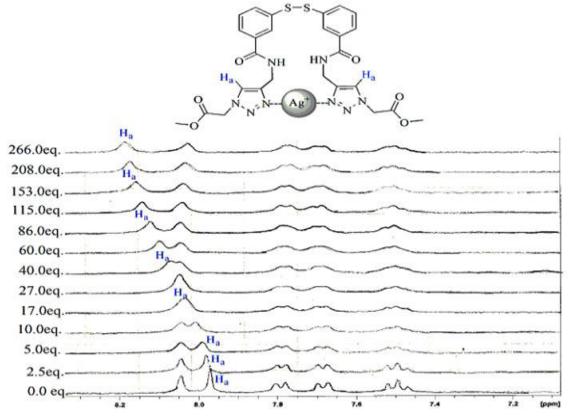


Figure 4: ^{1}H NMR Titration data of E20 with silver ion

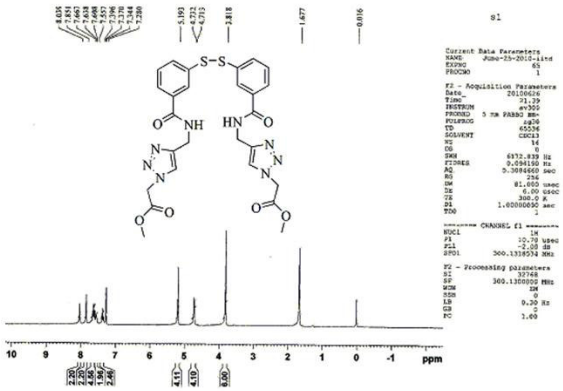


Figure 5: ^{1}H NMR (300 MHz, DMSO-d_6) spectrum of compound E20

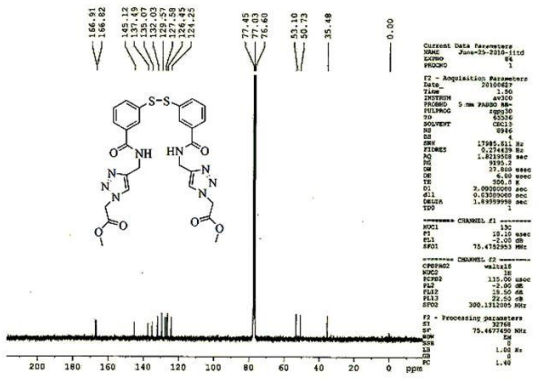


Figure 6: ^{13}C NMR (75 MHz, DMSO-d_6) spectrum of compound E20

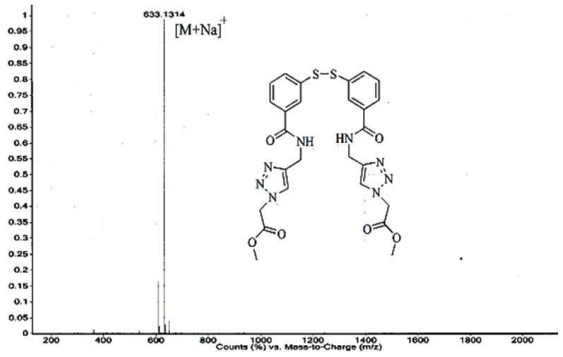


Figure 7: Mass spectrum of compound E20

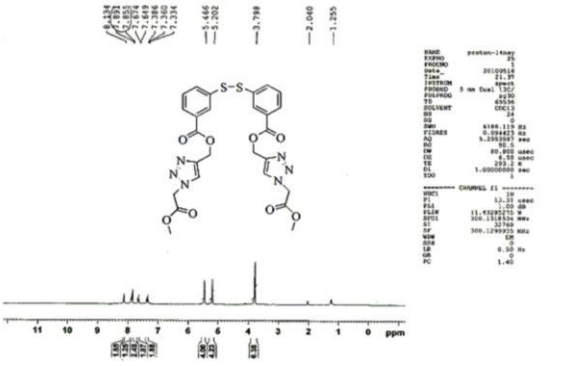


Figure 8: ^{1}H NMR (300 MHz, $CDCl_3$) spectrum of compound E21

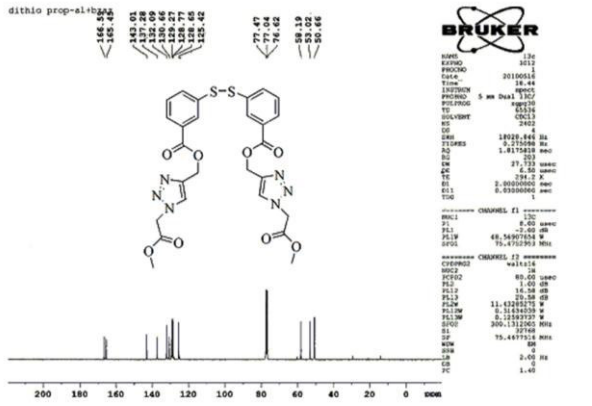


Figure 9: ^{13}C NMR (75 MHz, $CDCl_3$) spectrum of compound E21

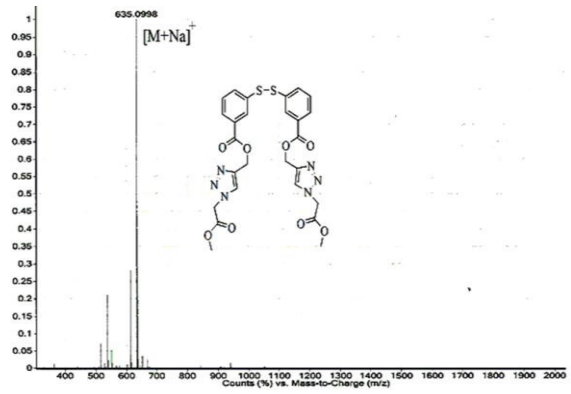
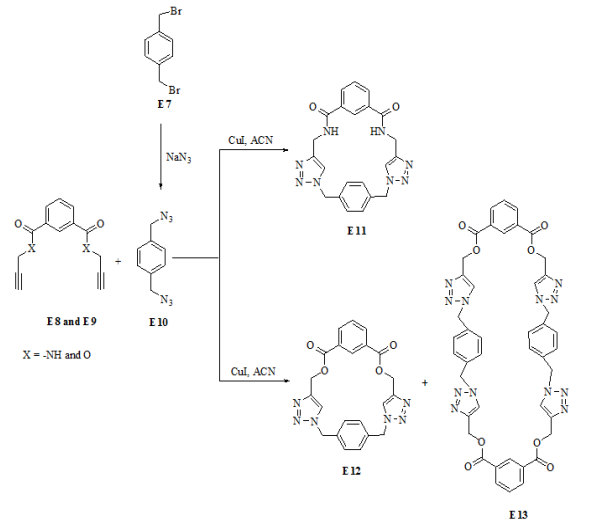
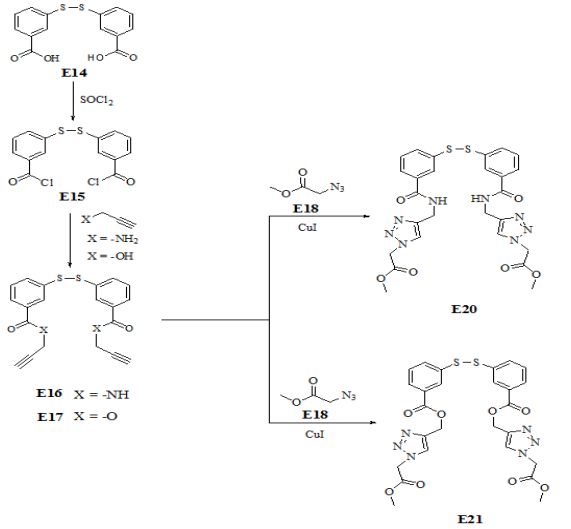


Figure 10: Mass spectrum of compound E21



Scheme 1: Synthesis of triazole based macrocycles E11 and E12



Scheme 2: Synthesis of triazole-based compounds E20 and E21

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Economic Ordering Policy for Deteriorating and Breakable Items with Two Warehouse Inventory and Demand

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Received: 03 Mar 2020 Revised: 05 Apr 2020 Accepted: 08 May 2020

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ABSTRACT

The proposed model represents the EOQ, optimal total cost and optimal time run with two-warehouse inventory, demands across the different class interval with these assumptions the breakability occurs for breakable and deteriorating items. First of all through the first-class interval then the deterioration happened later across the second assumed interval, the finite horizon planning, without shortage cost. Sensitivity analysis of the proposed data was performed using different values lies in the range of the break-ability and deterioration rates to achieve optimal total cost under economic order quantity where break-ability rates more than the deterioration rate, the represented figures explained the behavior of optimal total cost, EOQ within different times.

Keywords: Inventory-Deterioration-Break-ability -Optimal time run -Total Cost Function (TC)

INTRODUCTION

The inventory models with deterioration rate or a break-ability rate represented by many researchers as Abdullah, Muley [1] developed a model for non-zero inventory levels. Saha, Roy, and Kar, Maiti M [2] presented inventory based on demand with assumed constraint. Liang, Zhou [3] represented a developed inventory model with two period times. Chung, Huang [4] represented an inventory model for deteriorating items under some conditions limited shortage capacity under trade credit financing. Chang, Ouyang, and Teng [5] urbanized model to calculate EOQ for deteriorating items to obtain lot size. Das, Maity, and Maiti [6], Goal [7], Huang [8] proposed a model to determine economic order quantity under a conditionally permissible delay in payments. Huang [9], Huang [10] developed a model to determine the optimal retailer's replenishment decisions in the optimal quantity model. Hwang, Shinn [11] represents a developmental model with retailers, pricing for exponentially deteriorating items

with assumptions as permissible delay in payments, Lee, Hsu [12] developed the model under assumptions as two warehouse production, deteriorating items where the demand depends on the time Maiti [13], Rong and Mahapatra [14]. Rong et al. [15] developed a model with a two-warehouse inventory model for a deteriorating item with an assumed fuzzy lead time, Sana and Chaudhuri [16]. Zhou [17].In real life, the break-ability for breakable and deteriorating items occurs first when items transformed into stock after that the deterioration occurs within across remaining period, there are many items having deterioration and break-ability characteristics at the same time as the bulb and each item packed in a can made from glass as medicines and milk which are packed in the glass as olive, olive oil, these types of items require an advanced model to manage the items with two warehouse inventory close validity with minimum risk of break-ability characteristic at first and after that deterioration in the industry to determine the optimal economic ordering quantity, the optimal total cost of per unit in each those items across the different class interval. The proposed model concerned with all of the items, especially items which packed in the glass.

MATERIAL AND METHODS

Assumptions and notions
Assumptions

In this paper, the mathematical model is developed with the following assumptions

1) The planning horizon is finite.
2) The replenishment rate is infinite.
3) Single item inventory control.
4) Demand and deterioration and the break-ability rates are constant.
5) Deterioration rate more than break-ability rate.
6) Break-ability occurs as soon as the items are received into inventory within [0, t_w] and deterioration through [t_w, T].
7) The demand with [t_w, T] is more than demand within [0, t_w].
8) There is no replacement or repair of breakable items during the period under consideration.
9) The shortage is not allowed.
10) The lead time is zero.
11) The inventory level at the end of the planning horizon will be zero.
12) The cost factors are deterministic.
13) The total relevant cost consists of fixed ordering, purchasing, and holding costs.
14) The last order is only being placed to satisfy the balance in the stock of the last period.

Notation

D_w= The demand rate quantity in period [0, t_w].
D_T= The demand rate quantity in the period [t_w, T].
$D_T > D_W$
C = the present value of purchasing cost.
I_h =The holding cost.
Q_w= The order quantity in period [0, t_w].
TC_{Aw} = The total fixed ordering cost during [0, t_w].
TC_{hw} = The total holding cost during [0, t_w]

TC_{Pw} = The total purchasing cost during [0, t_w].
TC_{w}= The total relevant cost during [0,t_w].
C_T = The present value of holding costs during [t_w, T].
Q_T= The order quantity in period [t_w, T].
TC_{AT} = The total fixed ordering cost during [t_w, T].
TC_{hT} = The total holding cost during [t_w, T].
TC_{pT} = The total purchasing cost during [t_w, T].
TC_{T}= The total relevant cost during [t_w, T].

Parameters

T = The length of the finite planning horizon.
$I_W(t)$ = The inventory level at a time [0, t_w].
$I_T(t)$ = The inventory level at time [t_w , T].
T= the length of replenishment.
t_w = The time at which the inventory level reduces to W.
Θ = the constant break-ability rate units/unit time during [0, t_w].
φ = the constant deterioration rate units/unit time during [t_w, T].

Mathematical model

Let $I_W(t)$ is the inventory level at any time t,$0 \le t \le t_w$ Depletion due to demand and break-ability rate. The first-order differential equation that describes the instantaneous state of $I_W(t)$ over the open interval [0, t_w] is given by.

$$\frac{dI_w(t)}{dt} + \theta I_w(t) = -D_w, 0 \le t \le t_w \;\; 0 \le \theta \le 1 \qquad (1)$$

Let $I_T(t)$ is the inventory level at any time t,$t_w \le t \le T$ Depletion due to demand and deterioration rate. The first order differential equation that describes the instantaneous state of $I_T(t)$ over the open interval [t_w, T] is given by.

$$\frac{dI_T(t)}{dt} + \phi I_T(t) = -D_T \quad , t_w \le t \le T, \; I_T(t_W) = e^{-\phi t_W} \qquad (2)$$

$$I_W(t) = I_0(t) \int_t^{t_w} D_w e^{\theta u} = \frac{D_w}{\theta}(e^{\theta(t_w - t)} - 1), I_0(t) = e^{-\theta t}$$

$$I_W(t) = I_0(t) \int_t^{t_w} D_w e^{\theta u} = \frac{D_w}{\theta}(e^{\theta(t_w - t)} - 1), I_0(t) = e^{-\theta t} \qquad (3)$$

$$Q_W = \frac{D_w}{\theta}(e^{\theta t_w} - 1) \qquad (4)$$

$$I_T(t) = I_0(t) \int_{t_w}^{T} D_T e^{\phi u} = \frac{D_T}{\theta}(e^{\phi(T - t_w)} - 1), I_0(t) = We^{-\phi t_w}$$

$$I_T(t) = I_0(t) \int_{t_w}^{T} D_T e^{\phi u} = \frac{WD_T}{\theta}(e^{\phi(T - t_w)} - 1), I_0(t) = We^{-\phi t_w}$$
(5)

$$Q_T = \frac{WD_T}{\theta}(e^{\theta(T - t_w)} - 1)$$

According to Eq.2 the maximum inventory quantity at the begin each period is given as

$$Q_T = \frac{WD_T}{\theta}\left(e^{\theta(T-t_w)} - 1\right)$$

Fixed ordering cost

We assumed the number of replenishment is N so that the fixed ordering cost over the planning horizon under the inflation consideration is:

$$TC_{AW} = A \quad (6)$$

$$TC_{AT} = A \quad (7)$$

Purchasing cost

According to fig.1 of inventory level the purchasing cost of

$$TC_{PW} = \frac{CD_w(e^{tW}-1)}{\theta} \quad (8)$$

$$TC_{PT} = \frac{WCD_T(e^{\theta(T-t_W)}-1)}{\phi} \quad (9)$$

Holding cost excluding interest cost

We find the average inventory quantity to obtain holding cost

$$TC_{hW} = \int_0^{t_W} I_w(t)dt = \int_0^{t_W} \frac{D_w}{\theta}(e^{\theta(t_W-t)} - 1)dt = \frac{D_w I_h}{\theta^2}\left(e^{\theta t_W} - \theta t_W - 1\right) \quad (10)$$

$$TC_{hT} = \int_{t_W}^{T} I_W(t)dt = \int_{t_W}^{T} \frac{D_T}{\phi}\left(e^{\phi(T-t_W)} - 1\right)dt = \frac{WD_T I_h}{\phi^2}\left(e^{\phi(T-t_1)} - \phi(T-t_1) - 1\right) \quad (11)$$

$$TC_W = TC_{AW} + TC_{hW} + TC_{PW} \quad (12)$$

$$TC_T = TC_{AT} + TC_{hT} + TC_{PT} \quad (13)$$

Economic order quantity

Economic order quantity during [0, t_W]

To find EOQ by minimizing the total cost

$$Q_W = \frac{D_W}{\theta}\left(e^{\theta t_W} - 1\right)$$

By subsisting Eq. (6, 8, 10) in Eq. (12)

Then

$$TC_W = \frac{1}{t_w}[A + \frac{CD_W(e^{\theta t_W} - 1)}{\theta} + \frac{D_W I_h}{\theta^2}(e^{\theta t_W} - \theta t_W - 1)]$$

$$\frac{dTC_W}{dt_w} = \frac{-1}{{t_w}^2}\left(A + \frac{CD_W(e^{\theta t_W}-1)}{\theta} + \frac{D_W I_h}{\theta^2}(e^{\theta t_W} - \theta t_W - 1)\right) + \frac{1}{t_w}\left(CD_W e^{\theta t_W} + \frac{D_W I_h}{\theta}(e^{\theta t_W} - 1)\right)=0$$

Here

By using Taylor's series for $e^{\theta t_W}$ *about zero*

$$e^{\theta t_W} = 1 + \theta t_W$$

$${t_W}^* = [\frac{A}{D_W(C\theta + I_h)}]^{\frac{1}{2}}$$

$${Q_w}^* = \frac{D_W}{\theta}(e^{\theta {t_W}^*} - 1) \quad (14)$$

Economic order quantity during $[t_W, T]$

To find EOQ by minimizing the total cost function by taking derivation the total cost for second interval with respect to T.

$$Q_T = \frac{WD_T}{\phi}\left(e^{\phi(T-t_W)} - 1\right) \quad (15)$$

By substituting the Eq. (7, 9, 11, and 13) in the equation Eq. (15), then it can be rewritten as

$$TC_T = \frac{1}{T-t_w}[A + \frac{WCD_T(e^{\phi(T-t_W)}-1)}{\phi} + \frac{WD_T I_h}{\theta^2}\left(e^{\phi(T-t_1)} - \phi(T-t_1) - 1\right)$$

$$\frac{dTC_T}{dT} = \frac{-1}{(T-t_w)^2}[A + \frac{WCD_T(e^{\phi(T-t_W)}-1)}{\phi} + \frac{WD_T I_h}{\phi^2}\left(e^{\phi(T-t_1)} - \phi(T-t_1) - 1\right) + \frac{1}{T-t_w}[WCD_T e^{\phi(T-t_W)} + \frac{WD_T I_h(e^{\phi(T-t_1)}-1)}{\phi} = 0$$

$$(T-t_w)^* = [\frac{A}{WD_T(C\phi+I_h)}]^{\frac{1}{2}}=\mathrm{K}$$

$$T^* = [\frac{A}{WD_T(C\phi + I_h)}]^{\frac{1}{2}} + [\frac{A}{D_w(C\theta + I_h)}]^{\frac{1}{2}}$$

$${Q_T}^* = \frac{WD_T}{\phi}\left(e^{\phi(T-t_W)} - 1\right) \quad (16)$$

Lemma (1)

a) TC_W Has minimum value where $k_1 = D_W(\theta C + I_h)$

b) TC_T Has minimum value where $k_2 = WD_T(\phi C + I_h)$

Proof

a) $\frac{dTC_W}{dt_w} = \frac{-1}{t_w{}^2}\left(A + \frac{CD_W(e^{\theta t_W}-1)}{\theta} + \frac{D_W I_h}{\theta^2}\left(e^{\theta t_W} - \theta t_W - 1\right)\right) + \frac{1}{t_w}\left(CD_W e^{\theta t_W} + \frac{D_W I_h}{\theta}\left(e^{\theta t_W} - 1\right)\right)$

The second derivative of TC_W with respect to t_w

$$\frac{d^2TC_W}{dt_w{}^2} = \frac{2A}{t_w{}^3} - \frac{D_W(C\theta + I_h)}{t_w} + \theta(D_W(\theta C + I_h))$$

Let

$$k_1 = D_W(\theta C + I_h)$$

Then

$$\frac{d^2TC_W}{dt_w{}^2} = k_1(\sqrt{k_1} + \theta\sqrt{A}) > 0$$

Then TC_W has a minimum value
The first part holds on.

b)

$$\frac{dTC_T}{dT} = \frac{-1}{(T-t_w)^2}[A + \frac{WCD_T\left(e^{\phi(T-t_w)}-1\right)}{\phi} + \frac{WD_T I_h}{\phi^2}\left(e^{\phi(T-t_1)} - \phi(T-t_1) - 1\right)$$

$$+\frac{1}{T-t_w}[WCD_T e^{\phi(T-t_w)} + \frac{WD_T I_h\left(e^{\phi(T-t_1)}-1\right)}{\phi}$$

The second derivative of TC_W with respect to t_w

$$\frac{d^2TC_T}{dT^2} = \frac{2A}{(T-t_w)^3} - \frac{WD_T(C\phi + I_h)}{(T-t_w)} + \phi W(D_T(\phi C + I_h))$$

Let

$$k_2 = WD_T(\phi C + I_h)$$

$$\frac{d^2TC_W}{dT^2} = k_2(\sqrt{k_2} + \phi\sqrt{A}) > 0$$

Then TC_W has a minimum value
The second part holds on.

Sensitivity analysis

The assumption of the parameters of the total cost for the two inventory models as follows:

Example

$I_h = 0.05\$, C =, 20\$, A = 15\$, W = 1\ unit$

Fig.2 explained the first optimal runtime increased. Fig.3 the optimal second run time decreased when break-ability and deterioration rates increased. Fig. 4 explores the EOQ within first run time decreased when the first optimal runtime increased. Fig. 5 elaborates EOQ within second run time decreased when the second optimal runtime increased. Fig.6 illustrates that the total cost within the break-ability class interval decreased when the first optimal run time increased up to 0.5 after that increased. Fig. 7 represents the total cost within the deterioration class interval decreased when the deterioration time increased. The total cost within the second optimal runtime is feasible when the total of break-ability and deterioration rates less than 1.2 and take opposite that when the sum greater than 1.2. The impression of the output of the proposed model for the items is the committed policy of breakable and deteriorating items.

CONCLUSION

In this paper the deterioration, break-ability represented in the proposed model at different times to reduce the total cost of items. The assumed the daily demand is constant, developed the mathematical model by the above assumptions. The developed model can apply it in real life where the break-ability occurs before, the deterioration of items have these properties output of the mathematical model is minimizing the total cost of items and obtaining the economic order quantity for several break-ability rate values. The proposed model is having two cases of the first case. The model is fitted with less or more break-ability, obtained an economic order quantity is not satisfied the obtaining optimal total cost. The second with any value lies in the range of deterioration. The proposed inventory model can be investigated by minimizing the break-ability rate, suggest the safe system for the purpose of reducing the break-ability rate.

ACKNOWLEDGMENTS

We would like to thank Thamer University in Yemen for financial supporting also SRTM University in India. This work is supported by the Mathematical School of Sciences, India to develop the inventory model of deteriorating items.

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Table1. The sensitivity analysis

θ	Φ	D_W	D_T	t_W^*	Q_W^*	$(T-t_W)^*$	Q_T^*	TC_W^*	TC_T^*
0.0005	0.0006	50	100	2.236068	111.8659	2.419355	242.1112	3771.494	6897.589
0.045	0.046	100	150	0.39736	40.09336	0.103093	15.50064	1883.179	1727.016
0.05	0.051	150	200	0.308607	46.64999	0.070093	14.04378	2729.42	1610.184
0.15	0.16	200	250	0.156813	31.73426	0.018462	4.622208	3775.273	1653.843
0.25	0.26	250	300	0.109001	27.6249	0.009524	2.860683	4785.666	1567.488
0.35	0.36	300	350	0.084215	25.64058	0.005911	2.071169	5786.788	1352.718
0.45	0.46	350	400	0.068816	24.46229	0.004054	1.623135	6784.093	1006.327
0.55	0.56	400	450	0.058255	23.67939	0.002963	1.33444	7779.445	527.3962
0.65	0.66	450	500	0.05054	23.12063	0.002264	1.132922	8773.661	-84.4341
0.75	0.76	500	550	0.044647	22.70147	0.001788	0.984275	9767.157	-829.335
0.85	0.86	550	600	0.039995	22.37524	0.001449	0.870107	10760.17	-1707.4
0.95	0.96	600	650	0.036226	22.11405	0.001199	0.779669	11752.83	-2718.68

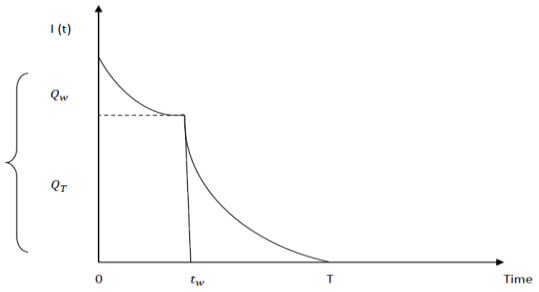

Fig.1Graphical representation of two warehouse inventory control diagram

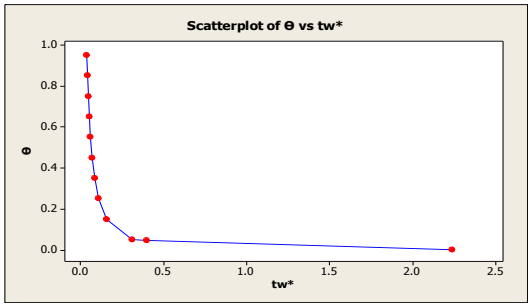

Fig.2. First optimal runtime VS the break-ability rate

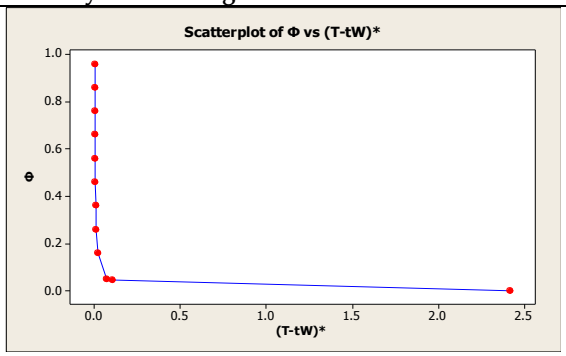

Fig.3. Second optimal runtime VS deterioration rate

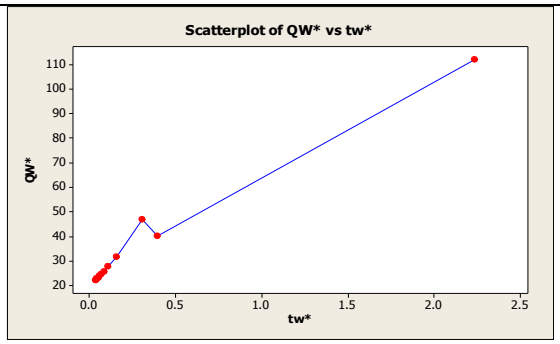

Fig.4. EOQ of the first interval VS first optimal runtime

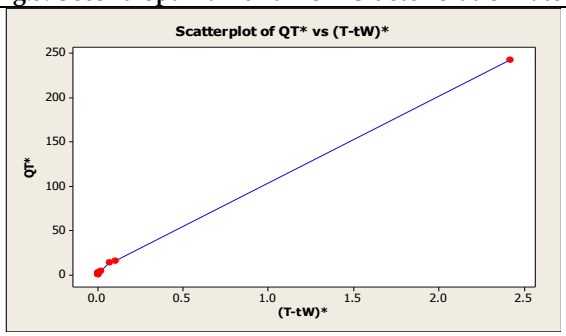

Fig.5. EOQ of second class interval VS optimal second runtime

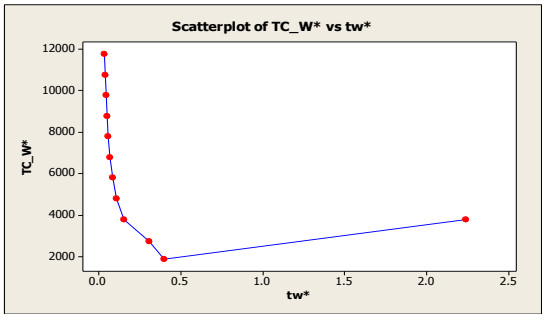

Fig.6. Optimal total cost VS first class interval

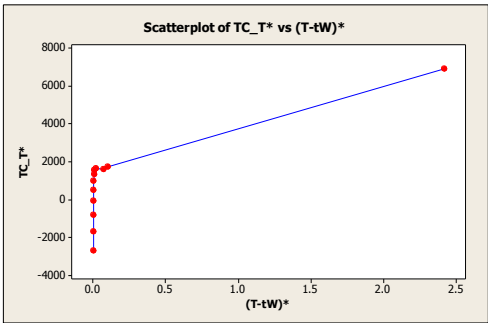

Fig.7. Optimal total cost second class interval

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Salary Prediction using a Unified Regression Model

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Received: 05 Mar 2020 Revised: 07 Apr 2020 Accepted: 09 May 2020

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ABSTRACT

Salary prediction engine is a tool that is developed using a particularprogramming code that can predict the salary of a person based oncertain values that is given to the prediction engine. The idea is to provide a machine learning model that predicts the most appropriate salary for a new employee and to check the validity of a potential employee's said salary based on his work experience and position in his previous job, if any. An employee's salary is the most important factor in determining their worth and their value and hence offering a fair andappropriate salary to a new employee at a concern is a major factor in determining their longevity there. In this paper, machine learning techniques such as are used to determine the salary of an employee. A particular dataset is used to train and predict the salary using various regression models and the most accurate and precise model isdetermined.

Keywords: Polynomial regression, Support vector regression, Decision tree regression, Random forest regression, Prediction Engine.

INTRODUCTION

The world is expanding rapidly, let it be the population, capital, business market and job requirements [1]. Due to these various factors like large population and job requirements, the salaries among various different sectors and types of industries among various places vary. In some of the companies or the institutions, the employees are underpaid and in some, they are overpaid and in some they are paid according to the pay scale of their particular company or institution. The difference in the salary for a similar role may differ across different places. For example, the American college teachers are paid handsomely whereas the college teachers of china are paid less as compared to the American college teachers [2].

With the increase in the amount of the companies, the competitiveness among them increases. Due to the competitiveness, various companies have various pay scales and the total number of employment increase and as a result the data related to the employee is generated in large amounts. In today's world the companies are setup for a large number of years and with every year the data increases and as a result these companies end up with a large amount of data [3]. The companies use these stored data to analyze and business decisions.

Such amount of large data cannot be handled and analyzed manually to make business decisions and also decisions regarding the employee's salary. In these cases, four machine learning regression techniques are used to develop a machine learning model or predictive engine that can predict the desired result. Some of the issues that a company might face while hiring a potential employee is determining whether the salary that the employee is demanding is a fair offer or not. Therefore, in such a situation the prediction engine helps in providing an accurate salary value based on which the person hiring can make easy decisions regarding the salary that has to be paid to the employee. Prediction engine [4] uses a particular dataset that is fed to it and it analyzes the given dataset and based on the learnings, takes the key values and predicts the solution with accuracy and precision.

Predicting the salary of a person may not be easy as many factors like his role or position in his previous company, the work experience of the employee i.e. for how long an employee has been working in the field and his highest achievement etc. In general, other factors like the qualification of the employee (educational) and the number of projects that the person has worked in is also taken into consideration. Due to these many conditions, predicting the salary of a person becomes difficult. In India, predicting salaries of various individuals depends on further different factors and the historical study about the data of India [5]. To make it simple the dataset that is used to train the model contains basic details like the highest position of the person in his previous company and the salary for the various positions are present. Each position is given a value under the level column.

The input that is given to predict the salary is this value. Say if a person has worked in a particular position for a particular amount of time the value between the corresponding position and the next position is taken as the input to predict the salary of the person. Some of the studies that are done to find solution for analyzing and developing salary range classifications is a Spanish case study [6] that helps in finding the features that are important in determining a person's salary range by using tree based ensembles. In this the data of over 4000 people are analyzed. Some of the models used in this are logistic regression, K-nearest neighbors, linear models, Multilayer perceptron etc. In this study various regression techniques like the support vector regression, random forest regression, decision tree regression and polynomial regression are used to develop models individually predict the salary of an employee by analyzing the dataset provided. The predicted salary may vary among different regression techniques, therefore the values generated by all the different models are compared among themselves and the expected values to find the technique that provide solution with the best precision and accuracy.

The predictive engine that is developed using the best regression technique is selected and presented as the solution to predict salary for an employee based on his previous role and his work experience. This technique can further be analyzed and developed to provide even more accurate predictions. A tree based ensembles is used to predict the salary with accurate salary classifier in the labor market with identifying the most rewarded jobs [7].Motivates the college students to know about the salary they will receive with their skills and completion of degree using random forest algorithm [8].The performance of students based on their studying habit is predicted using the k nearest neighbor and support vector machine classifiers [9].

Preprocessing

Overview of the Dataset

The proposed predictive engine which is to be build using regression technique uses the database to analyze and learns to predict the salary of an employee based on certain values. The database contains the following content i.e. position, level and salary. Based on these conditions the salary of an employee is predicted.

Description of the Dataset

The Dataset that is used for building the model is similar to Dataset that is uploaded in kaggle.com [10]. All the columns of the dataset used is similar to the dataset present in kaggle.com but has been modified slightly to increase the total number of elements present in dataset. The dataset has 60 elements spread across 3 columns mainly position, level and salary. In Machine learning the dataset elements can be differentiated in target attributes and non-target attributes. Target attributes are the attributes mainly based on which the final prediction are made. Target attributes are used as the learning curve by the model to predict the desired values. Here in the dataset which is used in problem the target attribute is the salary. The salary distribution across various levels is analyzes by the model and it learns to predict the supposed salary for a given level. The non-target attribute in the dataset used are the position and level. For every position there is an equivalent value given to the level. If an employee is said to have worked in a particular position for some years then the value of the level is taken from the value in between the value of the level for the corresponding level and the next level. Based on the value of the level the salary is predicted. Salary is taken as the dependent variable and level is taken as the independent variables. The graph is plotted using these variables and according to the input value of the level the corresponding value of the salary is predicted.

Non Target Attributes

Position: The position is the indication about employee's highest role in a company.
Level: It is the value given to the various level of the positions starting from 1 to 20. If the employee has worked for some years in a same position then the value of the level is taken from the value in between the value of the level of the current position to the next level value.

Target Attribute

Salary: - It contains the information regarding the pay scale of the employees. The salary increases from one position to another position. In the dataset that is used here has a salary range of 20000 to 2500000.

Process involved in the proposed prediction model

Developing the proposed prediction model involves applying machine learning techniques such as regression. There are mainly four regression techniques used i.e. polynomial regression, support vector regression, decision tree regression and random forest regression. The outcomes of the models developed using these regression techniques are taken individually and are compared for accuracy and precision. The most regression technique which provides the most accurate outcome is selected and in future can be improved further.

Polynomial Regression

Polynomial regression is a type of linear regression in which the relationship between the independent variable x and dependent variable y is plotted.
Linear regression equation: -

$$y = b_0 + b_1 x_1 \quad (1)$$

Multiple linear regression equation: -

$y = b_0+b_1x_1+b_2x_2+\ldots..+b_nx_n$ (2)

Polynomial regression equation: -

$y = b_0 +b_1x_1+b_2x_1^2+\ldots..+b_nx_1^n$ (3)

From the above equations it is seen that the value of x in polynomial regression equation is x1 throughout the equation. Only the power of x is increasing from 1 to n.

Support Vector Regression (SVR)

SVR is a type of supervised learning in which the function produces the output from the given input based on the predefined data. It is different from the other regressions. In other regression technique the main aim is to minimize the error but in SVR it is to fit error within a particular range. The line that is used or which helps in plotting the value that is targeted is known as the hyper plane. Then there are two other lines apart from the hyper plane that is known as the boundary line. Equation of a hyper plane which is a linear line and passes through the y axis is: -

$Wx + b = 0$ (4)

Equation for boundary lines are: -

$Wx + b = +e$ (5)
$Wx + b = -e$ (6)
$e <= y – Wx – b <= (+e)$ (7)
It is know that $y = Wx + b$ (8)
Therefore $y -Wx - b = 0$ (9)

Decision Tree Regression

Decision tree regression is an algorithm in which is represented in form of a tree structure. It is a non-linear model. The data from the dataset are broken down into smaller and smaller parts known as subsets. The end result of the process is tree with a leaf node and decision node. These nodes contains values and decision based on which the process is done. The top most decision node is the root node of a tree. Decision tree algorithm is based on greedy search and top down method without the ability of back tracking.

Random Forest Regression

Random forest regression is a type of supervised learning algorithm. It uses a combination of various machine learning algorithm and this technique is known as ensemble model. It uses combination of many machine learning algorithm so that it can develop a predicting model that has the best accuracy and precision. Some of the machine learning algorithm that it uses are support vector machine, decision tree, k nearest neighbor and naïve Bayes. Ensemble model can be classified into boosting and bagging (bootstrap aggregation). Random forest doesn't use boosting technique, it only uses bagging technique. Some of the advantages of random forest regression is that it is very accurate as compared to the other prediction engine model developed using other algorithm. It can run on any size of data. It has the ability of handling large data very well. It is very accurate is estimating the missing data as compared to the other machine learning techniques.

RESULT

To develop a salary prediction engine four machine learning algorithms such as support vector, random forest, polynomial and decision tree regression are used. The prediction engine developed using various algorithm provides various accuracy of the outputs. Random forest provides the best accuracy for the predicted salary. From the above table it is noted that decision tree predicts the exact salary for the values that are predefined in the dataset but for the values that are not predefined in the dataset the predicted salary is not accurate. Whereas the random forest regression provides a very accurate salary prediction for all values.

To further improve the overall accuracy of the prediction engine the system that is developed is a combination of decision tree regression and random forest regression. In this salary prediction engine that is developed the input values are checked for existence in the dataset. If the value is present in the dataset then decision tree regression is applied on the value to provide an exact salary prediction. If the value is not present in the dataset then random forest regression is applied on the value to predict a salary with the best accuracy. This model provides the least percent error of 1.01. Pseudocode for creating predictive engine using a remodeled algorithm:-
Check for the existence of the value in the dataset

If value is present in the dataset
Perform decision tree regression
Display the result

Else
Perform random forest regression
Display the result

CONCLUSION

The viability of four regression algorithms were examined for a salary production model and the most accurate algorithm was determined. Though Decision Tree and Random Forest Regression produced relatively accurate results, they do have their shortcomings. When the input level is a rational number, decision tree algorithm rounds it up to the closest whole number and produces inaccurate results but produces a result with maximum accuracy for whole numbers. The overall accuracy of the results from the Random Forest regression algorithm is relatively high, so the proposed system is the combination of them both. It has also been further observed that the proposed system has the least percent error and hence can be deemed the most accurate method. The future enhancement will be to extend this design to take into account more attributes that could determine the salary of an employee and also including different datasets into training the algorithm into producing more accurate results.

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Table 1. Predicted salary of various models for various values

Value	Polynomial	SVR	Decision tree	Random forest	Proposed system	Expected Salary
5	42199.0132	98229.37201	40000	39139	40000	40000
5.5	40198.8996	106324.878	40000	41029	41029	42000
7	37986.9201	121767.4224	50000	49507	50000	50000
7.5	40299.3312	123425.8614	50000	52911	52911	54000
10	99841.1149	135526.0959	110000	105283	110000	110000
10.5	125403.794	148902.9779	110000	119682	119682	122000
14	505838.215	555732.125	500000	472152	500000	500000
14.5	596662.176	667549.4379	500000	553362	553362	570000
18	1582385.08	1556070.438	1500000	1473500	1500000	1500000
Percent error	9.87	>>>	3.71	2.65	1.01	-

Index	Position	Level	Salary
0	ASE Trainee	1	20000
1	Ass Systems Engineer	2	25000
2	Systems Engineer	3	30000
3	IT Analyst	4	35000
4	Supervisor	5	40000
5	Business Analyst	6	45000
6	Manager	7	50000
7	Assistant Consultant	8	60000
8	Associate Consultant	9	80000
9	Consultant	10	110000
10	Senior Consultant	11	150000
11	Region Manager	12	200000
12	Principal Consultant	13	300000
13	Finance Director	14	500000
14	Corporate Counsel	15	750000
15	Partner Development ...	16	1000000
16	Research Scientist	17	1250000
17	Senior Research Sci...	18	1500000
18	Vice President	19	2000000
19	CEO	20	2500000

Fig. 1. An image of the Dataset

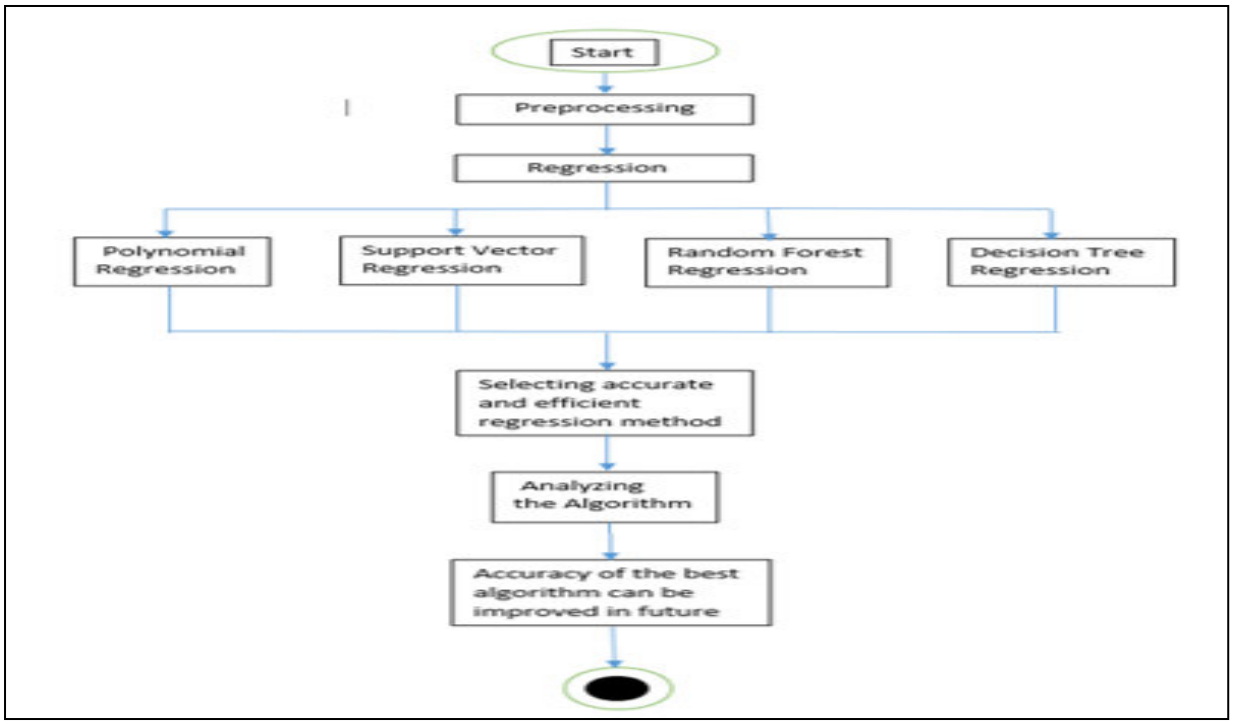

Fig. 2. An image of the flowchart representing the process

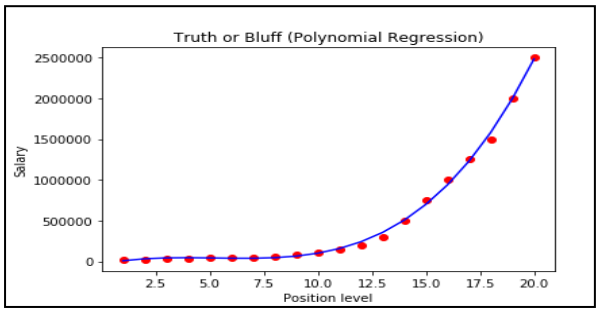

Fig. 3. Graph of support Polynomial regression

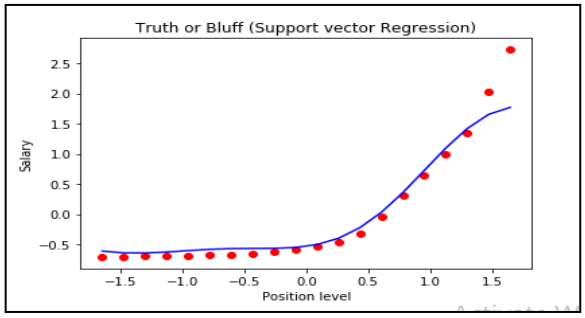

Fig. 4. Graph of support vector regression

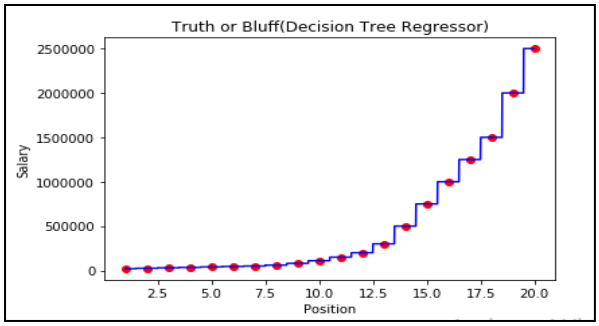

Fig. 5. Graph of Decision Tree regression

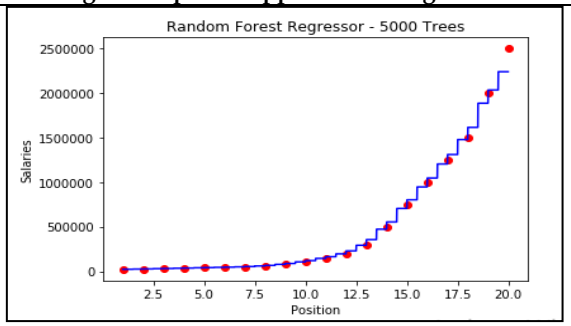

Fig. 6. Graph of Random Forest regression

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Comparative Study on Protein Content in Layer Egg with Different Species of Poultry Egg

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Received: 16 Feb 2020 Revised: 18 Mar 2020 Accepted: 20 Apr 2020

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ABSTRACT

The aim of this report was to investigate the protein content in layer egg with a comparision of different species of poultry egg.The protein content in layer egg is(3.131g),where as in native variety egg (2.953g), duck egg (2.652g), kadaknath egg (2.485g) and Muscovy egg (2.348g) is having .where as by t-testing it is observed that protein content is more in layer egg and less in Muscovy egg as compaired to other egg.The data obtained from the above study reveals that the native variety egg have little less content of protein (0.178g) and duck egg have much less content of protein (0.479g) while compaired with the layer egg.so it is analysed that the layer egg have more nutritive and protein characters as compaired to other species of poultry egg.Although these eggs have great nutrient and protein potential ,but the production of fresh kadaknath egg, duck egg and Muscovy egg for consumption is currently not seen in much greater.

Key words: Muscovy egg, kadaknath egg, physical and nutritive characters.

INTRODUCTION

Poultry eggs, as the whole nutrition reservoir for embryo development, have long been recognized as one of the major nutrient sources for humans (Otterillo, 1978). Undergoing the divergent evolution processes, eggs from different poultry species formed their unique properties from egg size to nutritional composition (Hoffmann, 2005). . Egg albumen as the major part of egg (taking up about 60%), consists of water (88%), proteins (11%), minerals and carbohydrate (1%) (Campbell *et al.,* 2003). The existence of various proteins in egg albumin gave it the .characters of foaming, emulsifying, gelling (review Poult. Sci.,2013) and egg albumin is also fast strong medical adhesive glue for wound healing (Xu and Liu, 2017). However, the information on egg albumen characteristics has been limited

mostly to chicken egg. Other usual poultry species, such as duck, goose, turkey, quail, and pigeon should also be paid attention to their egg properties, which would be helpful in technological and functional application of egg albumen from different poultry origins.

There have been extensive investigations for the use of albumen proteins in food processing, pharmaceuticals, health care, and others (Mine, 2002). However, proteomics analysis revealed that protein compositions in egg albumen of varied poultry species have large differences.(Sun and Liu,2017). The physical properties such as raw albumen viscidity and gel texture of heated egg albumen would also correspondingly vary. The viscidity of raw albumen, mainly due to the existence of ovalbumin, is the basic property for the usage in food processing (Abeyrathne, 2014). And heat-induced gel formation is another important property of egg albumen (Hoska, 2011). Gel is formed by the aggregation of denatured proteins that is induced by heat or other factors (Banerjee, 2012). Hardness is the major mechanical characteristic of gel and is defined as the maximal strength that needs to reach a special strain in cut, compression, or puncture trial (Peleg, 2006). . On the other hand, differences in protein composition of egg albumen can lead to varied nutritional values in eggs of different poultry species. Surpassing 90% of the dry substance of egg albumen is protein (Feeney, 1960) and egg albumen is considered as one of the most balanced amino acids resource for human demands (Swendseid, 1959). The nutritional value of amino acids in food mainly depends on the quantity and proportion of essential amino acids (EAA). World Health Organization (WHO) and Food and Agriculture Organization (FAO) in 1973 had recommended the EAA quantity in food, which is an important criterion to evaluate the nutritional value of food protein.

MATERIALS AND METHODS

Sample Collection

The eggs of chicken, duck, muscovy, kadaknath, native variety eggs are collected from different poultry farms at Balasore district. The breeds of these poultry were white leghorn layer (40wk old), jinding duck (50wk old), black king Muscovy (40wk old), black king kadaknath (50wk old) and native variety (40wk old). Eggs are transported 4degree C to our lab within 2days.

Measurement of Egg Quality

Specifically, egg weight was measured with weighing mechine accurate to 0.01 g. Then, the shape of egg (length/breadth) was measured with a vernier caliper accurate to 0.1 mm afterwards, eggs were broken to transfer the internal contents to a glass plate, and the heights of thick albumen were measured with a tri-pod micrometer. Egg yolk was separated from the albumen and was weighted by weighing mechine. The eggshells were then washed with water to remove residual egg white and were dried in fume hood and then weighted. Finally, eggshell thickness was measured with eggshell thickness gauge.

Determination of Protein Nutrients

The moisture content was determined by drying the albumen at 105°C to constant weight in oven.Before that the weight of petridish and wet albumin was measured in weighing mechine .Then after drying, again the albumin weight was measured and finally the moisture content was measured. The protein content in albumin was determined by lowery's method.In this method six testube was taken and before that standard solution and stock solution was prepared .Then the testube was arranged like 0.2, 0.4, 0.6, 0.8, 0 and last one is experimental tube .Then after wards, reagent-C and reagent–D was added and incubate for 15minutes and 20 minutes respectively.Finally the protein content in different species off eggs was measured in spectrophotometer at 660nm.

Statistical Analysis

Different species protein content was conducted to examine the difference of egg quality characters among five species of with the method of bar graph. The different egg quality traits and chemical composition of eggs were studied and the mean value was analysed using the t-test. Values of protein parameter were compared and significance of the test was calculated.

RESULTS AND DISCUSSION

External Egg Quality of Five Poultry Species

Duck had the heaviest egg weight (80.811), and native variety egg was the smallest (34.64). Due to the large egg size, duck egg also had the heaviest yolk, albumen and eggshell. Besides, duck eggshell was the strongest one with a breaking strength, while native variety eggshell was the weakest. Relatively high albumen heights were observed in duck (1.6mm), muscovy (1.3 mm), and layer egg (8 mm)

Internal Egg Quality of Five Poultry Species

The internal egg quality of five different species measures to be different. comparatively all the cell thickness ,yolk length and measurement is different .while it is observed that duck egg yolk length (5.3cm), layer egg yolk length (4.8cm), Muscovy egg yolk length (5.8cm), kadaknath yolk length (0.8cm) and native variety egg yolk length (0.7cm). Chemical analysis of protein content of different poultry species is comparatively different.it is observed that protein content of layer egg (3.131) is more while in Muscovy egg having less protein content (2.348). the mean and less amount of protein content (compaired with layer egg) with five different species of poultry egg (Table 1).

CONCLUSION

From the analysis, it is identified that layer egg has more protein content as compared to others .Egg are mainly rich in retinoic acid and omega -3 fatty acid which is good for our eyes and curing of our heart disease.

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Table 1. less amount of protein content (compaired with layer egg) with five different species of poultry egg

SPECIES NAME	PROTEIN CONTENT	LESS AMOUNT OF PROTEIN AS COMPAIRED TO LAYER EGG
Layer egg	3.131	3.131
Native variety egg	2.953	<.17
Duck egg	2.652	<.47
Kadaknath egg	2.485	<.64
Muscovy egg	2.348	<.78

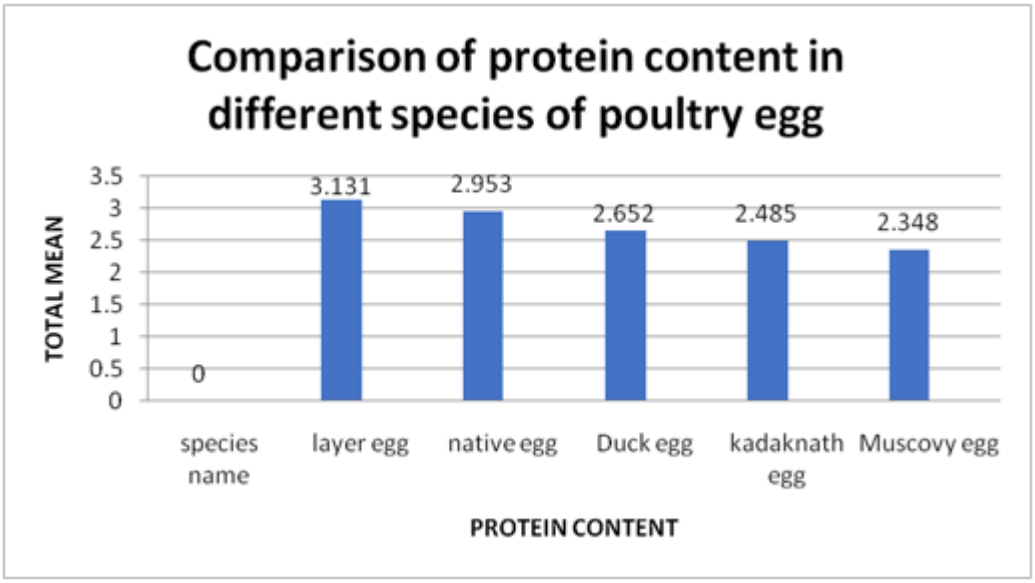

Figure.1. Graphical representation on total mean of different species of poultry egg

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Metagenomic Insights of Endophytic Bacteria in *Morinda pubescens* using NGS Technology

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Received: 05 Mar 2020 Revised: 07 Apr 2020 Accepted: 09 May 2020

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ABSTRACT

A high throughput technique of next generation sequencing (NGS) has become an inevitable sequencing tool to study the diverse microbial community in the environmental sample. In our work, this technique has been implemented for the metagenomics study of endophytic bacteria prevailing in the leaves of *Morinda pubescens*. The study profiles the complete data about the composition and diversity of microbial community by accessing PCR amplicon of 16srDNA sequences in the V3-V4 regions. A total paired ends of 376421 reads each with approximately 250bp sequence length are generated using IlluminaHiseq sequencing platform. The phred base quality score of the reads are checked and found to be 35.39 having an average G+C content of 54.49%. The taxonomic analysis of 11241 OTU's showed that the sequences belonged to 4 major phylaProteobacteria, BacteroidetesFirmicutes and Actinobacteria. The result reports the presence of dominance of genus *Brevundimonas*, *Bacteroides* followed by *Serratia* and *Propioni bacterium*, perhaps playing crucial role in promoting plant growth and metabolism.

Key words: Endophytic bacteria, IlluminaHiseq, Metagenomics, *Morinda pubescens*, OTU.

INTRODUCTION

The microorganisms are the most diverse and inexhaustible forms of life on earth. But yet understanding of their individual physiological diversity was limited largely to the species that can be grown only in cultures (Rinke et al. 2013; Solden et al. 2016). To an account, only 2-5% of the estimated 1.5 million endophytic microorganisms that supports the plant life have been documented. Plants are internally inhabited by its associated bacteria, known as

endophytes, which directly influence the responses mediated byplant cells(Hardoim et al. 2008) without affecting the host plant or evoking strong defense responses (Reinhold-Hurek and Hurek 2011). Bacterial endophytes that are symbionts of plants are beneficial through intervening processes of plant-growth promotion such as the biological nitrogen fixiation, phosphate solubilisation (Hallman et al. 2006) biocontrol of plant pathogens, phytoremediation (Strobel et al. 2004; Ryan et al. 2008;Staniek et al. 2008) and synthesis of plant hormones (De Melo Pereira et al. 2012). The fact that most microorganisms cannot be readilygrown in pure cultures awaits the microbiologist for a new technology applicable to identify the uncultured microorganisms. Metagenomics is a modern and fast growing field that gives entire ideaon the collective genome of microbial community extracted directly from environmental samples or natural habitats (Chen et al. 1995). With the advent of high throughput sequencing technology, the genetic study of microbial communities has become more reliable and easier. Also metagenomic analysis of the plant microbiome explored greater insights intofunctional informationof individual bacterial strains rather than the genome information (Berg 2009).

The present study focuses on the endophytic bacterial communities from the leaves of *Morinda pubescens* J. E Smith in culture-independent method. We have also isolated and characterised some of the culturable bacterial endophyte isolates from this plant with potential bioactive compounds (unpublished work). *M. pubescens* are medicinally important as astringent, deobstruent (Wang MY et al.2002; Kumaresan PT et al. 2009) and to treat dyspepsia (Muthu et al. 2006). The leaves are reported to act as a biosorbents in regulating ammonia pollution in wastewater treatment (Suneetha M et al. 2012). *M.pubescens* (Rubiaceae) leaves is distinguished for its traditional value and therapeutically used as anticonvulsant, analgesic, antiinflammatory, cytoprotective effect and antimicrobial activity (Deepti et al. 2011). Since the plant have many beneficial properties there may be key endophytic bacteria supporting the metabolism of the bioactive compound produced by this plant. So our work is centralised on identifying the bacterial communities and reporting the microbial colonisation in this medicinally important plant. For this, wehave used the NGS technology to revealboth the culturable and unculturableendophytic bacterial diversity in *Morinda pubescens*. Studies on the diversity of endophyticbacterial communities based on metagenomestratergy in another species *Morinda citrifolia* has been reported (Yang Liu et al. 2015)

MATERIALS AND METHODS

Sampling and surface sterilization of plant

The leaves of *Morinda pubescens* were collected from Thrissur (Lat 10°37′06′′N and Lon 76°12′17′′E), Kerala and transferred into sterile biosafety bag. The sample was thoroughly washed in tap water several times and surface sterilization was performed with 70 % ethanol for 2minsfollowed by 2.5% (W/V) NaOCl, solution for 10mins. Samples were then washed thrice with sterile distilled water for 10 mins. The effectiveness of the surface sterilization procedure were checked by plating the last rinsed water ofthe surface-sterilized tissues onto the agar plates to ensure there is no bacterial growth on plates after an incubation (37°C) period of 24 h and if any bacterial growth were found,the samples were discarded. After ensuring the effective surface sterilization, the tissues were gently disrupted with sterile mortar and pestle and genomic DNA of the endophytic bacterial isolates were extracted using QiagenDNeasy Plant Minikit (Cat no.69104) and lysozyme.

Extraction of DNA and PCR amplification

Disrupt 0.1g of the sample using sterile mortar and pestle and then add 400 µl AP1 buffer and 4 µlRNase into the homogenate then vortex and incubate the mixture for 10 min at 65°C. Centrifuge the lysate for 5 min at 20,000 x g (14,000 rpm) and pipette the lysate into a QIAshredder spin column and centrifuge for 2 min at 20,000 x g. Transfer the flow-through into a new tube without disturbing the pellet if present. Add 1.5 volumes of Buffer AW1 and mix well by pipetting. Transfer 650µl of it into Mini spin column kept in a collection tube of2 ml. Centrifuge for 1 min at

≥6000 x g (≥8000 rpm). Add 500 µl Buffer AW2, and centrifuge at 20,000 x g for 1 min and repeat this process again after discarding the flow through and then add 100 µl of elution buffer AE.Incubate for 5 min at room temperature (www.qiagen.com/handbooks). The extracted DNA was quantified and checked for purity using Qubit ® 2.0 fluorometer and Nanodrop (Thermo Fisher Scientific, U.S.A.) at A260/280nm and stored at −20 °C.

Illumina Library preparation

The genomic DNA of bacterial isolates from leaf tissue samples was standardized to concentration 10ng/µL.PCR amplification of V3–V4 conserved regions of 16S rRNA gene sequences was carried out using the forward and reverse primers (341 F: 5′ CCTACGGGAGGCAGCAG 3′ and 806 R: 5' GGACTACHVGGGTWTCTAAT 3′). The PCR library preparation was carried out using QiagenTaqPCR Master-mix Kit (Cat. No.201900). Concisely, PCR reaction were assembled into 50 µL volume containing 4µL of genomic DNA template, 25µLTaq DNA polymerase (1 U), Qiagen PCR buffer, PCR- grade dNTPs mix (10mM), 2.0 µL each forward and reverse primer and nuclease free sterile water. PCR reactions were started initially with denaturation step for 2 min at 94 °Cfollowed by 30 cycles at 94 °C denaturation for 30s, 55 °C primer annealing for 45 s, and 72 °C for 1 min extension and ended with a final extension step at 72 °C for 5 min. The PCR products were then analysed via 2% agarose gel electrophoresis and purified DNA were recovered using gel extraction kit QIAquick(Qiagen, Mississauga, Ontario, Canada). Thesequencing of the samples wascarried out on the IlluminaHiSeq sequencer by loading the sample DNA onto the paired-end flow cell of the system which performs dual index sequencing with automated cluster generation (Illumina guide 2013).

Sequence data processing

The paired-end sequence thus formed contains V3-V4 region and some portion of conserved region. As a preliminary step the conserved region were removed from paired-end reads to study V3-V4 metagenomics alone. Using Trimmomatic tool, the unwanted sequences were trimmed from original paired-end data and a consensus V3-V4 region sequence is constructed using FLASH program to improve genome assemblies. Chimeras and singletons are removed using the de-novo chimera removal method UCHIME which is implemented in the open source metagenomicstool VSEARCH. The raw data containing forward and reverse reads were merged using both QIIME and MG-RAST pipeline alignment method to evaluate their accuracy in assigning taxonomic units.

Characterisation of bacterial community composition

The pre-processed consensus V3-V4 sequences were clustered in Operational taxonomic units (OTUs) based on sequence similarity using algorithm implemented inUclust program having dissimilarity threshold of 3 % and similarity cutoff of 97 %. QIIME program, a quantitative insight in microbial ecology was used to perform downstream analysis such as microbial community analysis (Caporaso et al. 2010). Using PyNAST pro-gram the representative sequence was identified for each OTU aligned against Greengenes database (DeSantis et al. 2006). AlsoSILVA OTUs database raised byRDP classifier was used to quantify the microbial diversity, richness, composition and taxonomy classification of inferred OTUs.

RESULT

Sequence data analysis

The paired-end reads obtained from the sample were in the format of two FASTq files labelled as NMT_R1.FASTq and NMT_R2.FASTq. The Bioproject (PRJNA516334) was registered with the NCBI GenBank, with theBiosample accession number SAMN10787442. Using IlluminaHiseq sequencing platform, the highest reads obtained is 376421 from the leaf sample. The raw data obtained from the sequencing process was transferred to FASTA files. The GC

content of the sequence is 54.49. All the consensus sequence formed after the removal of unwanted sequences has an average contig length of approximately 350-450bp (refer graph 1). The readsafter the withdrawal of Chimeras using UCHIME from 373823 pre-processed sequenceswere compiled and clustered into Operational taxonomic units using UCLUST algorithm. A sum total of 198830 OTUs were analyzed from pre-processed sequences out of which 187589 singletons were removed and finally 11241 operational taxonomic units were selected for the taxonomic classification as mentioned in table no.1

Sequence processing and analysis

In the present work, we have usedtwo pipelines MG-RAST and QIIME to process the sequences, with high demand computational soft-ware package. The obtained raw data pointed that the reads covered V3-V4 region successfully has an average size of ~ 250 bp. The forward and the reverse reads were merged and again the sequences were processed using the QIIME and MG-RAST pipeline. The sequencesprocessed in both pipelines were found to be more or less similar. The errors formationwith ambiguous base, unusual length and low quality scores within the reads may occur hence it was necessary to remove such reads. Reads were trimmed based on quality scores and singletons were excluded from the sequence datasets to further reduce the frequency of error. The chimeric sequences developed during PCR amplification of DNAwere removed by QIIME incorporated with UCHIME was reported to be the best to perform a comparative study with the reference database (Schloss et al. 2011). As a result analyses such as clustering and assemblage of OTUs, taxonomy assignment andthe comparison of multiple samples were done with highly efficient bioinformatics tool.

Composition and diversity characterization of bacterial community

The taxonomic classification from phylum to genus of all the OTUs sequences were performed according to the program QIIME and RDP classifier against SILVA OTUs database. This study gives the profile of bacterial community enrichment in the leaves of *Morinda pubescens*. At phylum level, it was found that the dominance of proteobacteria followed by bacteroidetes and Firmicutes and at class level, gammaproteobacteria, alphaproteobacteria, bacteroidia and actinobacteria. The α-diversity analysis of the data indicates the richness and Simpson diversity index of the bacterial endophyte belonging to the most prominent phyla as shown in table 2.

DISCUSSION

Our study reveals for the first time the presence of bacterial community prevailing in the medicinal plant *Morinda pubescens* using IlluminaHiseq sequencing protocol.High-throughput sequencing discloses the diversity of bacterial communities in the leaf at the genus level. By using appropriate primer pair, to amplify a stretch of V3-V4 region of 16S rRNA gene, sequencing can be performed accurately. The correct choice of using various bioinformatics tools was equally important and challenging to reduce the errors hosted by the host DNA. The de-novo chimaera removal method VSEARCH implemented by UCHIME was used to improve the overall quality of the reads. The result draws special attention to the utility of thesequencing platform for its high resolution microbiota profiling (N90% at genus level) of endophytic bacterial communities. The four most abundant phyla Proteobacteria, Bacteroidetes, Firmicutes and Actinobacteria (refer graph 2) were detected at similar abundances by MG-RAST and QIIME pipeline. NGS uses the shotgun 16S rRNA gene sequencing which discloses the overall richness and diversity of microbial communities in plant tissues in both the culture dependent and independent method. The α-diversity analysis indicates that the species richness is more in Actinobacteria but the species eveness is more in Proteobacteria and also the Simpson diversity index and Shannon index varies within the most prominent phyla. Conclusively, four prominent phyla Proteobacteria, Bacteriodetes Firmicutes and Actinobacteria were identified to colonise *Morinda pubescens* plant tissues were shown to have many beneficial bioactive compounds (unpublished). The study elucidates the genus such as Brevundimonas, Bacteroides, Serratia, Propionibacterium by OTU distribution (refer graph 3). Culture-

independent methods applied in this work will provide us a notion of endophytic bacteria prevailing in this plant to isolate that can promote plant growth or may have potential bioactive compounds with immense value.

Funding

This work was supported by the grants given to Neenu A Santhosh by CSIR-UGC, New Delhi, India.

Conflict of interest

The authors declare no conflict of interest regarding the content and writing of the manuscript.

ACKNOWLEDGEMENTS

The authors acknowledged the Agrigenome, Labs Pvt Ltd.for the use of Illumina HiSeq and DST-FIST sponsored Lab, department of botany and Zoology, St. Thomas College (autonomous), Thrissur for their assistance.

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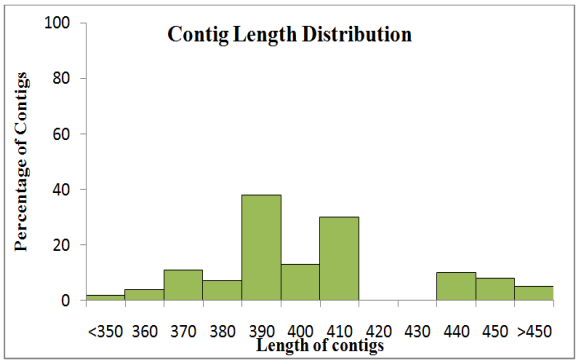
Table 1.Summary of OTUs

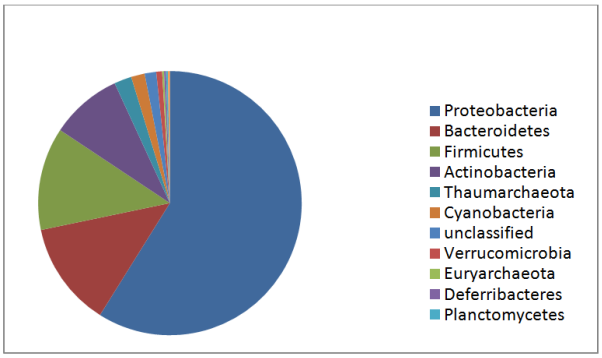
Consensus sequences	374752
Chimeric sequences	929
Total Pre-processed Consensus	373823
Total OTUs Picked	198830
Total Singleton OTUs	187589
Total OTUs after Singleton removal	11241

Table 2: Diversity indices among different major phyla

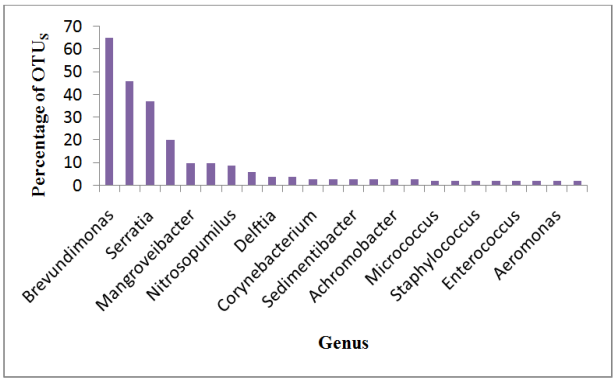
Phylum	Margalef index (d)	Eveness J'	Shannon diversity index H' (loge)	Simpson index 1-Lamdba'
Proteobacteria	0.7411	0.9975	1.383	0.7616
Bacteroidetes	0.8936	0.8936	1.386	0.7771
Firmicutes	0.9334	0.9334	1.349	0.7642
Actinobacteria	0.9551	0.9551	1.385	0.7834



Graph 1. Contig length distribution of the sequence



Graph 2: OTUs classification at phylum level



Graph 3. OTUs classification at genus level

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Plant Resources used in Kartika Purnima for Worshipping Lord Kartikeya in Odisha, India

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Received: 16 Feb 2020 Revised: 18 Mar 2020 Accepted: 20 Apr 2020

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ABSTRACT

Nature is important and valuable thing. Biodiversity is gift of nature which fulfills all the basic requirements which we need. Biodiversity refers to variety of plants and animals of the world. Plants have major role and are great contributors to earth. It has been observed that large number of plants used in worshiping God and Goddess. The India is famous country for Hindus. India is a place, where many festivals celebrated all around the year like Holi, Diwali, Dussehra, Ganesh Chaturthi and Kartika Purnima etc. Among these Kartika Purnima is one the famous festival of Hindus. The festival observed in the month of Kartika (i.e October and November) on the full moon day of the Hindu liner calendar. According to the Hindu calendar Kartika is the eight lunar month. The data represents on plants and plant parts used in Kartika Purnima which are collected through the interaction with priest and knowledgeable old person who are involved in Kartika Purnima particles. In this survey a total numbers of 53 plants which are belongings to 35 families were recorded.

Keywords: Biodiversity, Botanical, Hinduism, Kartika, Plant, Worshiping

INTRODUCTION

The Hinduism is the traditional religion of India, they believe in the Vedas. Everything which we need to fulfill our desire that directly or indirectly comes from plant. Plant plays important role in Hindu mythology. The Vedic Aranyan goddess of forest is today still worshipped in rural India as Vana-devi. Plants also have traditional value. In many festivals plants are largely used (Dalasingh et al., 2018; Dash et al., 2019). Kartika Purnima is one of the festivals celebrated by Hindus, Jain and Sikhs on full moon day in the month of Kartika (i.e October and November) . According to the Hindu lunar calendar Kartika is the eight lunar month. The festival is celebrated by worshiping the

lord Kartikeya (Kurien, 2006). The festival is also known as Tripuri Purnima and sometimes it also called Deva-deepawali, festival of light. In Odisha, on the day of Kartika Purnima people celebrate Boita Bandana. Boita stands for boat or ship. A history behind this festival is Kalinga, tradesman and mariners known as Sadhavas travelled on boitas (Boat) to trade with distance island nations that share borders with the Bay of Bengal like Indonesia, Java, Sumatra, and Bali. This festival observed fifteen days after Dipawali (Mishra, 2013; Verma, 2013; Mandal et al., 2020).

MATERIALS AND METHODS

This data represents plant and plant part used in Kartik Purnima for worshiping the lord Kartika in different village areas, cities, pandals of Odisha was carried out during the time of Kartika Purnima. The data were recorded about the importance of various plant and plant part used for worshiping lord Kartikeya. The data were collected through the interaction with specialized person like priest who worship god in pandals and in village areas. The botanical name of plants are documented and identified with the help of "The Flora of Odisha" as well as with the help of Department of Botany, Centurion University of Technology and Management, Odisha, India. Finally the plant specimen was deposited in the department of Botany (Saxena and Bhahmam, 1994; Haines, 1921).

RESULT AND DISCUSSION

The present study represents the plant and plant parts used in Kartika Purnima in different villages, areas, sites and pandals of Odisha. A total number of 53 plant species belongs to 35 families were recorded. The Hinduism has been called the dominant religion in India. This paper represents different plant species that were used in worshipping of lord Kartikeya. Many plants are associated with several rituals and used for all religious purposes among the Hindus. *Aegle marmelos* (L.) Corr. is important leaves used for worshipping god Kartikeya. Flowers like *Nelumbo nucifera* Gaertin. and *Calotropis gigantean* R.Br. are mostly used for worshipping and favorite flower of lord Kartikeya. There are about 10 leaves which are used for decoration as well as worshipping, 14 different types of flowers, seeds, cereals are used, 12 types of fruits and 17 other plants are used for making prasad and worship. Details of the recorded 53 plant species in terms of their botanical name, local name, family, habit, plant part and form of use are given in the table 1.

Sl no.	Botanical Name	Local Name	Family	Habitat	Part used	Form of uses
Leaves						
1	*Osimum sanctum* L.	Tulsi	Lamiaceae	Herb	Leaves	Worshipping
2	*Mangifera indica* L.	Amba	Anacardiceae	Tree	Leaves	Worshipping
3	*Ficus benghalensis* L.	Bara	Moraceae	Tree	Leaves	Worshipping
4	*Piper betel* L.	Pana	Piperaceae	Climber	Leaves	Worshipping
5	*Musa paradisiacal* L.	Kadali	Musaceae	Tree	Leaves	Worshipping
6	*Ficus religiosa* L.	Asta	Moraceae	Tree	Leaves	Worshipping
7	*Shorea robusta* Gaertn.f.	Sala	Dipterocarpaceae	Tree	Leaves	Worshipping
8	*Ziziphus mauritiana* Lam.	Barakoli	Rhamnaceae	Tree	Leaves	Worshipping
9	*Phyllanthus emblica* L.	Amla	Phyllanthaceae	Tree	Leaves	Worshipping
10	*Aegle marmelos* (L.) Corr.	Bela	Rutaceae	Tree	Leaves	Worshipping
Flowers						
1	*Tagetes erecta* L.	Gendu	Asteraceae	Herb	Flower	Used in garland
2	*Polianthus tuberose* L.	Rajanigandha	Asparagaceae	Herb	Flower	Used in garland
3	*Hibiscus rosa sinensis* L.	Mandara	Malvaceae	Shrubs	Flower	Used for worshipping

4	*Cascabela thevetica* (L.) Lppold	Kaniara	Apocynaceae	Tree	Flower	Used for Worshipping and in garland
5	*Nelumbo nucifera* Gaertin.	Padma	Nelumbonaceae	Herb	Flower	Used in garland
6	*Plumeria rubra* L.	Champa	Apocyanaceae	Tree	Flower	Used in garland
7	*Calotropis gigantean* R.Br.	Arakha	Apocyanaceae	shrub	Flower	Used in garland
8	*Clitoria ternatea* L.	Aparajita	Fabaceae	Climber	Flower	Used in garland
9	*Tabernaemontana divaricata (L.) R.Br. ex Roem. & Schult.*	Tagara	Apocynaceae	Shrub	Flower	Used in garland
10	*Nyctanthus arbortritis* L.	Gangasiuli	Oleaceae	Tree	Flower	Used for worshipping
11	*Impatiens balsamina* L.	Haragaura	Asteraceae	Herb	Flower	Used for worshipping
12	*Saraca asoca* (Roxb.) de Wilde	Ashoka	Fabaceae	Shrub	Flower	Used in garland
13	*Allamanda cathartica* L.		Apocynaceae	Tree	Flower	Used in garland
14	*Delonix regia* (Boj.ex Hook.) Raf.	Krushnachuda	Apocynaceae	Tree	Flower	Used in garland
Fruits						
1	*Musa paradisiacal* L.	Kadali	Musaceae	Shrub	Fruit	Used in Prasad
2	*Cocos nucifera* L.	Nadia	Arecaceae	Tree	Fruit	Used in Prasad
3	*Vitis vinifera* L.	Angur	Vitaceae	Tree	Fruit	Used in Prasad
4	*Pyrus malus* L.	Seu	Rosaceae	Tree	Fruit	Used in Prasad
5	*Citrus reticulata* Blanco	Kamala	Rutaceae	Tree	Fruit	Used in Prasad
6	*Cucumis sativus* L.	Kakudi	Cucurbitaceae	Climber	Fruit	Used in Prasad
7	*Psidium guajava* L.	Pijuli	Myrtaceae	Tree	Fruit	Used in Prasad
8	*Ananas comosus* (L.). Merr.	Sapuri	Bromeliaceae	Tree	Fruit	Used in Prasad
9	*Punica granatum* L.	Dalimba	Lythraceae	Tree	Fruit	Used in Prasad
10	*Annona squamosa* L.	Ata	Annonaceae	Tree	Fruit	Used in Prasad
11	*Litchi chinensis* Lam.	Lichi	Sapindaceae	Herb	Fruit	Used in Prasad
12	*Mangifera indica* L.	Amba	Anacardiaceae	Tree	Fruit	Used in Prasad
Others						
1	*Sesamum indicum* L.	Rashi	Pedaliaceae	Herb	Seed	Used in Prasad
2	*Vigna radiate* (L.) R. Wilczek	Muga	Fabaceae	Herb	Seed	Used in Prasad
3	*Vigna mungo* (L.) Hepper	Biri	Fabaceae	Herb	Seed	Used in Prasad
4	*Brassica napus* L.	Sorisha	Brassicaceae	Herb	Seed	Used in Prasad
5	*Terminalia chebula* Retz.	Harida	Combretaceae	Tree	Fruit	Used in Prasad
6	*Terminalia bellirica* (Gaertn.) Roxb.	Bahada	Combretaceae	Tree	Fruit	Used in Prasad
7	*Santalum album* L.	Santala	Santalaceae	Tree	Wood	Used for worshipping
8	*Saccharum officinarum* L.	Akhu	Poaceae	Grass	Stem	Used for worshipping

9	*Oryza sativa* L.	Dhana	Poaceae	Grass	Seed	Used for worshipping
10	*Bambusa vulgaris* Schrad.	Baunsa	Poaceae	Tree	Stem	Used for worshipping
11	*Cucurma longa* L.	Haladi	Zingiberaceae	Shrub	Rhizome	Used in Prasad
12	*Shorea robusta* Gaertn.f.	Sala	Dipterocarpaceae	Tree	Wax (jhuna)	Used for worshipping
13	*Solanum tuberosum* L.	Alu	Solanaceae	Herb	Tuber	Used for prasad
14	*Cuminum cyminum* L.	Jeera	Apiaceae	Herb	Seed	Used in Prasad
15	*Piper nigrum* L.	Golamaricha	Piperaceae	Climber	Fruit	Used in prasad
16	*Zingiber officinale* Rosc.	Ada	Zingiberaceae	Herb	Rhizome	Used in prasad
17	*Colocasia esculenta* (L.) Schott	Saru	Araceae	Herb	Tuber	Used in prasad

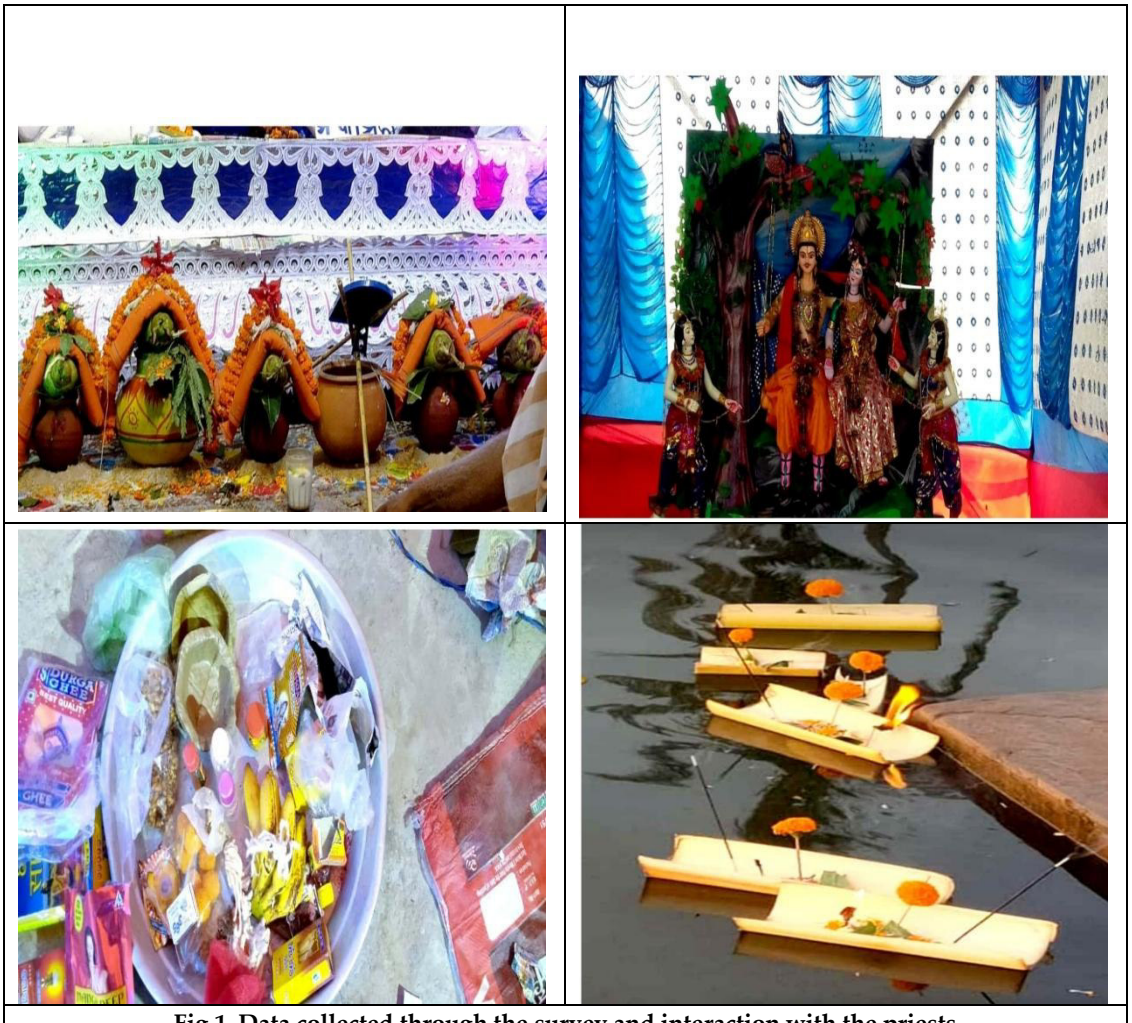

Fig.1. Data collected through the survey and interaction with the priests

CONCLUSION

The study on religious plant and plant part used in Kartika Purnima for worshipping God Kartikeya represents about the importance of plant in human life. The present study helps to understand how the Hindu community of Odisha is contributing towards the conservation of plant and forest in general of their own interest to safeguard their inherent socio-cultural and religious activities, such moment of monitoring and utilizing the plant species for the sake of worshipping and socio-social and convictions uncover a solid immensity in the present worry of biodiversity preservation.

ACKNOWLEDGEMENT

The authors are thankful to villagers, old people and priests who given the information for this work and kind support, special thanks to priest Nilakantha Panda , Ghanashyam Tripathy for their valuable help and co-operation during this survey work.

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Efficacy of Plant Derived Materials on Oviposition, Adult Emergence, Survival of Pulse Beetle in Black Gram

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Received: 05 Mar 2020 Revised: 07 Apr 2020 Accepted: 09 May 2020

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ABSTRACT

The experiment was conducted to test botanicals and oils against pulse beetle in black gram, where five pairs of adult beetle were released for egg laying and it was observed that mortality of adult beetles was higher in black gram treated with deltamethrin 11 EC @ 2.5ml/kg (6.67 nos.) followed by turpentine oil @ 4ml/kg (4.67 nos.) and acetone @ 4ml/kg (4.33 nos.) while in botanical, *viz.,* pungam oil @ 5ml/kg, neem oil @ 5ml/kg and castor oil @ 10ml/kg the mortality ranged from 3.67-3.00 on 5 days after treatment . Deltamethrin 11 EC @ 2.5ml/kg recorded less number of pulse beetle eggs and adult emergence (12.33 nos. and 1.67 nos., respectively) followed by turpentine oil @ 1ml/kg, pungam oil @ 5ml/kg, castor oil @ 10ml/kg and neem oil @ 5ml/kg in egg laying (28.67– 38.33nos.) and adult emergence (5.33–8.67 nos.).

Keywords: Black gram, Pulse beetle, Botanicals

INTRODUCTION

Pulses are rich in protein content which ranges from 17 to 24 per cent. The demand for pulses is increasing 3 per cent every year. Apart from human and animal nutrition they also play an important role in sustaining soil fertility with their unique ability to fix atmospheric nitrogen through symbiosis with Rhizobium. The pulse crops are attacked by more than 150 insect pests. It is recorded that 55- 60 per cent loss in seed weight and 45.50 to 66.30 per cent loss in protein content of pulses is due to infestation caused by pulse beetle or bruchids (Solanki and Mittal, 2018**).** The pulse

beetles have been observed to be the most important pest in pulses during storage (Sarwar *et a1.,* 2005). These includes three bruchid species, *Callosobruchus maculatus* Fab., *C. chinensis* Linn. and *C. analis* which infest most of the pulses.

Generally, infestation starts in the field but population builds up in storage as the insect feeds inside the seed and emerge as an adult and causes secondary infestation inflicting heavy losses. The infested seeds are rendered unfit for human consumption as well as for sowing purposes (Bhalla *et al.,* 2008). These insects can damage 100 per cent of stored seeds causing weight losses up to 60 per cent (Khaire *et al.,* 1993). Therefore, it is necessary to reduce such storage losses by controlling the insect pests on stored grains . In India, synthetic chemical insecticides and fumigants are used to protect the pulses from the infestation of pulse beetle in storage. But the use of chemical insecticides causes several problems like resistance and toxic residues in food. However, the use of chemical pesticides not only involved potential health hazards, residues, pollution, contamination, but also beyond the financial capability of the farmers (Khaire *et al.,* 1993). It is an urgent need to find out a safe and sound alternative to chemical insecticides to protect the stored products in storage.

MATERIALS AND METHODS

The homogeneous culture of pulse beetle *C. maculatus* was maintained under laboratory conditions in black gram (VBN 6). For this purpose 3 kg infested seeds was kept in plastic containers and were covered with muslin cloth and kept in incubator at 27.5°C ± 1°C. Only freshly emerged adults were used in the experiments. All the emerged adults were removed and fresh culture was maintained at regular intervals.

Twenty grams of black gram seeds were treated with following plant materials (T1- Neem oil 5ml/kg, T2- Castor oil 10ml/kg, T3- Deltamethrin 11 EC 2.5ml/kg, T4- Acetone 4ml/kg, T5- Pungam oil 5ml/kg, T6- Turpentine oil 1ml/kg and T7- Control) and kept in plastic containers. The completely randomized block design (CRD) was adopted with three replication. Five pairs of adult beetle were released in each container, covered with muslin cloth and were observed at one, three and five days after treatment to record the mortality of adult beetles in different treatments. All the adults were removed after 5 days. The number of eggs laid on grains in each treatment were counted. Fifteen days onwards treatments were observed daily to record the number of beetles emerged in each treatment and survival percentage was worked out.

The data obtained from experiments were subjected to ANOVA (Analysis of variance). The data on percentage and population in numbers were transformed into arcsine and square root values, respectively before statistical analysis. The data obtained were analyzed in completely randomized design (CRD) and the means were separated by least significant means (Gomez,K.A and A.A.Gomez,1984)

EXPERIMENTAL RESULTS

The results revealed that mortality of adult beetles at one day after treatment (DAT) was higher in black gram treated with deltamethrin 11 EC @ 2.5ml/kg (3.33 nos.) followed by turpentine oil @ 4ml/kg (2.67 nos.), neem oil @ 5ml/kg and acetone @ 4ml/kg (2.00 nos.), castor oil @ 10ml/kg (1.67 nos.) and pungam oil @ 5ml/kg (1.33 nos.) which were on par and control recorded no mortality (Table 1 and Fig 1). On 2 DAT higher mortality was in deltamethrin 11 EC @ 2.5ml/kg (4.33 nos.) compared to other treatments (3.00 -1.67 nos.) and control (0.33 nos.). The same trend was noted on 5 DAT also. Results on egg laying, adult emergence and survival differed significantly among all treatment (Table 2). Among the treatments, deltamethrin 11 EC @ 2.5ml/kg recorded less number of pulse beetle eggs (12.33) followed by other treatments which were on par (28.67- 45.67) and control (79.33) (Fig 2).

The adult emergence was low in seeds treated with deltamethrin 11 EC @ 2.5ml/kg (1.67 nos.) followed by pungam oil @ 5ml/kg and turpentine oil @ 1ml/kg (5.33 nos.), whereas in castor oil @ 10ml/kg, neem oil @ 5ml/kg and acetone @ 4ml/kg, the emergence was 7.33, 8.67 and 12.33, respectively. Survival percentage was low in all the treatments *viz.,* deltamethrin 11 EC @ 2.5ml/kg (12.00%), turpentine oil @ 1ml/kg (14.67%), acetone and pungam oil @ 5ml/kg (16.00%), neem oil @ 5ml/kg and castor oil @ 10ml/kg (17.00%) whereas in control it was high (24.33%).

Bhargava and Meena (2002) studied the efficacy of some vegetable oils against pulse beetle, *C. chinensis* on cowpea and found that all the oils caused significant mortality in adults three days after treatment and the mean mortality varied from 60.0 to 80.2 per cent in different oils. They also reported that the oils also inhibited the oviposition in treated seeds as against untreated control. Yadav *et al.* (2004) worked with 9 edible/non-edible oils (10 ml/kg seed) against *C. maculatus* in green gram and found that the mortality was significantly higher in oil-treated seeds compared to the control. They also observed a gradual reduction in the efficacy of the oils with delay in time. Castor oil was the most effective treatment, recording no adult emergence, less seed damage and reduced seed weight loss.

Choudhury (1992) worked out the residual effect of eight vegetable oils (groundnut, sesame, linseed, soybean, neem, castor, safflower and coconut) on chickpea against *C. chinensis* and reported that all oil treatments showed significant reduction in the number of eggs laid, adult emergence and seed damage. Biswas and Biswas (2005) conducted a laboratory experiment on pre-storage seed treatment of gram (*Cicer arietinum*) with the oils of aripple (*Lantana* sp.), karanj (*Pongamia pinnata*), eucalyptus, neem, palas (*Butea monosperma*), citronella (*Cymbopogon* sp.) and annona (*Annona* sp.) against *C. chinensis* and reported that citronella and neem oil at 2.5 and 5.0 ml/kg of seed effectively controlled *C. chinensis* population by reducing oviposition rate and these treatments also recorded the least seed damage and weight loss due to pulse beetle infestation, as well as the highest percentage of gram seed germination.
The present study reveals that mortality of adult beetles was higher in black gram treated with deltamethrin 11 EC @ 2.5ml/kg followed by turpentine oil @ 4ml/kg where as in botanicals, *viz.,* pungam oil @ 5ml/kg, neem oil @ 5ml/kg and castor oil @ 10ml/kg the mortality ranged from 3.67-3.00 numbers of adults on 5 days after treatment . Deltamethrin 11 EC @ 2.5ml/kg has more effect on fecundity of pulse beetle and adult emergence followed by turpentine oil @ 1ml/kg, pungam oil @ 5ml/kg, castor oil @ 10ml/kg and neem oil @ 5ml/kg..

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Table 1. Efficacy of plant derived materials on adult mortality of pulse beetle

Treatments	Mortality of adults (Number)		
	1 DAT	3 DAT	5 DAT
T1- Neem oil @ 5ml/kg	2.00 (1.58)^{ab}	2.00 (1.58)^{b}	3.33 (1.79)^{b}
T2- Castor oil @ 10ml/kg	1.67 (1.46)^{a}	2.00 (1.58)^{b}	3.00 (1.72)^{b}
T3- Deltamethrin 11 EC @ 2.5ml/kg	3.33 (1.95)^{a}	4.33 (2.18)^{a}	6.67 (2.58)^{a}
T4-Acetone @ 4ml/kg	2.00 (1.56)^{ab}	2.67 (1.74)^{ab}	4.33 (2.08)^{ab}
T5- Pungam oil @ 5ml/kg	1.33 (1.34)^{a}	1.67 (1.46)^{b}	3.67 (1.88)^{b}
T6- Turpentine oil @ 1ml/kg	2.67 (1.74)^{ab}	3.00 (1.86)^{ab}	4.67 (2.16)^{ab}
T7- Control	0.00 (0.71)^{c}	0.33 (0.88)^{c}	1.67 (1.28)^{c}
SEd	0.20	0.23	0.23
CD (0.05)	0.43	0.49	0.50

Figures in the parentheses are square root transformed values
Means followed by same alphabet in a column do not differ significantly
DAT- Days after treatment

Table 2. Efficacy of plant derived materials on oviposition, adult emergence, survival of pulse beetle

Treatments	No. of eggs laid	Adult emergence	Per cent survival
T1- Neem oil @ 5ml/kg	38.33 (6.19)^{bc}	8.67 (3.02)^{bc}	17.00 (24.31)^{a}
T2- Castor oil @ 10ml/kg	37.67 (6.14)^{bc}	7.33 (2.79)^{bc}	17.00 (24.23)^{a}
T3- Deltamethrin @ 11 EC 2.5ml/kg	12.33 (3.50)^{a}	1.67 (1.39)^{a}	12.00 (20.17)^{a}
T4- Acetone @ 4ml/kg	45.67 (6.75)^{c}	12.33 (3.57)^{c}	16.00 (23.56)^{a}
T5- Pungam oil @ 5ml/kg	34.33 (5.83)^{bc}	5.33 (2.39)^{b}	16.00 (23.49)^{a}
T6- Turpentine oil @ 1ml/kg	28.67 (5.33)^{b}	5.33 (2.34)^{b}	14.67 (22.42)^{a}
T7- Control	79.33 (8.87)^{d}	21.00 (4.63)^{d}	24.33 (29.51)^{b}
SEd	0.48	0.38	2.15
CD (0.05)	1.04	0.82	4.61

Figures in the parentheses are square root (Number) and arc sine (Per cent) transformed values
Means followed by same alphabet in a column do not differ significantly

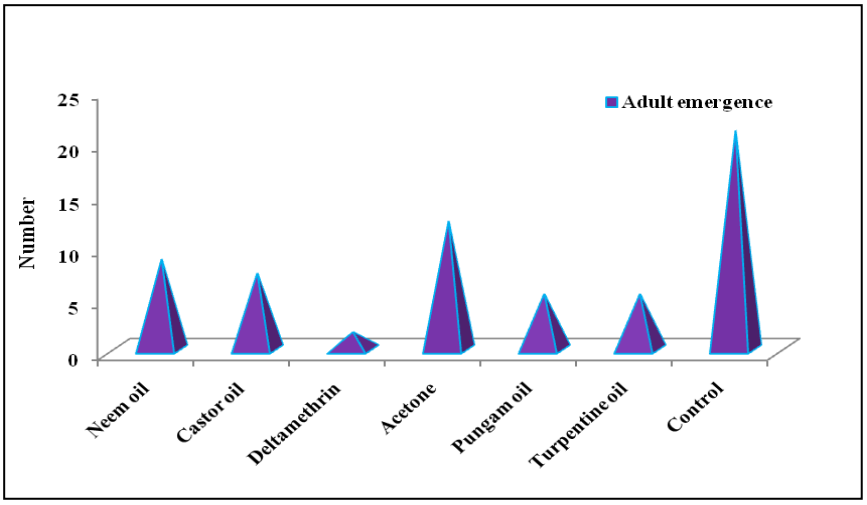

Fig.1. Efficacy of plant derived materials on adult emergence of pulse beetle

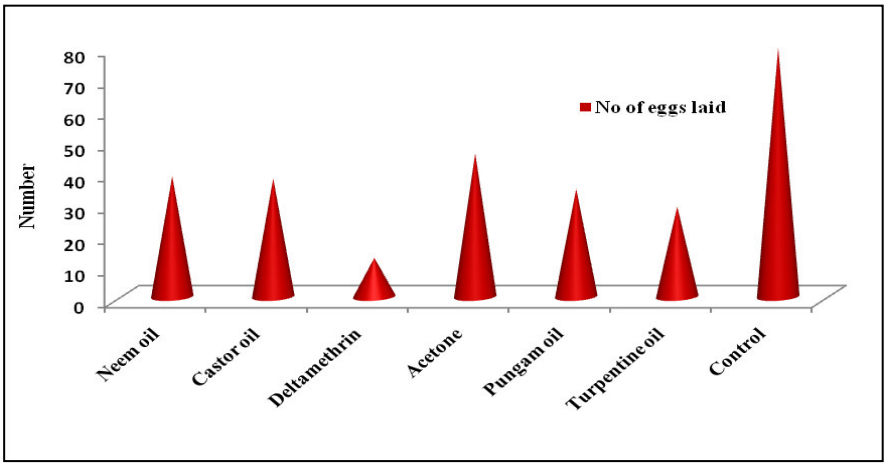

Fig. 2. Efficacy of plant derived materials on oviposition of pulse beetle

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Molecular Interaction in Binary Liquid Mixtures by Ultrasonic Technique

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Received: 14 Feb 2020 Revised: 16 Mar 2020 Accepted: 20 Apr 2020

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ABSTRACT

Density (ρ), ultrasonic velocities (U) and dynamic viscosity (η) for binary mixture of cyclohexane and Nitro-benzene is experimented at frequency range 2 MHz, MHz, 6 MHz and 8 MHz at temperature 308.15 K and at atmospheric pressure over various compositions. The density (ρ) and viscosity (η) are calibrated using Specific gravity bottle and Ostwald's glass capillary viscometer respectively. The velocity (U) is measured using ultrasonic interferometer. The thermo dynamic parameters such as internal pressure (π_i), free volume (V_f), Gibb's free energy and acoustical parameters such as adiabatic compressibility (β), inter molecular free length (L_f), acoustic impedance (Z), relaxation time (τ) and their excess parameters have been calculated. Molecular interaction among the composite liquids explained from these calculations.

Keywords: Ultrasonic velocity, density, viscosity, adiabatic compressibility, free volume, internal pressure, Rao's constantand molecular interactions.

INTRODUCTION

In recent years effort has been made with measurement and interpretation of the ultrasonic properties of liquids and liquid mixtures at different temperatures and frequencies [1-2]. The ultrasonic studies are of great importance in helping to understand the nature and extent of the patterns of molecular aggregation that exist in liquid mixtures, resulting from intermolecular interactions [3-4]. The sign and magnitude of excess parameters have been used to investigate the interactions between the components of a system [5-7]. Molecular interactions are interactions between electrically neutral molecules or atoms. Other than atomic bonds these are electrical in nature and consist of attractive forces (orientation, induction, and dispersion forces) and repulsive forces. Ultrasonic waves have their extensive applications in various fields like non-destructive tests for solids and liquids in medical and engineering, pharmaceutical, polymer and chemicals, metallurgical industries etc. Ultrasonic investigations of binary mixtures

have been taking place since decades by so many scholars [8-12] under various heads like acoustic, thermodynamic, molecular interactions etc. Ultrasonic investigation of liquid mixtures consisting polar and non-polar components is of considerable importance in understanding intermolecular interaction between the component molecules and they find applications in a number of industrial and technological processes. In the present paper the author submitting part of the studies as the effect of frequency on ultrasonic velocity(v) at 308 .15 K in the mixtures of two organic liquids Cyclohexane and Nitrobenzene. The effects on Adiabatic compressibility (β), Inter molecular free length (L_f) and Internal pressure (π_i) were studied. Results were tabulated and the relations among the parameters are represented diagrammatically.

MATERIALS AND EXPERIMENTS

The liquid mixtures of fixed concentration (6:4) in mole fraction were prepared by taking analytical reagent grade and spectroscopic reagent grade chemicals with minimum assay of 99.9% and obtained from E-Merck Ltd (India).The densities and viscosities of the liquid mixture was measured with specific gravity bottle and Ostwald viscometer pre calibrated with water.Multifrequency ultrasonic interferometer model no. M-84 was used to measure the ultrasonic velocity at frequencies 2 MHz, 4 MHz, 6 MHz and 8 MHz.

THEORY

The derived and excess values are calculated by using the fallowing relations.

Adiabatic compressibility (*β*);

Adiabatic compressibility, the parameter which represents the ability to change volume of a liquid sample is

$$\beta = (\rho\, U^2)^{-1} \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Intermolecular free length (L_f);

The formula for outer to outer distance between the interacting molecules is called intermolecular distance, which can be calculated by using the formula

$$L_f = K\sqrt{\beta} \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Free volume (V_f);

The free volumes of the binary mixtures have been computed using its relationship with the ultrasonic velocity and viscosity as given below

$$V_f = \left(\frac{MU}{K\eta}\right)^{\frac{3}{2}} \qquad \ldots\ldots\ldots\ldots (3)$$

Where K is a constant, which is independent of temperature and its value is 4.28 X 10^9 for all liquids. M is the molecular weight.

Acoustic impedance (*Z*);

The ultrasonic velocity is influenced by the acoustic impedance (Z), which is given by the relation

$$Z = \rho.U \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

Internal Pressure (π_i);

Internal pressure, can be calculated by using the relation [13,14]

$$\pi_i = bRT\left(\frac{K\eta}{U}\right)^{\frac{1}{2}}\left(\frac{\rho^{\frac{2}{3}}}{M^{\frac{7}{6}}}\right) \quad \text{.....(5)}$$

Where, *b* stands for the cubic packing factor, which is assumed to be 2 for all liquids and solutions. *R* is the gas constant.

Gibb's free energy (ΔG);

Gibb's free energy, can be calculated by using the equation

$$\Delta G = 2.30.KT.exp\left(\frac{KBT\tau}{h}\right) \quad \text{.....(6)}$$

Where, $\boldsymbol{\tau}$ is the viscous relaxation time, T is the absolutetemperature, K_B is the Boltzmann's constant and *h*is thePlanck's constant.

Excess thermodynamic parameters

With the help of excess parameters, the extent of deviation from the ideal behaviour of binary mixture can be estimated. The difference between the thermodynamic function of mixing for a real system and the value corresponding to a perfect solution at the same frequency, temperature, pressure and composition is called the thermodynamic excess function, denoted by Y^E.

Excess value Y^E for each parameter can compute, by using the general formula

$$Y^E = Y - (Y_1 X_1 + Y_2 X_2) \quad \text{..... (7)}$$

Where,$Y^E$is the parameter under consideration, X_1&X_2 are mole fractions of component liquids and Y_1& $Y_2$are parameters of component liquids.For example, if we take the parameter as free volume, then $Y^E$is the excess free volume of the mixture, $Y$is the free volume of the liquid mixture, $Y_1$is the free volume of cyclohexane and Y_2 is the free volume of nitro-benzene.

RESULT AND DISCUSSION

The experimental values of density, viscosity and ultrasonic velocity at different frequencies are presented in table-1. Calculated values of acoustic and thermodynamical parameters are presented in table-2 and table-3. The excess parameters explaining the nature and strength of molecular interactions, are tabulated in table-4 and table-5. When frequency increases, velocity decreases indicating weakening of intermolecular interaction.The molecules in the present mixture are close to each other and the vibrations are transmitted through the molecules to a large distance increasing the apparent free volume. Free volume decreases very slowly with increase in frequency, indicating weak molecular interaction. Free volumedecreases with increase in frequency, which shows the interaction in the mixture being stronger and the expansion is less. The interaction is less because of steric hindrance in nitrobenzene mixture. When molecules are subjected to larger frequencies they vibrate rapidly, increasing the interaction between the molecules, which is of dispersive type. This reduces the free volume and hence the above observation.

Internal pressure is minimum as Nitrobenzene has a high dipole moment, but the complex structure of nitrobenzene molecules leads to less intermolecular forces and for the same reason also gives a less force of cohesion. Internal pressure increases very slowly with increase in frequency. This is because, when frequency increases, molecular motion increases and hence the molecular interaction increases. Gibb's free energy increases slowly with increase in frequency. Increase in ΔG suggests shorter time for rearrangement of molecules in the mixture. This may be due to the fact that, when frequency increases, the energy imparted to the molecules expedites the rearrangement procedure.

Excess values of the binary mixtures result from the contributions due to the physical, chemical, and structural characteristics of the component liquids. Excess free volume is positive due to expansion in volume because of additivity.Excess free length is positive indicates weak interaction. This may be due to the non-polar nature of molecules or steric hindrance or expansion in volume from additivity.Adiabatic compressibility changes in the same way as free length changes. Excess internal pressure is negative at all frequencies. Excess Gibb's free energy is positive, interaction being stronger, shorter time is requiredfor rearrangement of molecules in the mixture.

CONCLUSION

Variation of ultrasonic velocity with frequency in the binary mixture of cyclohexane and nitrobenzene enabled us to study the thermodynamic parameters and their excess values. These variations indicate the nature of the interaction between the components of the mixture. Although cyclohexane is nonpolar the intermolecular interaction is evident through theexcess values of the thermodynamic parameters. The negative values of excess velocity suggest that the binary liquid mixture is less compressible than the corresponding ideal liquids and the positive values indicate the reverse action. It has been observed that, the change in velocity with changein frequency is conspicuous. This leads to large variation in the parameters and their excess values with change in frequency.

ACKNOWLEDGMENTS

The authors sincerely thank management of Ajay Binay Institute of Technology, Cuttack for their strategic help and constant encouragement in carrying out the Research and Development activities.

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Table-1: Experimental values of Density, Viscosity and Velocity of binary liquid mixture.

Frequency	Density	Viscosity	Velocity
2 MHz	856.69	0.6459	1170.2
4 MHz	856.69	0.6459	1168.5
6 MHz	856.69	0.6459	1166.1
8 MHz	856.69	0.6459	1164.5

Table-2: Calculated values of free length, free volume and adiabatic compressibility of binary liquid mixture.

Frequency	Free Length	Free Volume	Adiabatic Compressibility
2 MHz	0.5930	3.069	8.524
4 MHz	0.5938	3.062	8.549
6 MHz	0.5951	3.053	8.584
8 MHz	0.5959	3.046	8.608

Table-3: Calculated values of Gibb's Free Energy, Acoustic Impedance, Internal Pressure, and Relaxation time of binary liquid mixture.

Frequency	Gibb's Free Energy	Acoustic Impedance	Internal Pressure	Relaxation time
2 MHz	0.695	1.002	312.79	0.734
4 MHz	0.696	1.001	313.01	0.736
6 MHz	0.698	0.999	313.33	0.739
8 MHz	0.699	0.998	313.55	0741

Table-4: Excess values of velocity, adiabatic compressibility, free length and free volume of binary liquid mixture.

Frequency	Excess Velocity	Excess Adiabatic Compressibility	Excess Free Length	Excess Free Volume
2 MHz	- 124.02	1.965	0.0828	0.3911
4 MHz	- 124.46	1.975	0.0831	0.3885
6 MHz	- 126.00	1.999	0.0839	0.3820
8 MHz	- 126.54	2.009	0.0842	0.3793

Table-5: Excess values of acoustic impedance, internal pressure, relaxation time and Gibb's free energy of binary liquid mixture.

Frequency	Excess Acoustic Impedance	Excess Internal Pressure	Excess Relaxation Time	Excess Gibb's Free Energy
2MHz	-0.3297	-52.687	0.1105	0.0723
4 MHz	-0.3299	-52.639	0.1114	0.0727
6 MHz	-0.3312	-52.441	0.1135	0.0738
8 MHz	-0.3316	-52.379	0.1145	0.0743

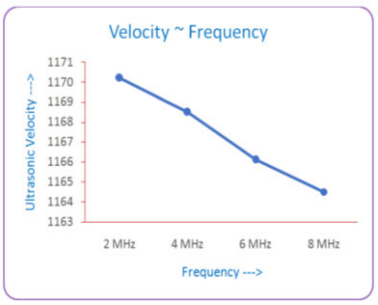

Fig. 1: Variation of ultrasonic velocity with frequency.

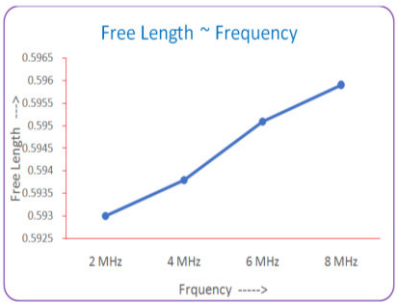

Fig. 2: Variation of free length with frequency

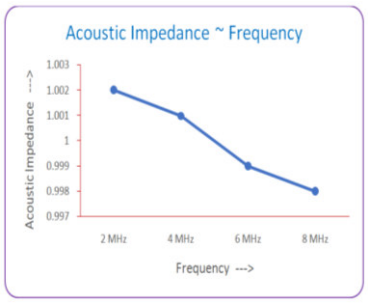

Fig. 3: Variation of acoustic impedance with frequency.

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Synthesis, Spectral Studies and Antimicrobial Screening of Copper Metal Complexes with Substituted Phenyl Thiourea

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Received: 07 Mar 2020 Revised: 09 Apr 2020 Accepted: 11 May 2020

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ABSTRACT

Complexes of Cu have been prepared by reacting metal acetate with o-Chlorophenylthiourea, o-Methyalphenylthiourea as ligands and their complexes were screened for their IR, NMR and Antifungal, Antibacterial studies. Schiff bases and their coordination complexes have acquired great significance in the field of inorganic research mainly because of their biological Activity. Metal complexes play an essential role in agriculture, pharmaceutical and industrial chemistry.

Keywords: Synthesis, Spectral Studies, Antibacterial and Antifungal activity, Analytical and Physical data, Copper Metal Complex

INTRODUCTION

Metal complexes of thiourea derivatives have been of interest because of their formation from widely applied ligands, and their enhanced properties [1-3]. Thiourea derivatives coordinate with metal ion via sulphur[4], nitrogen or in some cases oxygen donor atom 5 to form complexes. These compounds have shown to have anti-tumour [6] anticancer [7-8], anti-fungal, anti-bacterial [9] anti-corrosion[10] and plant-growth regulatory properties. Their corresponding metal complexes have shown to be greater efficacious. Some metal-thiourea derivatives have additionally been used as a catalyst [11] and florescence enhancement. The Schiff's base and their metal chelates is effective anticancer, antitumor, anti tuberculosis, antipyretic agent as well as anti fertility. Schiff's base possess industrial application as catalysts, dyes, fiber, perfumes an aesthetic, plant growth inhibitors cosmetics corrosion inhibitors, oxygen absorbents, polymers, lubricating agents, for removing metal impurities of oil and drying accelerators.[12-15] The metal complexes with Schiff's base derived from chalcone and thiosemicarbazone have been extensively studied and have exhibited medicinal properties[16].Copper (II) complexes have found possible medicinal uses in the treatment of many diseases including cancer. [17] Semicarbazone of various transition

metals have been investigated owing to their coordination capability and pharmaceutical activity [18-20]. Schiff's bases make them more effective in attaining high coordination structure.[21]

Copper-thiosemicarbazone complexes have significantly higher growth inhibitory activity than the uncomplexed ligand and have lower IC50 values against tumor cells than other reported topoisomerase-II inhibitors [22]. The antitumor activity of 1, 2-naphthoquinone-2-thiosemicarbazone (D) and that of its metal complexes of copper (II), palladium (II) and nickel (II) was investigated by Chen *et al*.[23] against MCF-7 human breast cancer cells. The results revealed that these complexes are effective antitumor chemicals in inhibiting MCF-7 cell growth. Another field in which thiosemicarbazone metal complexes are receiving a great deal of attention is their use as carrier for radiotracers such as Cu [24]. Copper complex showed greater activity against Pseudomonas aeruginosa as compared Co(II) complex.[25] Schiff base derived from sulfonamide and their copper(II) complexes have been screened for their *in* vitro antibacterial activity against bacterial strains, *Escherichia coli, Shigella flexneri, Pseudomonas aeruginosa, Salmonella typhi*.[26]

MATERIALS AND METHODS

Synthesis of substituted phenylthiourea's-(1a-d) o-Chloro-phenylthiourea

O-Chlorophenylthiourea was prepared by the method of Douglas's and Dains [27] ammonium thiocyanate (1.5g, 20mmol) was taken into a 250 ml round bottomed flask. To this benzoyl chloride (2.8g, 20mmol) in acetone 50ml was added slowly with continuous stirring and then refluxed for thirty minutes and then cooled. It was then treated dropwise with an acetonic solution of o-chloroaniline (2.5g, 20mmol) with stirring and refluxing and the resulting mixture was further refluxed gently for one and half an hour. The resultant mixture was then poured into water which yielded desired β-benzoyl-α-arylthiourea as precipitate. This was filtered and dissolved in boiling 10% sodium hydroxide solution on a water bath and then again filtered. The filtrates when acidified by dilute HCl and made basic with liquid ammonia give on cooling the desired white crystalline O-Chlorophenylthiourea. All the other substituted phenylthioureas were synthesized similarly and analytical, physical data of these compounds are listed in Table 1.

Synthesis of metal complexes of substituted phenylthioureas

Bis [O-chlorophenylthiourea] copper (II) (2a-d)

O-Chlorophenylthiourea (0.74g, 4mmol) was dissolved in ethanol (20ml) and heated on a water bath. This solution was slowly treated with an aqueous solution of metal salt Cu $(OCOCH_3)_2.H_2O$ (0.61g, 2mmol) in 2:1 molar ratio was added slowly with stirring. Reaction mixture was refluxed on water bath for 2-3 hrs and left overnight to yield the crystalline black colored complexes which was then filtered under suction washed with water till the filtrate was colorless. The product was finally washed with dilute ethanol and dried over fused calcium chloride in vacuum desiccators.

Yield	84%
M.P.	340°C
IR(KBr) υ_{max} cm^{-1}	3490(–NH_2 str.), 3010(Ar-H str.), 1540(>C=N str.), 1600 (>C=C),1050 (-C-S)
^{1}H NMR δppm ($CDCl_3$)	2.0 (–NH_2 s), 7.1-7.3 (m, Ar-H, 8H),

All the other substituted phenylthiourea metal complexes were synthesized similarly and analytical, physical data of these compounds are listed in Table 2.

RESULTS AND DISCUSSION

Complexes of Cu (II) with substituted phenyl thiourea as ligands.

Bis [O-Chlorophenylthiourea] Copper (II) (2a-d)

The substituted phenylthiourea (1a-d) was prepared by the method of Douglas's and Dains [27]**.** This hot solution was treated with an aqueous solution of metal salt in 2:1 molar ratio with stirring give final product (2a-d). The product was finally washed with dilute ethanol and dried over fussed calcium chloride in a vacuum desicator.

All the synthesized compounds gave single spot on TLC. The names and m. p. of all synthesized compounds (1a-d), and (2a-d) are recorded in Table-03and 04, respectively. The synthetic steps are illustrated in the following Scheme

Substituted phenylthiourea's (1a-d)

In the IR spectra of Substituted phenylthiourea's absorption band at 3350-3270 cm-1 due to NH_2, 3200-3170 cm^{-1} and 1200-1170 cm^{-1} due to –NH, and >C=H str. Vibration is observed. Similarly in the ^1H NMR spectra singlet at 4.0-4.2 ppm, 2.0 ppm and broad signal at 6.4-7.0 ppm due to –NH, -NH_2, and aromatic protons.

Metal complexes of substituted phenylthiourea's (2a-d)

In the IR spectra of metal complexes of Substituted phenylthiourea's the absorption bands at 3350 cm-1 due to –NH_2 and (symmetric) and at 3410 cm-1 due to –NH2 (asymmetric) modes. But slight displacement of these frequencies is due to increased positive charge on nitrogen atom arising from the donation of electron pair from sulphur of thioamide moiety which is involved in chelation.[28] In the ^1H NMR spectra signal of -NH_2 appearing from δ-2.0 ppm and multiple from δ6.8-7.4 due to aromatic protons is observed.

Thus it may be concluded that each of the ligand behaves towards metal in a neutral bidentate manner coordinating through nitrogen and sulphur atom. All compounds are crystalline dark colored solids, have fairly high melting points and are sparingly soluble in common organic solvents and are insoluble in water. The analytical data of the complexes indicate that they have 1:2 stoichiometry and forms ML_2 type complexes. The IR and ^1H NMR data of substituted phenylthiourea's and their metal complexes are summarized in Table-5 and 6.

Antibacterial and Antifungal Activities

Representative Substituted phenylthiourea ligands and their metal complexes were screened for their antibacterial activity and antifungal activity at 60, 80, and 100 ppm concentrations.

ACKNOWLEDGMENT

An author is thankful to HOD department of chemistry, university of rajas than jaipur for providing research facilities. CDRI Lucknow and seminal applied science pvt. Ltd jaipur for providing research facilities.

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Table: 1. Analytical and physical data of substituted phenylthiourea's.

Comp.	Y	Mol. formula	color	Mol. Wt.	m.p.	Yield	Elemental analysis % (calcd.) C H N S
1a	2-CH_3	$C_8H_{10}N_2S$	White	166.24	140	58	57.61,6.06,16.85,19.29
1b	4-F	$C_7H_7FN_2S$	Cream	170.21	150	64	49.40,4.15,16.46,18.84
1c	2-Cl	$C_7H_7ClN_2S$	Cream	186.66	135	65	45.04,3.78,15.01,17.18
1d	4-OH	$C_7H_8ON_2S$	Brown	168.22	155	68	49.98,4.79,16.65,19.06

Table: 2 Analytical and physical data of substituted phenylthiourea's metal complexes

Comp.	Y	Mol. Formula	color	Mol. Wt.	m.p.	Yield	Elemental analysis % (calcd.) C H N S
2a	2-CH_3	$C_{16}H_{18}N_4S_2Cu$	White	394.02	340	76	48.77,4.60,14.22,16.28
2b	4-F	$C_{14}H_{12}F_2N_4S_2Cu$	Cream	401.95	360	68	41.83,3.01,13.94,15.96
2c	2-Cl	$C_{14}H_{12}Cl_2N_4S_2Cu$	Cream	434.86	175	72	36.67,2.78,12.88,14.75
2d	4-OH	$C_{14}H_{14}O_2N_4S_2Cu$	Brown	397.96	180	78	42.25,3.55,14.08,16.11

Table : 3 Substituted Phenylthiourea (1a-d)

Comp. No.	Name	M. P. (^{0}C)
1a	2-Methyl phenylthiourea	140
1b	4-Fluorophenylthiourea	150
1c	2-Chlorophenlthiourea	135
1d	4-Hydroxyphenylthiourea	155

Table : 4. Bis [O-Chlorophenylthiourea] Copper (II) (2a-d)

Comp No.	Name	M. P. (^{0}C)
2a	Bis[2-Methylphenylthiourea] Copper (II)	340
2b	Bis[4-Fluorophenylthiourea] Copper (II)	360
2c	Bis[2-Chlorophenylthiourea] Copper (II)	175
2d	Bis[4-Hydroxyphenylthiourea] Copper (II)	180

Table: 5. The IR data of substituted phenylthiourea's and their metal complexes

IR (KBr : ν max cm^{-1})								
Comp. No	**>NH_2**	**>NH**	**Ar-H**	**>C=N**	**>C=C<**	**M-N**	**>C=S**	**C-S**
1a	3340	3250	3010	-	1605	-	1250	-
1b	3410	3190	3040	-	1650	-	1190	-
1c	3414	3210	3150	-	1610	-	1110	-
1d	3420	3170	3021	-	1650	-	1120	-
2a	3400	-	3010	1610	1610	428	-	1020
2b	3350	-	3050	1580	1550	430	-	1090
2c	3400	-	3065	1585	1540	430	-	1156
2d	3390	-	3085	1650	1480	420	-	1140

Table: 6. The 1H NMR data of substituted phenylthiourea's and their metal complexes

^{1}H NMR spectral data(δ,ppm)						
Compd. No.	**Mol. formula**	**Ar-H (m)**	**NH(s,1H)**	**- NH_2**	**-CH_3**	**-OH**
1a	$C_8H_{10}N_2S$	6.3-6.8	4.0	2.0	-	-
1b	$C_7H_7FN_2S$	6.4-6.7	4.0	2.0	-	-
1c	$C_7H_7ClN_2S$	6.3-7.0	4.0	2.0	-	
1d	$C_7H_8ON_2S$	6.2-6.4	4.0	2.0	-	5.0
2a	$C_{16}H_{18}N_4S_2Cu$	7.0-7.1	-	2.0	2.3	-
2b	$C_{14}H_{12}F_2N_4S_2Cu$	7.0-7.2	-	2.0	-	-
2c	$C_{14}H_{12}Cl_2N_4S_2Cu$	7.1-7.3	-	2.0	-	-
2d	$C_{14}H_{14}O_2N_4S_2Cu$	6.8-7.1	-	2.0	-	5.0

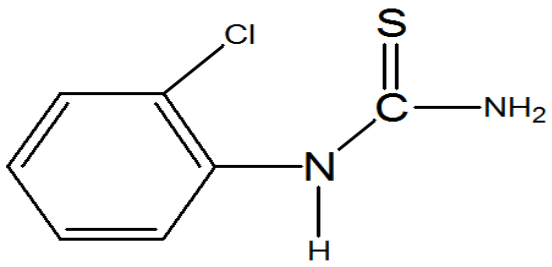


Fig.1. O-Chloro-phenylthiourea

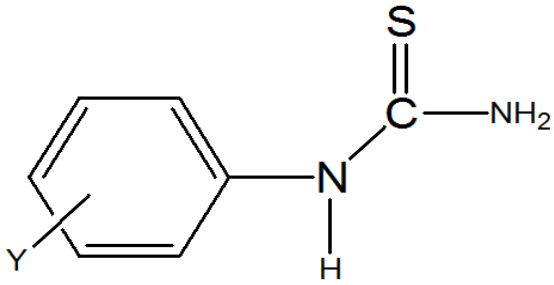


Fig.2. substituted phenylthioureas

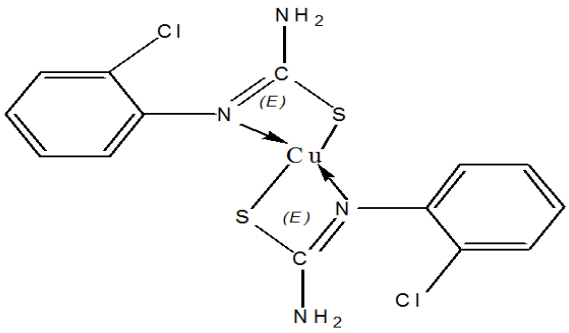


Fig.3.Bis [O-chlorophenylthiourea] copper (II)

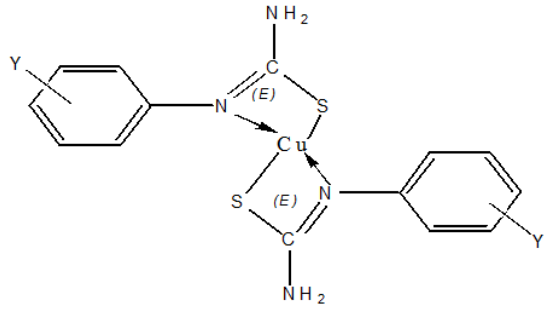
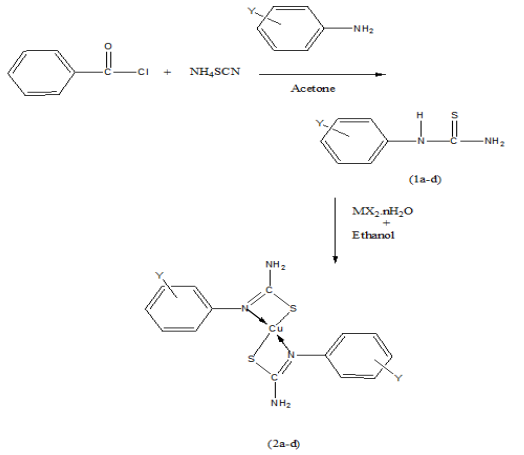


Fig.4. Bis [O-Chlorophenylthiourea] Copper (II) (2a-d).



Y = CH_3, F, Cl, OH

Fig.5 Bis [O-Chlorophenylthiourea] Copper (II) (2a-d).

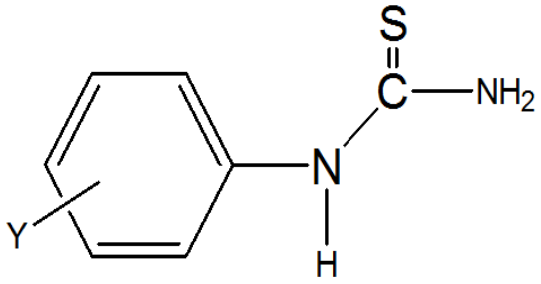


Fig.6.Substituted Phenylthiourea (1a-d)

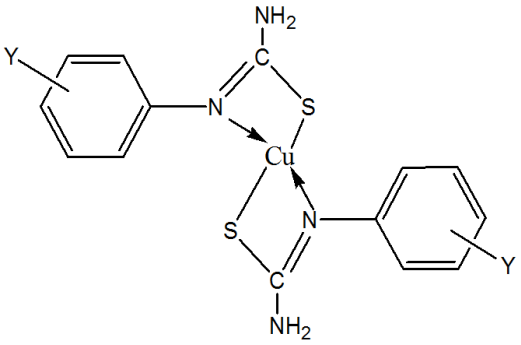


Fig.7 Substituted phenylthiourea's (1a-d)

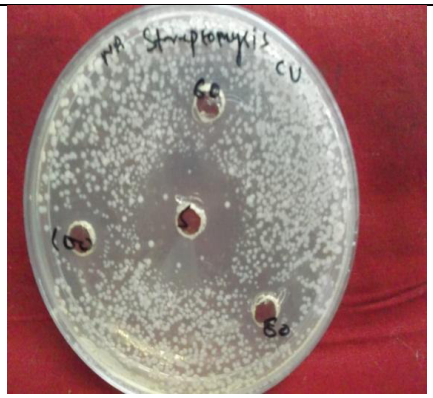


Fig.8.Antibacterial specie *Streptomyces*

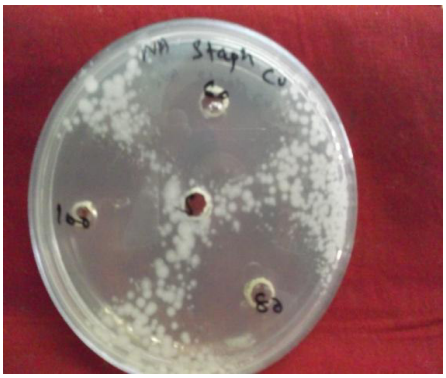


Fig.9.Antibacterial specie *Streptococcus aurous*

Fig.10.Antibacterial species *E. coli*

Fig.11.Antibacterial species *Bacillus cereu*

Fig .12.Antifungal species *Trichoderma reesei*

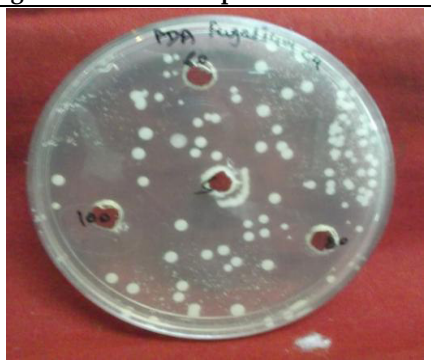


Fig.13.Antifungal species *Fusarium*

Fig .14.Antifungal species *Penicillum*

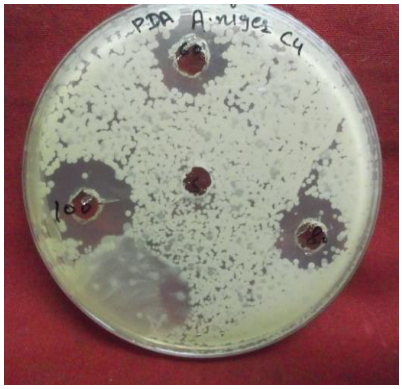


Fig.15. Antifungal species *A. niger*

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Comparative Studies of Haemin Crystal of Mammals - Structural and Statistical Analysis

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Received: 15 Feb 2020 Revised: 17 Mar 2020 Accepted: 20 Apr 2020

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ABSTRACT

A laboratory study was made to identify the structural difference in some of the mammals i.e., *Felis catus, Canis lupus familiaris, Bos taurus, Capra aegagrus hircus, Oryctolagus cuniculus, Homo sapiens*. During this investigation some farm and domestic mammal's blood was collected from the locality (Sundarpada) and examined. After the collection of blood samples, hemin crystals were made from all blood samples. After that when it is compared with the hemin crystal of human being some significant and also some non-significant results were obtained. Statistical calculation was also done to find the structural difference (mean value of the length and mean value of the breadth of the mammals were calculated and standard deviation was calculated) and it was also found that there is a huge difference in their length whereas the breadth maintain consistency.By forming hemin crystals of any blood (unknown sample) it is easy to identify that from which animal the blood belongs to. It is also useful to find differences of bloods of different species depending upon the shape and size of the crystal.

Keywords: Hemin crystal, blood sample of different mammals, structural difference.

INTRODUCTION

Hemin is an iron that contain porphyrin with chlorine which form by a heme group, such as heme b that is found in the haemoglobin of human blood.The hemin can be made from hemoglobin by the method known as Teichmann test, when hemoglobin is heated with glacial acetic acid (saturated with saline) thus, can be used to detect blood traces.Hemin was first crystallized and discovered from blood(Teichmann, 1853). Haemin was first crystallized from blood Teichmann (1853) and he discovered that these blood pigments can form microscopic crystals. Thus, crystals of hemin that are appearing reffered to as Teichmann crystals.

The reddish brown color crystalline chloride of heme, C34H32N4O4FeCl, produces when hemoglobin reacts with glacial acetic acid and sodium chloride in one of the laboratory examination in the presence of blood.The crystals are characteristic in appearance, and their formation has for many years represented one of the most important tests for blood coloring matter.Teichmann's crystals are also chemically known as hydrochloride of hematin. When hemoglobin or dry blood is heated with a few drops of glacial acetic acid and if required a small crystal of NaCl, there are formed yellowish, microscopic crystals called hemin or Teichmann's crystal. These hemin crystal which is hydrochloride of heme, are veryimportant characteristic of blood and that's why furnish us with a reliable and delicate test for blood. Hemin crystal formation is important because they can be used to recognize or analyse the blood sample that are collected by the forensic department. If any unknown blood sample is given, then by forming hemin crystal of that blood we can ensure that whether the blood belongs to any human or animal.

Hemin is protoporphyrin IX containing a ferric iron (Fe3+) ion with a coordinating chloride ligand. Chemically, hemin differs from related heme compound and hematin is the coordinating ion is a chloride ion in hemin, whereas the coordinating ion is ahydroxide ion in hematin. The iron ion in heme is ferrous (Fe 2+), whereas it is ferric (Fe3+) in both hemin and hematin. It can form incorrect as a result of hemolysis or vascular injury. Several proteins in human blood binds to hemin, such as hemopexin and serum album.For present experiment blood are collected from different mammals available in the locality such as- cat, dog, cow, goat, rabbit etc. in EDTA vial by the help of Veterinary worker.Then the blood had been taken to laboratory and hemin crystal of that blood sample had been prepared and the measurement of its length and breadth is taken.When sodium chloride and few drops of glacial acetic acid along with some blood are warmed or heated on a slide a typical microscope reddish brown crystal is formed. (Das. P. 2012).

This process carrying out various tests with blood collected from the camel *Camelus bactrianus,* and the result was obtained by Teichmann's test a new form of hemin crystal. This appear to be renew the original idea of Teichmann (1853), "who attributed to them diagnostic properties and characteristics as regards the blood of different animals," (Formad, 1888).While reviewing the literature one should find two phases of the subject under inquiry. One deals with the reliability of the hemin test in forensic chemistry and the other was concerned with the possible specific character of hemin crystals as made from the blood of different animals.While working with different blood samples, he got different results, (Heine *et.al.,*1912), and for maintain that this test is absolutely true Whilst had made many concepts, (Ewing *et.al.,*1904).

It was stated that there was no predictable way of differentiating the various types of blood,(Preyer*et.al.,*1871). A more modern writer stated that there is a difference in the hemin crystal of different animal bloods, to be attributed to contamination, (Rohmann *et.al.,*1908). Other workers, saw difference but the general opinion remains same as Preyer (1871) expressed it, that it is unreliable. With the help of these different phases, various experiments have been done to investigate both the conformation of Teichmann's test and its possible specific. As a general test for old and less quantities of blood the reliability of the Teichmann's method would depend mainly on the details of the technique employed. One of the factors has been stated as contributing to its success or failure, has received considerable attention, namely, the quantity and the kind of chloride used, (Ewing *et.al.,*1904).A satisfactory result while working on a twenty years old sample of human blood by using five percent solution of sodium chloride beside using the salt grains is been found, (Strassmann *et.al.,*1920).

A method is given in which 2 drops of a solution is used and that solution containcontain about 0.1% each of potassium chloride, iodine, and bromide, in glacial acetic acid, is well known, (Nippe *et.al.,*1912). Two parts of a saturated aqueous solution of salt along with three parts of acid,one part of same along with three parts and one part along with ten parts had been used (Bokarius et.al., 1981). Good results were obtained by him when he uses 5 parts of a sodium chloride solution mix in glycerine along with 20 parts of glacial acetic acid.This type of alternation in the technique helps to change the amount of chloride present and also carefully studied by them and it is quite evident from the results obtained that an extra amount of salt unclear the reaction. However, it has been found that the main

point is the amount of heat applied. With due attention to the heating it has been found possible to secure excellent results without the addition of salt for there is present in the blood a sufficiency of sodium chloride. The following technique has been used as these are found satisfactory. Heme is a prosthetic group having a large number of essential proteins, that plays a vital role in oxygen transportation, storage activity and in electron shutting. It has been stated that the activation of Toll like receptor 4 (TLR4) is one of the medium in which the danger signal of free heme is detected.(Piazza *et.al.*,Feb2011).

A laboratory study was conducted to assess the structural difference in some common mammals. During investigation some farm and sanctuary mammal blood were examined. After that when compared with that of human haemin crystals some significant and also non- significant result were obtained. Statistical analysis was done to determine the structural differences. (Das*et.al.*,2012). Heme contains some proteins that are associated with peroxidase activity. Protein like haemoglobin, cytochrome C, myoglobulin, micro-peroxidase other than peroxidases have been shown to exhibit weak peroxidase like activity. The weak peroxidase like activity in haemoglobin like molecule is because of heme moiety. Molecular dynamics studies to show the unfolding path of Ba-Glb (a truncated haemoglobin from Bacillus anthracis) and the role of heme moiety to its unfolding paths. (Sharma *et.al.*, 2013).

The physiochemical properties of Hgb Zurich were investigated. This new haemoglobin is characterized by an electrophoretic mobility between Hgb A and Hgb S at p 8.6. Similarly, it works between Hgb S and Hgb A on amberlite IRC50 and also on carboxymethyl cellulose chromatography. (Bachmann *et.al.*,1962). Hb Koln was found to precipitate rapidly during mechanical shaking. The rate of precipitation of Hb Koln is 56 times faster than that of Hb S. The kinetics of precipitation of the patient's hemolysate which is a mixture of Hb Koln and Hb A, showed a biphasic curve indicating that Hb Koln precipitates independently from Hb A. The instability of Hb Koln maybe attributed to the conformational change in the vicinity of heme. The mechanical shaking has been used as a new technique for detection and quantitation analysis of haemoglobin Koln and other unstable haemoglobins. (Asakura*et.al.*,1975).

By doing study on different blood samples, Kerr and Victor (1926)stated that in majority of cases, especially with fresh stain and if case be exercised,there is no difficulty in getting haemin crystals. Haemochromogen test is a simple technique; that obtained in the cold by adding the solution (Takayama 2). If it is desired to heat the slide, there is no danger of overheating as there is in the case of haemin crystals. The crystals are large, easily seen, and are of a very characteristic appearance and colour. The nature of the crystals may be confirmed by means of spectroscope. (Kerr*et.al.*,1926). The aim and objective of this work is to study the specific characters of hemin crystal of some mammals and difference in shape of haemin crystal of some mammals in comparison to that of human being with the analysis of standard deviation in size and correlation.

MATERIALS AND METHODOLOGY

Blood samples were collected from different mammals from Sundarpada area. A drop of fresh blood is taken and smear is formed of a thin film upon a slide and allowed to dry the slide in the air. A drop of glacial acetic acid is added and covered by a cover glass. The slide is then heated upon a water bath or spirit lamp for one minute, then removed and cooled by adding a further drop of acid. 0bserve the slide under the microscope which shows the characteristics features of haemin crystals and photographed by the help of microscope.

RESULTS

The study showed that hemin crystal of *Bos taurus* was observed brown color rectangular with sharp edges projecting: *Canis lupus familiar is* it was spindo rhomboidal plates: *Felis catus* was rectangular with highly sharped edges, breadth region is somewhat projected outward: *Capra aegagru hirus* was thin rectangular crystals with sharp

edges breadth portion is inwardly projected: *Oryctolagus cuniculus* was spindle shaped and in *Homo sapiens* it was found rhomboidal plates or rectangular shape. Following are the measurments of length and width obtained from various haemin crystals. The measurements was done in ocular stage micrometer. Where one ocular is equal to mm, i.e. 1 ocular = 0.025mm.

By doing standard deviation it is found that breadth shows consistency in measurements in all six mammalian species. But length shows variations. It indicates that as per as breadth concern it maintains consistency.

DISCUSSIONS

Heine (1912) works with numerous samples and got various result. He stated that all organisms had a different haemin structure. Similarly, Whilst Ewing (1904) he also follows Heine's method and stated that, this test is absolutely reliable. Another scientist Preyer (1871) he stated that there was no reliable way of differentiating the various types of blood. A modern writer Rohmann (1908) says that there is difference in the haemin of various animal bloods, to be attributed to contamination. Other worker, note a difference but the general opinion remains as Preyer expressed it, that it is unreliable**.** One of the factors quoted by Ewing (1904) as contributing to its success or failure, has received consideration attention, namely the quality and kind of chloride used. A satisfactory result was obtained by Strassmann (1920) from a 20 years old sample of human blood by the use of 5% solution of sodium chloride in place of the salt grains. A method is introduced in which 2 drops of a solution is used, which contain about 0.1% each of potassium chloride, iodine, and bromide, in glacial acetic acid, is well known, (Nippe1912).

Bokarius (1918) was collected good result by using 5 parts of a saturated sodium chloride solution in glycerine along with 20 parts of glacial acetic acid. The blood of the different animals such as cat, horse, dog, rabbit, sheep, camel, and man, were tried with the above method, and a very remarkable contrast was obtained in the appearance of the haemin crystals. Rohmann(1908) stated that the contaminants, i.e., other bodies present in the blood than haemoglobin, is unimportant. The difference between haemin from the blood of camel and haemin from the blood of human is most marked. Other bloods, whilst they do not give such a contrast show marked differences. It would appear that with a more remarkable technique as that suggested, an experienced worker could obtain satisfactory results of a specific character. Das (2012) had also done the comparative studies of haemin crystal of different mammals. She also got the similar result like other that all the mammals have different haemin crystals with different shape and size. In this present work also revels same results that all mammals' blood studied has different haemin crystals and vary from each other on the basis of shape and size.

CONCLUSION

Haemin crystals of different mammals are observed. In case of cow's hemin crystal, it is different from that of human hemin crystal and it is non-significant. Similarly non-significant difference has been seen in goat's blood of *Capra aegagrus hirus*. But haemin crystal has been seen significant difference in comparison with *Felis catus*, *Canis lupus familiaris* and *Oryctolagus cuniculus* to that of *Homo sapiens* blood. Standard deviation in length is seen higher deviation whereas there is very less deviation in breadth, which is negligible.

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Table- 1 comparative hemin crystals length and breadth with ± SD of different mammals

Species Name	Mean ± SD length (mm)	Mean ± SD breadth (mm)
Homo sapiens	0.19 ± 0.007	0.05 ± 0.007
Canis lupus familiaris	0.19 ± 0.02	0.07 ± 0.02
Bos taurus	0.24 ± 0.027	0.05 ± 0.011
Felis catus	0.26 ± 0.008	0.04 ± 0.008
Capra aegagrus hirus	0.30 ± 0.04	0.09 ± 0.016
Oryctolagus cuniculus	0.19 ± 0.030	0.05 ± 0.015

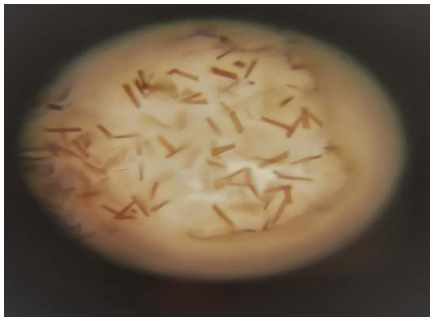


Fig.1.Hemin crystal of *Bos taurus*

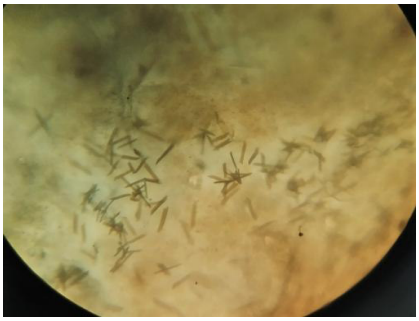


Fig.2.Hemin crystal of *Canis lupus familiaris*

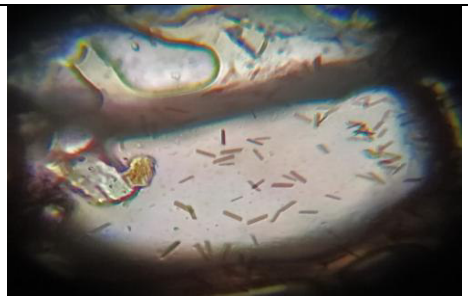


Fig.3.Hemin crystal of *Felis catus*

Fig.4.Hemin crystal of *Capra aegagrus hirus*

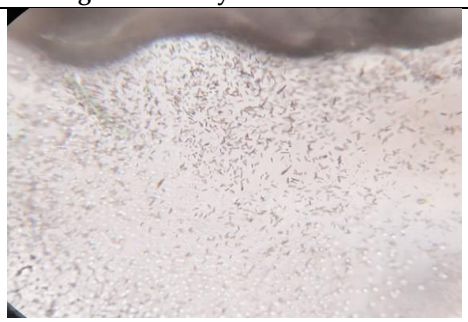
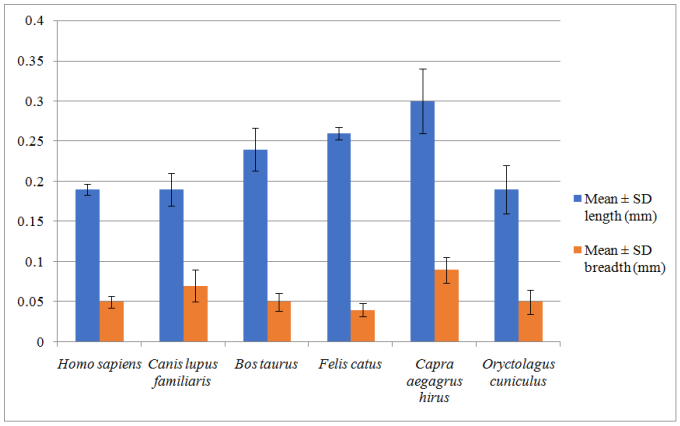


Fig.5.Hemin crystal of *Oryctolagus cuniculus*

Fig.6.Hemin crystal of Homo sapiens



Graph. 1 comparative hemin crystals length and breadth with ± SD of different mammals

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Comparative Analysis of Haematological Parameters of *Labeo rohita* and *Channa striata* Fishes from Different Habitat

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Received: 15 Feb 2020 Revised: 17 Mar 2020 Accepted: 20 Apr 2020

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ABSTRACT

Alive fresh water fishes *Labeo rohita* and *Channa striata* were collected from river and pond respectively and their haematological parameters were studied thoroughly. The aim of this study was to compare the haematological profile of two different fish species from two different habitat and to establish the similarities and differences between these species. The blood parameters viz., RBC and WBC count, Hb, PCV, MCV, MCH and MCHC values were analysed. These results clearly showes that the haematological parameters showed slight fluctuation between the two species. RBC and PCV results from the experiment were higher in *Channa striata* as compared to *Labeo rohita*.

Keywords: River water and pond water fishes, haematological parameters, statistical analysis.

INTRODUCTION

Aquaculture is one of the fastest growing food producing sectors which play an important role in providing nutrition over the year (Rani et al., 2016). From the ancient period fish is used to be considered as a source of nutritious food because it is rich in protein, vitamins, essential amino acids and fatty acids. Omega three fatty acids, which is abundantly found in fishes that help in prevention of coronary heart diseases and other cardiovascular diseases. Fish culture is increasing to compensate the shortage of animal protein all over the world (Leaf et al., 2008). The Fish will be badly affected under intensive culture condition. These awaited drawbacks enforced the fish pathologist to seek for other alternatives. In fish culture immune stimulants are used to prevent the diseases and could solve the problems of massive antibiotics use (Ortuno, et al.,2002). These awaited drawbacks enforced the fish pathologist to seek for other alternatives. The use of immune stimulants in fish culture for the prevention of diseases in a promising new development and could solve the problems of massive antibiotics use (Ortuno, et al.,2002).

The Rohu (*Labeo rohita*) (Hamilton, 1822) is a large, silver coloured fish. It belongs to the family Mostly found in rivers of south Asia. In India they mostly found in Northern and Central and Eastern India. It also found in the rivers of Pakistan, Bangladesh, Nepal and Myanmar. . It is an omnivore species because it eats mainly zooplankton during the early stage of its lifecycle but as it grows, it eats more and more phytoplankton. Rohu reach sexual maturity between 2-5 years of age and spawn during the monsoon season. fish is an important aquaculture freshwater species. *Channastriata* (Bloch, 1793) is a species of snakehead fish and simply called as mudfish. It is also known as the common snakehead or snakehead murrel belongs to the family channidae. It is mostly found in south and southeast Asia. Adults are dark brown in colour with faint black bands in its entire body but fry are reddish orange in colour. It mostly survives in the pond by burrowing in the mud. It is a carnivore fish.

Physiological and pathological changes in fishes can be monitor by using haematological characters (Satheeshkumar et al., 2012). The information about metabolic disorders, deficiencies and chronic stress are determined by haematological parameters (Bahmani et al., 2001). Haematological parameters changes depending upon the aquatic environment, fish species, age, and sexual maturity and health status (Patriche et al. 2011; Radu et al. 2009). Different blood parameters have been established by different investigators In fish physiology and pathology (Rey V'azquez. et al., 2007, Satheeshkumar et al., 2012, Fazio et al., 2013). Haematological study helps in determining disturbances and diseased condition caused in fishes. Blood parameters are considered as the pathological indicators of the whole body. it plays an important role in diagnosing the structural and functional status of fish when exposed to toxicants (Adhikari et al., 2004). Environmental factors influence Fish haematological parameters such as RBC, WBC, Hb and PCV values (Pandey,1977). The aim of the present study is to obtain basic knowledge of the haematological parameters of river water fish *Labeo rohita* and pond water fish *Channa striata*.

MATERIALS AND METHODS

The experiment was started on October, 2019 and completed on February 2020. The samples i.e.10 individuals of *Labeo rohita* were collected alive from Mahanadi river, Cuttack and 10 individuals of*Channastriata* werecollected alive from the pond. Then the samples were taken and brought to the laboratory where they were identified, weight by using digital weighting machine and measured by using meter tape.

Blood sample collection

The blood samples were drawn from caudal vein of the fish by using 2.5 ml syringe and needle 0.5in.Then the blood sample was transferred in a 2ml tube containing K3 EDTA as an anticoagulant. The collected blood sample were immediately subjected to haematological analysis.

Analysis of blood haematological parameters

The blood was diluted with appropriate diluting fluids for RBC and WBC counts and were determined using Neubauer haemocytometer and calcutated (Blaxhall and Daisley. 1973). The haemoglobin was determined by acid haematin method (Sahli, 1962).PCV was estimated by using Wintrobe's tube (Mukherjee, 1988). Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC) were calculated (Seiverd CE, 1964).

RESULTS AND DISCUSSION

Are influenced by their physico chemical conditions of the habitat (Bala, S. et al., 1994).The haematological parameters of *Labeo rohita* and *Channastriata* are given below in the table. On the basis of the data obtained from the

table the haematological parameters of two species are RBC 2.41×10^6 to $2.95\times10^6/mm^3$; WBC 4.13×10^6 to $10.73\times10^3/mm^3$; haemoglobin 8.21 to 9.34 g/dl; PCV 36.8 to 41.4 %; MCV 142.92 to 154.33 fl; MCH 28.18 to 41.49 pg; MCHC 19.73 to 27.13 %. From the given data it is clearly shows that the haematological parameters showed slight fluctuation between the two species. RBC level were higher in *Channa striata*($2.95\times10^6/mm^3$) as compared to *Labeo rohita* ($2.41\times10^6/mm^3$). This value was lower in some other fresh water fishes like *Clarias batrachus* (Sudha Summar war and Santosh Verma, 2012). Comparison between these two fishes showed that WBC of *Labeo rohita* ($10.73\times10^3/mm^3$) was more than *Channa striata* ($4.13\times10^3/mm^3$) . The species with high level of WBC will be able to fight against the infection more effectively than other species. Their levels indicate immune responses and the ability of the animal to fight infection (Douglass and Jane 2010) .

Highest RBC and PCV concentration were observed in*Channastriata* followed by *Labeo rohita.* Low PCV or haematocrit (Hct) probably indicate the anemic condition (Wedemeyer et al., 1976). The differences in the blood parameters between fishes were observed due to difference in their size (Satheeshkumar et ai., 2012). MCV, MCH, MCHC were calculated indirectly with reference to RBC ,Hct ,Hb . So the changes in RBC, Hct, Hb are directly linked with the blood parameters. The haematological parameters of fishes. In 1988 Sakthivel reported the RBC count in *Cyprinuscarpio* was $3.58 \pm 0.39 \times 10^6/mm^3$ when the protein level was 38% in their diet. In my experiment the RBC of *Labeo rohita* was $2.41 \pm 0.57 \times 10^6/mm^3$ and *Channa striata* was $2.95 \pm 0.45 \times 10^6/mm^3$. Sakthivel's experiment value is more as comparison with my work.

Southamani et al., (2015) reported that *Labeo rohita* had RBC count $1.20\times10^6/mm^3$, this value shows significantly low in comparison to my work 2.41×10^6. Ahmad et al.,(2015) observed that the RBC value decreased from normal value which is $3.42\pm0.47\times10^6/mm^3$during pre-monsoon and monsoon condition in case of *Labeo rohita*. Very high WBC value observed by Sakthivel (1988) during the 38% protein dietary condition, which means the fish may be infected or highly stressed condition. Southamani et al., (2015) reported that *Labeo rohita* had WBC count $14.1\times10^3/mm^3$ which is very much high in comparison to my work $10.73\times10^3/mm^3$. Ahmad et al., (2015) reported that the WBC value of *Labeo rohita* was high during monsoon condition. Southamani et al (2015) reported that *Labeo rohita* had 9 g/dl Hb and my work shows 9.34g/dl which is slightly high, which indicates that the fish have high oxygen carrying capacity. PCV value changes with varying in the protein level in the diet (Sakthivel, 1988). *Labeo rohita* as 23.9% PCV value (Southamani et al., 2015), but my work shows 36.8% as PCV value.Southamani et al., (2015) MCV was 95 fl but my work shows 154.33fl which is very much high. My work indicate they may have low protein in their diet (Sakthivel, 1988).

Exposure of *Channa striata* to insecticide results in disturbances in haematology (G.Sasikala et al., 2011).RBC count was $2.44\pm0.031\times10^6/mm^3$, WBC was $16.840\pm0.623\times10^3/mm^3$ and Hb 11.8±0.753 g/dl according to Deshmukh (2016) but the RBC value and Hbdecreased and WBCincreasedwhen exposed to insecticide.My work shows the RBC value that is $2.95\pm0.57\times10^6/mm^3$ which agree with the previous worker.WBC and Hb obtained in my experiment was comparatively low as compared to Deshmukh (2016). My experiment on *Channastriata* shows slight fluctuation in RBC,Hb,PCV,MCHC as compare to Malathi et al., (2012) but there is a great difference found in WBC,MCV and MCH. Increase in the protein level in diet cause the decrease in the MCVvalue (Sakthivel 1988).

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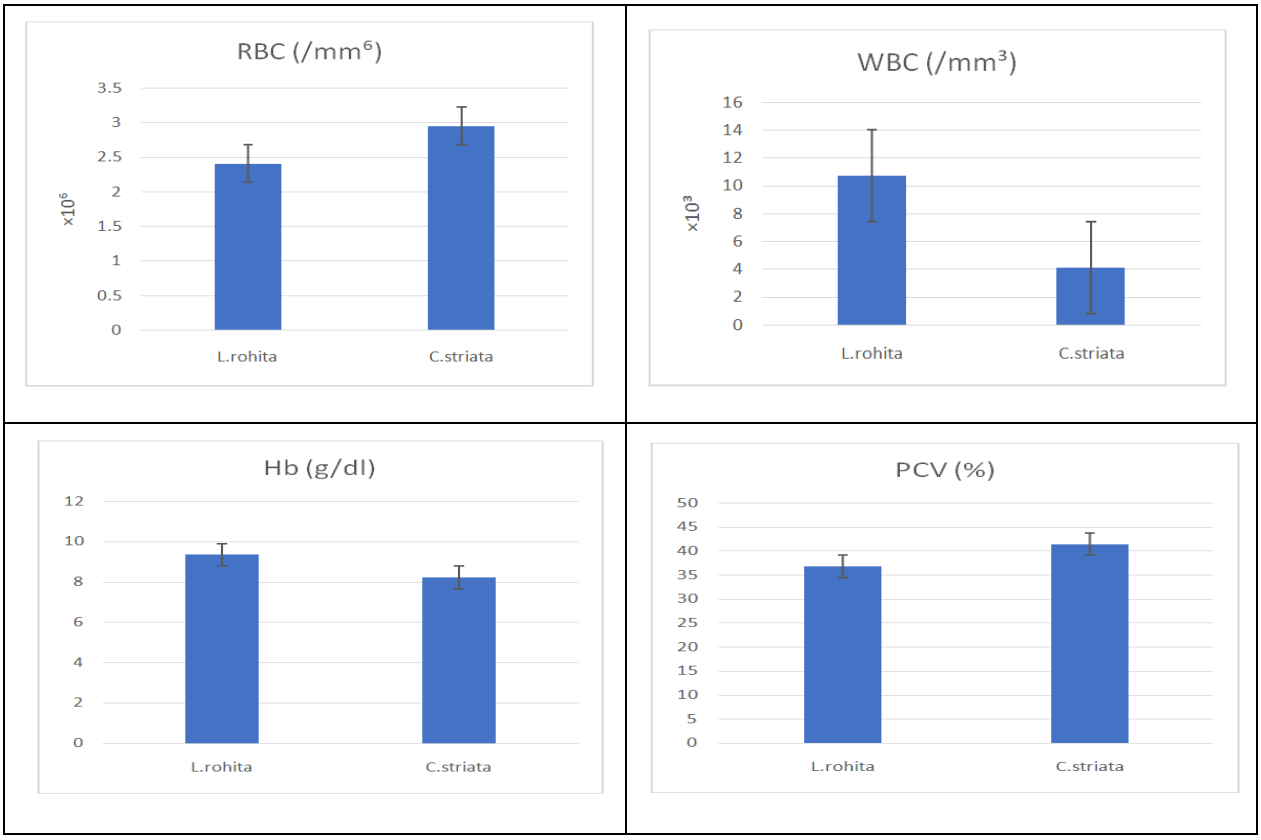
Table. 1.The haematological parameters of *Labeo rohita* and *Channa striata*

Biochemical compounds	*Labeo rohita*	*Channa striata*
RBC(×10⁶/mm³)	2.41±0.57	2.95±0.45
WBC (×10³/mm³)	10.73±1.60	4.13±0.59
Hb g/dl	9.34±1.42	8.21±1.27
PCV(%)	36.8±8.26	41.4±3.8
MCV(fl)	154.33±18.79	142.92±21.51
MCH(pg)	41.49±13.96	28.18±4.45
MCHC(%)	27.13±9.40	19.73±1.46



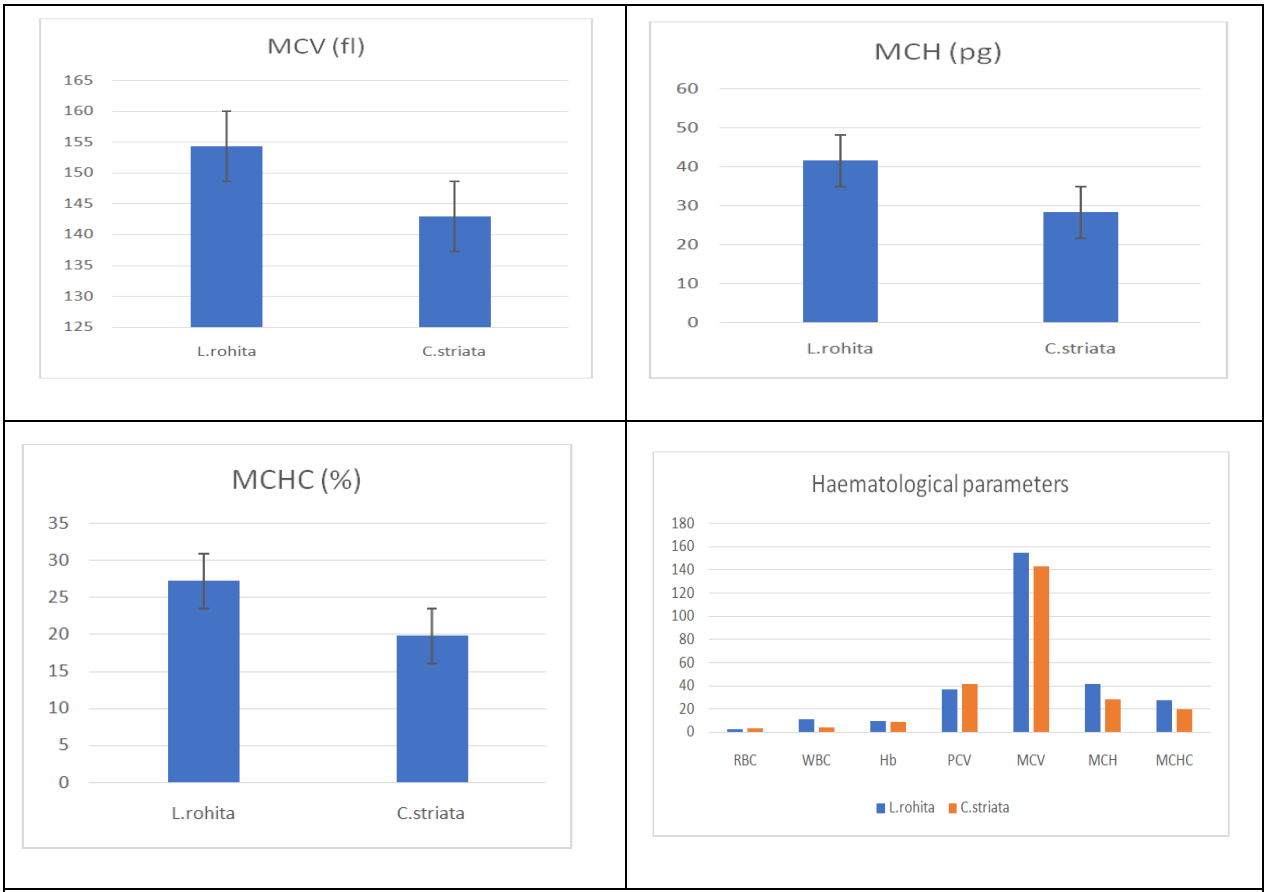


Fig.1.Comparative haematological parameters of *L.rohita* and *C.striata*

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

An Observational Study on Cognitive, Behaviour and Emotional Learning Pattern Among Children with Autism Spectrum Disorder

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Received: 11 Mar 2020 Revised: 13 Apr 2020 Accepted: 15 May 2020

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ABSTRACT

An observational study is an empirical but nonexperimental investigation of the effects caused by a treatment. It mainly designed to detect the reasonable hidden bias. Children diagnosed with autism spectrum disorder (ASD) exhibit behaviour that varies from home environment to therapy session. They produce lesser level of cognitive, adaptive behaviour and emotional functioning comorbid with lack of learning capacity. Understanding the daily activities of children with developmental delays or disabilities such as autism is essential and challenging for researchers and clinical practitioners. This study is an attempt to produce the observational data on cognitive, behaviour and emotional functioning of 12 children with ASD between the age of 5 to 10 years in the home and therapy environment. A direct observational method was carried out and the collective perceptive indicating the core features of these children'slearning patterns and also importance of developing appropriate effective interventions for their treatment plan was discussed in this study.

Keywords :Autism, ASD, Disability,Intervention,Learning style, Observation

INTRODUCTION

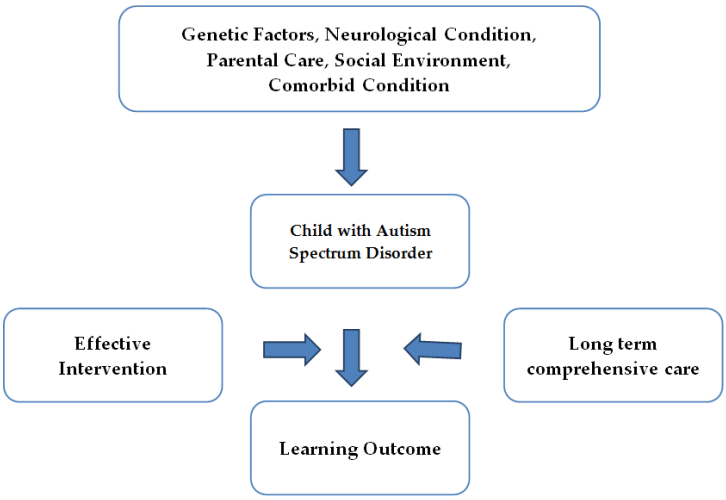
ASD is a neuro developmental condition which predominantly affect the individual social communication, emotional reciprocity with restricted repetitive stereotyped behaviour. These symptoms are evident prior to the age of 3 years (Faras H et al 2010). Early screening and identification of developmental delays such as ASD can be treat at

the earliest for the benefit of the child. Behavioral interventions predominantly incorporate language, communication and developmental approach of the children with ASD (Pasco G 2018). Cognitive behavioral intervention is widely used in children with ASD to treat anxiety, social deficits and to promote thehigh functioning abilities in the social environmental context(White S W et al 2010). Most of the interventions and trainings take place in the therapy room or class room set up where the structured socio environment, training materials and the certified therapists, psychologists are available for the comprehensive treatment. As the clinical psychology services are tailor made and modifiable as per the client requirements there are some home based interventions also found to be effective for the early treatment plan for children with ASD(Perera H et al 2016). Emotional dysregulation is defined as the failure to regulate emotions appropriately whether it could be positive emotion or negative emotion (Samson A C et al 2013). It is been studied that children with ASD can be emotional trained in empathy, emotional expression and can identify the complex emotion by the effective interventions (Golan O & Baron-Cohen S 2006; Baron-Cohen S et al 2009).

Observational studies provide critical descriptive data and information on long term effect at the cost effective way (Gilmartin-Thomas, J. F et al 2018). Observation based studies play an important role in investigating treatment outcomes of the selected individuals (Ligthelm, RJetal 2007). Studies like direct observation method detect the hidden bias (Cochran, W.G. 1965) and provide the critical analytical data(Rosenbaum P R 2001). The core features of autism may be identify by the parents observation on the child developmentallevel hence it is highly alarming for the health care professionals such as psychologists, counseling/clinical psychologists, psychiatrists , social workers and special educators to create awareness and psycho educate the parents on the same.The learning pattern is differ to every individual based on their intelligence level,(Vermunt, J. D., &Donche, V. 2017) but the special children especially children with ASD exhibitslow learning or no learning at all due to neurological condition. To empirically state the learning pattern on cognitive, behaviour and emotional aspects in therapy and home based environmentthis study was carried out.

Theoretical Framework: The Learning Patterns Perspective On Children With ASD

20th century innovativetechnology and teaching patterns have changed the way we learn and perceive the inputs. Studies show that creative visual explanation increase the learning outcome of the students (Bobek, E., & Tversky, B. (2016) and it is widely accepted for children with ASD as well. Focused interventions on behavioral, joint attention, picture exchange communication, modeling are showing significant improvement in the child adaptive functioning(Autism Spectrum Disorders: Guide to Evidence-based Interventions 2012). Having understanding of the ASD's neurological and psychological condition evidence based method this model was formed.



Aim

1. To qualitatively study the cognitive, behavioral and emotional learning patterns of children with autism spectrum disorder.
2. To propose the scientific methods that improve learning pattern of children with autism spectrum disorder.

Study Design

Direct observational method was used in this study

Study Places

1)Children with ASD home environments and
2) Therapy center :National Institute for Empowerment of Persons with Multiple Disabilities (Divyangjan) (NIEPMD), Chennai

Study Duration

3 Months

Sampling Technique

Purposive sampling technique

Number of participants

12 mothers having child with ASD

Inclusion criteria

- Age – 5 to 10 years
- Intelligent quotient – 70 and above
- The child with minimum level of verbal communication (1 word or 2 words sentence)
- Child with mild level of Autism spectrum disorder
- Children with parent / guardian are included

Exclusion criteria

- Children in the category of moderate and severe level of autism
- Children with any co-morbid condition such as Intellectual Disability, ADHD, childhood psychosis, CP etc.

Study Method

The selected 12 mothers having children with ASD werebriefed about their voluntary participation and the right to withdraw from the study. Anonymity and confidentiality were assured.The mothers of the selected samples were instructed to observe the child behaviour in deeply for the period of 3 months and unstructured interview was carried out after the observation period. The same technique was implanted with the therapists and the overall qualitative report was summarized in this study.

Therapy Environment learning

In therapy set up the children are exposed multimodal training and therapy such asbehavioral interventions, special education, physiotherapy, occupational therapy, speech and language training. In therapy the children arealso exposed to the social condition where the communication, functional abilities found to be increasingover the time of

interventions. The imitation and social modeling takes place in the group therapies and they perform wellduring the session. The specific behavioral interventions such as floor time therapy, picture exchange communication therapy, music therapy stimulate the brain functioning activities with sensory integration. The cognitive training of peg grading board, sorting board, picture construction and coloring enhance the thinking and analysis ability of the children with ASD. These learning patterns occurs through intensive intervention in the therapy set up and the therapists and clinical psychologists reported the respective child's improvement. Though the ASD children lack in involuntary emotional expression they try to exhibit the emotional reciprocity by social imitation. The therapists also encourage the parents of children with ASD to train them in the home environment to have the continuous development. The special educators reported the enthusiastic participation of the children during the class session which indicates the social involvement and longingness towards learning. The learning process takes very slowly in children with ASD but the targeted goal is achieved by intensive care of the special educators. The report mentioned in this study is well supported by the (Randi, J., Newman, T., &Grigorenko, E. L.2010).

Home Environment learning

In the home environment set up the children are narrowed to restricted activities and sometimes they even unattended. The cognitive and emotional learning occurs very little or no learning at all takes place. The parents reported their inefficient of providing training materials, spacious room and therapies like physio, speech and language. They reported the increase in temper tantrums and lack of sitting tolerance. They stated that the improvement level is seen clearly over the period of time due to the effect of therapies but in home situation it happens ineffectively. However they try to manage the personal, financial and work life in spite of having special child which is very much appreciable. A Study by (Karthikeyan S et al 2019) indicates the psychosocial problems faced by the mothers having children with disability especially in the case of Autism spectrum disorder hence the special training is very much essential for parents having children with disability.

DISCUSSION

The evolution of research in autism has changed the way we provide the treatment method to the individual who are in need. New theories and innovative researches will enlighten the possible way of treatment and intervention plan. Cognitive behavioral and emotional enhancement intervention will combine together produce the effective outcome in children with ASD. This observational study is a pilot workwhich will influence the broader research aspects in the area of autism spectrum disorder.

CONCLUSION

Special education and comprehensive intervention will graduallyincrease the adaptive functioning of the children with ASD. This study will support as anevidence based report for the observational method on the learning patterns among children with ASD.

Limitations and Future Directions

It is a qualitative attempt to express the way of learning patterns in home and therapy based set up in children with ASD. It will give an idea for developing appropriate intervention module for the benefit of both children with ASD and their parents.

ACKNOWLEDGMENT

The corresponding author would like to express the sincere gratitude to the Department of Clinical Psychology, National Institute for Empowerment of Persons with Multiple Disabilities (Divyangjan) for their constant support and cooperation throughout the study.

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Conservation of Rare Plant Species in Thal Kedar Sacred Forest of Pithoragarh, Kumaun Himalaya Uttarakhand

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Received: 10 Mar 2020 Revised: 12 Apr 2020 Accepted: 15 May 2020

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ABSTRACT

Forests have always been central to Indian civilization and believed as a community which has been viewed as a model for societal and civilization evolution. The present work is a documentation of sacred Thal Kedar forests for its phytodiversity with IUCN status of plants. Total 09 species were identifies which were under IUCN threat category out of which 01 was CR, 02 were EN, 02 were Vu and 04 species were categorised under NT status. There is an urgent need to protect these natural heritage sites to conserve unique plant diversity, not only for ecosystem health but also for the benefit of the indigenous tribes who heavily depend on local plants diversity for their day to day requirements.

Keywords: Indian Himalaya, Sacred forest, Belief system, IUCN status, plant diversity,

INTRODUCTION

Areas of land or water having special spiritual significance to peoples and communities'[1] [2].Sacred forests represent a traditional effort to conserve biodiversity[3].Until recent, these forest patches have been viewed as cultural curiosities. However, a closer look at these cases shows that sacred forests have specific beliefs maintained by strong institutional authorities and this uniqueness of these forests limit excessive extraction of natural resources of forest products and wildlife [4]. Forests have always been central to Indian civilization. It represented the feminine principle in "Prakrti". The forest as a community has been viewed as a model for societal and civilization evolution. The Indian civilization was guided by the diversity, harmony and self-sustaining nature of the forest. The forest thus nurtured an ecological civilization in the most fundamental sense of harmony with nature. The Vedic sages realized that the pure water, air etc. are the roots of to good health and happiness, and hence they considered all these as gods and Now a day's various types of environmental laws like Forest act 1972, Wildlife protection Act 1970, Water protection and pollution act 1980, Environmental protection Act, 1986 have been enacted for the protection and

preservation of environment. As per IUCN Red List version 2019-2: Table 6b 18 July 2019 in India total 76 plant species are in Critically endangered(CR) category, 176 under Endangered (EN), 147 under Vulnerable (VU) and 48 under Near threatened (NT) were classified (www.iucnredlist.org). As per recent IUCN red list data 2019, best estimates of percentage threatened species (with lower and upper estimates) for each group are cycads 63% (63-64%), selected dicots **(**magnolias, birches, cacti, southern beeches and teas) 36% (30-46%), conifers 34% (34-35%), study is focused on the documentation of IUCN category plants found in sacred forest Thal kedar.

METHODOLOGY

Extensive field visits were conducted for documentation of IUCN category plants. Thal Kedar Sacred forest is tribute to Lord Shiva, situated near Badabe village in Pithoragarh district at an altitude of 2450m asl covered with forest of *Quercus floribunda* having area of 195 h. Local communities such as Bhatt, Joshi, Ram, Oli, and Negi worship Lord Shiva. The field surveys were conducted within the selected sites habitats for the quantitative assessment of vegetation. In each site, Trees were sampled by randomly laid 10x10m quadrats; shrubs by 5x5m quadrats and herbs by 1x1m quadrats. For the collection of data from these quadrats, standard ecological methods followed. The vegetation data analyzed quantitatively for frequency, density, evenness and Shannon-Weiner Index (H′) for the various species and for the forest sites by using Curtis expressions [4].

RESULTS AND DISCUSSION

Present study was conducted in Thal Kedar sacred forest of Pithoragarh district of Uttarakhand, India. As given in below table 1 fig 1& 2. The sacred forests are known by different nomenclature in various parts of the country. In Uttarakhand sacred forest were studies for their plant wealth, soil profiling, ethno botanical study, traditional practices and conservation purposes by various researchers [5 -15].

Present study shows a rich reservoir of plants under IUCN category from Thal kedar sacred forests. Total 09 plant species recorded from sacred forests (photo plate 1), which were listed in IUCN category. Out of total 09 species shrubs were 01 and herbs 08, while 01 species was CR, 02 were EN, 02 were Vu and 04 species categorised under NT status (table 1). As per extensive review it is concluded that habitat destruction is a major cause for plant threat followed by over exploitation [16]. A detail observation through imaging (fig 2), forest area can be divided in three parts at basic level. Part A: having large tress, part B: having open land and part C: having mix vegetation of shrubs, herbs and small trees, all these levels makes this area unique for good plant growth and germination supported by Altitude (2001m asl to 2450m asl) and slop angle of study area ranged from 12.63^0 to 29.60^0. Slop angle of a hill very much significant for better soil health, sun intensity on area, rain water distribution, minimum soil erosion and seed dispersal phenomenon. Phytosociological parameters also very much affected by above said factors of an area, present study shows maximum density by *Viola biflora* (1633 ind/ha) and minimum by *Paris polyphylla* (200ind/ha), while *Zanthoxylum armatum* shows good range of evenness and diversity index, 0.053 and 0.213 respectively (Table 2).

CONCLUSION

Present day word is experiencing rapid floral species loss due to various anthropogenic factors such as over-exploitation, habitat destruction, forest fire and pollution. Present study of Sacred Thal Kedar forest comprises a diversified flora with a good environmental and edephic factors. The micro climatic conditions of sacred forests which are playing an important role as socio religious institutions as well as reservoir of biodiversity. Studies showed the role of these sacred sites in reducing soil erosion, preventing landslides and in conferring ecosystem stability. Therefore there is an urgent need to protect these natural heritage sites to conserve regional plant diversity,

not only for ecosystem health but also for the benefit of the indigenous tribes who heavily depend on local plants diversity for their day to day requirements.

ACKNOWLEDGEMENT

The authors are thankful Head, Department of Botany D. S. B. Campus, Nainital for necessary research facilities.

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Table 1: IUCN status of the plants documented from Thal kedar sacred forest, Kumaun Himalaya

Plant species	Family	Habit	IUCN category	Threats	Flowering/Fruiting
Paris polyphylla Sm.	Liliaceae	H	CR	HD	May-September
Epipactis heleborine (L.) Crantz	Orchidaceae	H	EN	HD	July-October
Goodyera repens (L.) R.Br.	Orchidaceae	H	EN	HD	August-October
Swertia angustifolia Ham. ex D. Don	Gentianaceae	H	VU	HD	October-November
Pedicularis pectinata Klotz.	Scrophulariacae	H	NT	HD	August-September
Zanthoxylum armatum DC.	Rutaceae	S	VU	OE	March- May
Taraxacum officinalis Weber	Asteraceae	H	NT	HD	April -June
Peperomia tetraphylla (Forst. f.) H.	Piperaceae	H	NT	HD	July-October
Viola biflora L.	Violaceae	H	NT	OE, HD	June - September

(CR= Critically endangered, EN= Endangered, NT= Near threatened, VU= Vulnerable, OE= Over exploitation, HD= Habitat destruction, H= Herb, S= Shrub, T= Tree, C= Climber)

Table 2: Phytosociological analysis of plants from Thal Kedar forests

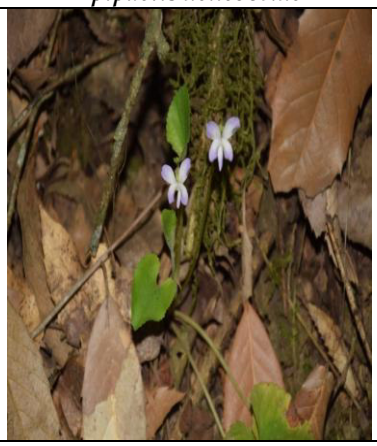
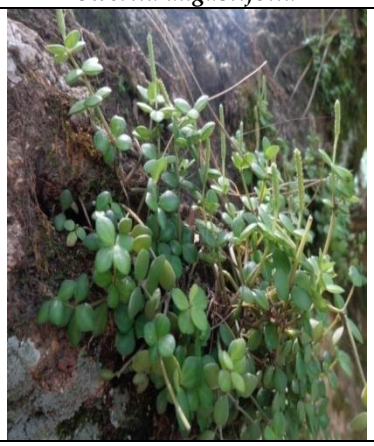
Plant	F	D(ind/ha)	E	(H′)
Epipactis heleborine	36.67	1133.3	0.011	0.54
Goodyera repens	40.00	866.6	0.009	0.044
Paris polyphylla	23.33	200.0	0.002	0.010
Pedicularis pectinata	50.00	1200.0	0.011	0.057
Peperomia tetraphylla	20.00	400.0	0.005	0.023
Swertia angustifolia	60.00	1133.3	0.011	0.054
Viola biflora	50.00	1633.3	0.014	0.072
Taraxacum officinalis	42.33	866.3	0.004	0.044
Zanthoxylum armatum	46.67	933.3	0.053	0.213

(F= Frequency, D= Density, E= Evenness, H′= Shannon-Weiner Index)

Fig.1: Thal Kedar sacred forest, Pithoragarh, Uttarakhand

Fig.2: Google earth image of location. A- large trees population, B- Open land with less vegetation and C- mix vegetation having tree, shrub and herbs, P1-P2- showing sloppy elevation (12.63⁰ to 29.60⁰).

Photo plate 1

		
Taraxacum officinale	***Goodyera repens***	***Zanthoxylum armatum***
		
Epipactis helleborine	***Swertia angustifolia***	***Paris polyphylla***
		
Viola biflora	***Peperomia tetraphylla***	***Pedicularis pectinata***

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

A Study on Biochemical Assessment of the Locally Accessible Exotic Red-Bellied Pacu (*Piaractus brachypomus)* in CUTM Campus, Bhubaneswar

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Received: 16 Feb 2020 Revised:18 Mar 2020 Accepted: 20 Apr 2020

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ABSTRACT

Fish are gill-bearing aquatic craniate animals that lacks limbs with digits.Fishes are found inalmost all aquatic environments may it be hot springs,the Atlantic,high elevated lakes(17,000 feet),under the sea(23,000 feet),salty and fresh water.Fishes are the sources of higher amount of protein and other organic products.Hence,they are widely being accepted as a good source of protein and other essential elements for the maintenance of the healthy body.Fish are of great interest to human for many reasons. Most important and obvious reason of fishes being of high interest is their role as a moderate but important part of the world's food supply. The present study deals with the analysis of the biochemical content of the alien Red-bellied pacu.The fish was collected from the local fish market near the Mahanadi River bank, Cuttack. The measurements such as the length and weight were estimated in the CUTM Laboratory. The species being an exotic species the proximate biochemical parameters such as protein, carbohydrate and minerals content were estimated which will be of greater use in the species selection for fish farming in Odisha.

Keywords: Fish, Red-bellied Pacu, Biochemical analysis, Protein, Carbohydrate, Minerals

INTRODUCTION

Odisha is one of the coastal states lying in the eastern margin of the Indian Peninsula that shares 480 Km of the coast line with the Bay of Bengal (Beura,2009). Fish is a high protein food consumed by the large percentage of population because of its high palatability,low cholesterol and tender flesh.It is the cheapest source of animal protein and other

vital nutrients required in the human diet particularly in the low and middle grade income groups,because of which it is referred to as poor man's protein. According to the statistics unveiled by Ministry of Animal Husbandry, Dairying and Fisheries inland captive fisheries accounted for 8.9Metric-ton (Singh,2018). India is currently the second largest producer of fisheries after China. Fish and fish products have presently emerged as the largest group in agricultural exports from India. India's share of global fish production was around 6% according to FAO.Fish production also contributes around 1% to India's GDP and over 5%to the agricultural GDP.Fish is a low-fat high quantity protein and filled with Omega-3 fatty acid and vitamins such as D and B2 (riboflavin).Fish is a rich source of calcium and phosphorus and a great source of minerals such as iron, zinc, iodine, magnesium and potassium (Falls,2012). Fish oil is also one of the most important natural sources of polyunsaturated fatty acids having two important X-3 PUFAs,EPA(Eicosapentaenoic Acid) and DHA(Docosahexaenoic Acid) which have been proven to have useful effects on human body(Imad, S. P. and Malex, B. 2008). Fishes are known to have necessary nutrients required for supplementing both infant and adult diets. Evidences suggest that high content of polyunsaturated fatty acids in fish flesh and fish oil are beneficial in reducing serum cholesterol(Huynh,2007).The present study is to estimate the biochemical composition from the muscle of locally available exotic Red Pomfret(*Piaractus brachypomus*).

The morphological taxonomy revealed that *Piaractus brachypomus* (Machadoallison1982,Goudling and Carvalho 1982,Ruizcarus and Davis 2003)belongs to family Characidae.It is a large species of pacu,which is a close relative of the furious Piranhas and silver dollars natively belonging to the Amazon Basin in tropical South America, commonly known as Red pomfret or Rupchandi or Pacu.The species is newly introduced and that much of work has not been carried out.The estimation of protein,carbohydrate and minerals from the flesh of fish will be helpful in the future as the fish is widely being introduced as exotic species because of its high growth rate and for ornamental purpose.People are being more sensitive to healthy eating than in the past,so consumption of white meat like fish has increased due to high nutritional content, to meet the needs of the population aquaculture practices are considered today as one of the promising sources of animal protein.

MATERIALS AND METHOD

Collection of Sample

The specimen of *Piaractus brachypomus* weighing 425gms was procured from the local fish market near the Mahanadi River Bank, Cuttack. Sample was treated with 5% $KMNO_4$ for five minutes for dormant disinfection.Then the sample was put in crushed ice in insulated container and brought to the CUTM laboratory for further analysis.

Preparation of the Sample

The specimen was again weighed in the CUTM laboratory in the weighing balance, which approximately weighed about 425gms.The specimen was placed on a dissecting tray, the morphology measurements were taken prior to dissection.The exoskeleton (scales) was taken out by the help ofscalpel (from the dissection box).The fresh muscles tissues were extracted by the help of the scalpel, bone cutter, scissor and the for-cep. The extra moisture from the muscles tissues was eliminated by soaking it by the help of the blotting paper.The extracted muscle was again weighed in the weighing balance. The muscle was placed in a petri-dish was oven dried at 95℃ to 105℃ for 24 hoursto dry out the moisture content completely. The rudiments present in the petri-dish was weighed in the weighing balance.The rudiments were crushed by the help of the mortar and pistol to obtain a fine powdered form.The powder was again weighed and stored properly in air tight container for further use.

Biochemical Estimation

Protein

Protein was estimated following the method of Lowry et al. (1951). The stock solution was prepared prior to the experiment day.10ml of egg albumin was added to 100ml of distilled water,from the solution 10ml was taken out and added to 50ml of distilled water and stirred well. Buffer solution (100ml) was prepared fresh on the experiments day by adding 10ml of buffer (9.2pH) with 90ml of distilled water.7 centrifuge tubes were taken(1,2,3,4,5,6a,6b), and out of these tubes one test tube was taken with 1ml of distilled water which wasthe marked as no-1.A series of 4 test tubes measuring (0.2,0.4,0.6,0.8)ml of stock solution and adding (0.8,0.6,0.4,0.2)ml of distilled water by continuous pipetting and making the solution 1ml in each test tubes respectively.Then in the test tube marking no.6(a) 10ml of the previously prepared buffer solution was taken along with 0.5gm of the sample powder (prepared and stored prior to experiment).From the solution present in no.6(a), 1ml was taken out into a centrifuge tube by the help of pasture pipette and the tube was centrifuge in the centrifuge machine at 8000rpm for 20 minutes to separate the supernatant fluid and the sediment.

The supernatant fluid was again collected by pipetting in another centrifuge tube marked as no.6(b).Fehling solution was freshly prepared in the laboratorywhere the Fehling-A and Fehling-B are present in a ratio 50:1.40ml of Fehling-A(39.2ml) and Fehling-B(0.8ml)and mixed well to prepare the Fehling's solution.From the freshly prepared Fehling solution 5ml of the solution was added to the series of 6 centrifuge tubes[1 to 6(b)].The tubes were placed in the hot air oven for 15minutes at 37℃.Meanwhile Fehling-D 4ml solution was freshly prepared by adding 2ml (Fehling-D) and 2ml (distilled water).From the solution 0.5ml was added by pipetting into each test tube and again kept in the oven at 37℃ for 30 minutes,so that the colour will develop because of the addition of the reagent. At last theabsorbance was measured in the spectrophotometer at 630nm and the results were recorded to plot the graph.

Carbohydrate

Carbohydrate estimated following Hedge and Hofreiter (1962)method.The basic principle underlying this method is that carbohydrates are first hydrolyzed into simple sugar using dilute hydrochloricacid.Inhot acidic medium glucose is dehydrated to hydroxymethyl furfural.This compound forms with anthron a green color product with an absorption maximum at 620nm.Anthronereagent was freshly prepared by dissolving 100mg anthrone in 100ml of ice cold 95% H_2SO_4(Sulphuric Acid). The standard glucose stock solution was prepared by dissolving 100mg in 100ml of water then the working standard was prepared by taking out 10ml from stock diluted to 100ml with distilled water.Store it in the refrigerator for fresh use. Weigh 0.1g of the sample powder into a boiling tube.Hydrolyzed by keeping it in a boiling water bath for 3 hours by adding 5ml of 2.5 N-HCL and cool to room temperature.Neutralize it with solid sodium carbonate until the effervescence ceases, so that the volume becomes 100ml and centrifuge it, then collect the supernatant (1ml) aliquots in a centrifuge tube marking it no.7 for analysis.

Prepare the standards by taking 0,0.2,0.4,0.6,0.8 and 1ml of the working standard in centrifuge tubes marking them no.1 to no.6. "0" serves as blank containing 1ml of distilled water only.Add distilled water to 4 centrifuge tubes having markings no.2 to no.5 so that the volume becomes 1ml.Add 4ml of anthrone reagent to each tube, heat them in the boiling water bath for 8miutes,cool rapidly and observe the green to dark green colour change.Measure the optical density in a spectrophotometer at 620nm.Draw a standard graph by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis.From the graph calculate the amount of carbohydrate present in the sample tube.

Minerals

Minerals were estimated by following the XRF method.X-rayfluorescence(XRF) is an analytical technique that can be used to determine the chemical composition of a wide variety of sample tube including solids, liquid, slurries and loose powder.The basic principle underlying the XRF method is that,it is an atomic emission method, similar in this respect to emission spectroscopy(DES),ICP and neutron activation analysis (gamma spectroscopy).Such methods measure the wave length and intensity of light (Xray in this case)emitted by energizedatoms in the sample.

RESULTS AND DISCUSSION

Protein is an integral part in the human body,so much so that every cell in your body contain it.The major components in your skin,organs and muscles is protein.Fish is one of the best dietary source of animal protein because it gives you a healthy amount of protein but has less saturated fat than red meat.In the present work the proximate protein composition of *Piaractus brachypomus* was recorded 0.890mg/ml(Table 1)which is lower than the protein composition of the albumin stock solution.Fish as a protein diet can be consumed by each and every individual but the egg albumin though contains higher protein content cannot be consumed by every individual because the egg also contains high amount of cholesterol,which is preferably not advised for those who are at risk for or are being treated for high cholesterol.Carbohydrate was found to be present in ample amount in the muscle of *Piaractus brachypomus*(Table 2).The procedure for the sample powder was repeated 5 times. Minerals such as phosphorous, potassium, iron, zinc was found in the muscle which was revealed by the XRF test (Figure 3 & 4).The specimen not only contains protein,it also contains several dietary minerals such as iron,phosphorous etc. which are beneficial to human.Minerals that are required are the essential components which are required in enzymatic biochemical activities in the body.

Phosphorous helps in the formation of bones and teeth.It plays a major role in how the body uses carbohydrates and fats.It is also needed for the body to make protein for the growth, maintenance and repair of cells and tissues. Iron components helps improving the compressive strength. Zinc being an important element for DNA synthesis, immune function, metabolism and growth is present in the muscle tissue in abundant amount.Potassium is one of the seven macro-nutrients, whichhelps in reducing the overall mortality by 20%. It is highly essential because it reduces the risk of stroke, helps lower blood pressure, preserves bone mineral density and reduces the formation of kidney stones.

CONCLUSION

The biochemical contents of fish provide the information on physiological and nutritive value of fishes but also helps in better management practices in inland fisheries.Conclusively,it can be suggested that not only taste,size,freshness and other related external appearance are to be taken under consideration for marketing and consumption of fish but also the proximate biochemical composition also plays a vital role in the selection of edible fishes. The result obtained in the present study has provided justified scientific information and detailed knowledge of the composition which can be included in the daily diet of an individual.Thespecies is newly introduced and much work has not been carried out,the study will throw some light on the beneficial qualities of the fish.It will be of great interest in the market for its higher growth rate,taste and nutritious value.The availability of the fish in the natural fresh water is low so it is recommendable to the fish farmers to cultivate the fish in large scale.

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Table 1. Protein estimation of collected sample for 1st experiment.

TEST TUBES	STOCK SOLUTION (ml)	DISTILLED WATER (ml)	RESULT
1	NIL	1	0
2	0.2	0.8	0.268
3	0.4	0.6	0.822
4	0.6	0.4	1.366
5	0.8	0.2	1.405
6(b)	Solution containing the sample powder		0.890

Table 2. Carbohydrate estimation of the collected sample for 1st experiment

TEST TUBE	WORKING STANDARD (ml)	DISTILLED WATER (ml)	RESULT
1	NIL	1	0
2	0.2	0.8	0.141
3	0.4	0.6	0.275
4	0.6	0.4	0.308
5	0.8	0.2	0.400
6	1	NIL	0.586
7	1 ml of sample (supernatant) solution		0.424

Table 3. The estimated carbohydrate and protein obtained 5 times after the experiment.

NO. OF THE EXPERIMENTS	ESTIMATED CARBOHYDRATE	ESTIMATED PROTEIN
1	0.424	0.890
2	0.426	0.892
3	0.424	0.901
4	0.430	0.890
5	0.428	0.903
MEAN	0.4264	0.8952
STANDARD DEVIATION	0.0026	0.0063

Table 4. Data obtained from the sample by XRF method.

COMPOUND	PERCENTAGE	PARTS PER MILLION
P_2O_5	18.780	187800
SO_3	27.800	278000
Cl	4.127	41270
K_2O	48.262	482620
Fe_2O_3	0.263	2630
Co_3O_4	00	00
ZnO	00	838.2
Br	00	239.0
Rb_2O	0.188	1880
Y_2O_3	00	00
SnO_2	00	400.5
Er_2O_3	0.429	4290
CO_2	00	00
Re	00	28.9

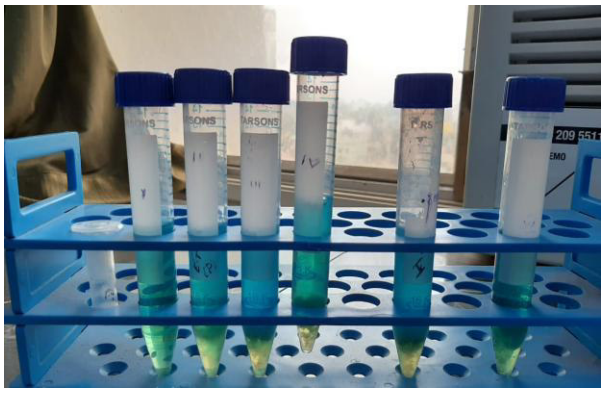

Figure 1. Procedures carried out during protein estimation

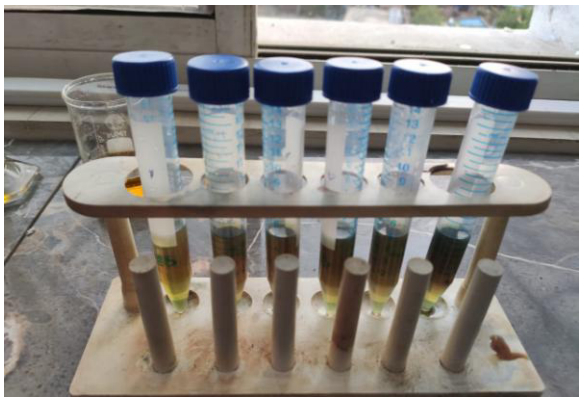

Figure 2. Procedure carried out during carbohydrate estimation.

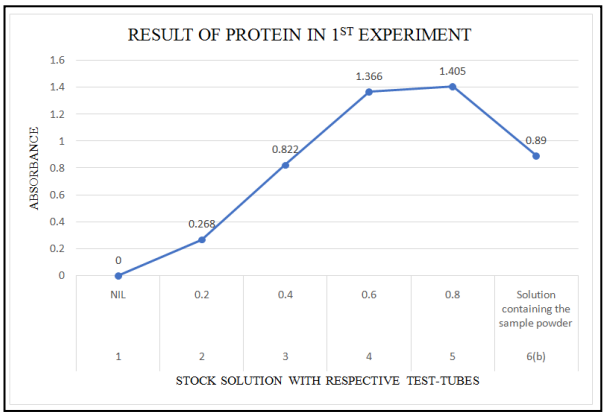

Figure 3. The graph shows Protein estimation of collected sample for 1st experiment

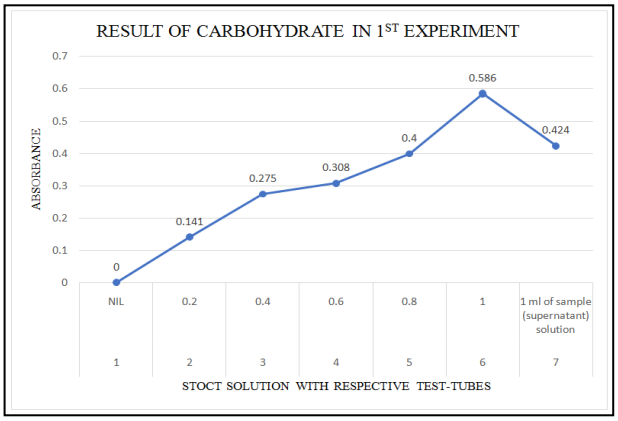

Figure 4. The graph showsCarbohydrate estimation of the collected sample for 1st experiment

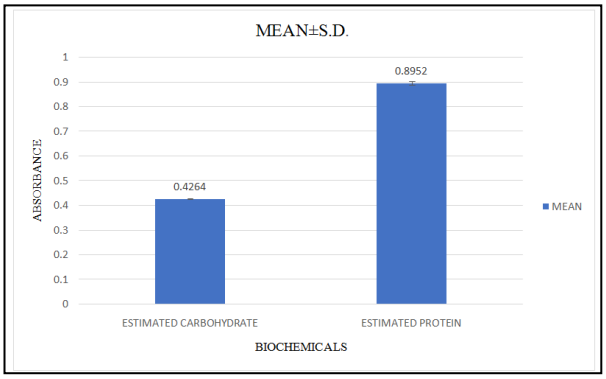

Figure 5. The graph shows estimated carbohydrate and protein obtained 5 times after the experiment

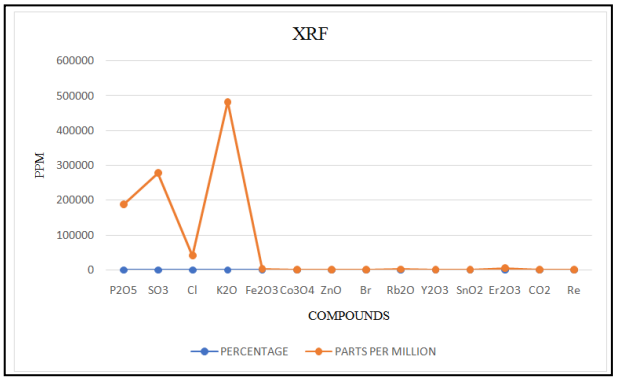

Figure 6: Graphical representation of the chemicals present in the sample obtained by the XRF method

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

High Blood Pressure as an Early Predictor of Mortality among Patients with Acute Hemorrhagic Stroke

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Received: 07 Mar 2020 Revised: 09 Apr 2020 Accepted: 11 May 2020

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ABSTRACT

Hypertension is a common risk factor for premature disability and death among patients with acute hemorrhagic stroke. The diagnosis and management of hypertension among stroke patients is complex due to the multifactorial etiology and the associated heterogenous consequences. Our aim was to determine the frequency of early mortality among acute hemorrhagic stroke patients admitted with the patients with high blood pressure. This prospective cohort study was conducted at a medical science institute of Islamabad from 31st January to 30th July 2015. A total of 126 stroke patients were enrolled and their detailed history and blood pressure (BP) was recorded. Early mortality was noted by monitoring in hospital death within 7 days of admission. SPSS Version 17.0 was used for statistical analysis. There were 126 patients with a mean age of 50.35 ± 28.72 years, 48(38%) female patients and 78(62%) patients were male. The overall early mortality rate among the enrolled stroke patients was 42%, while 58% patients were discharged after treatment completion. Blood pressure was significantly associated with stroke incidence & associated mortality ($p<0.05$). The mean systolic and diastolic BP was comparatively higher among expired patients than those discharged. In conclusion, high BP among stroke patients is significantly associated with death. Therefore, it might be a significant predictor of early mortality among hemorrhagic stroke patients.

Keywords: Early Mortality, Blood Pressure, Hypertension, Acute Hemorrhagic Stroke.

INTRODUCTION

Stroke is the leading cause of disability and the fourth leading cause of death among the people of developed countries, affecting 795,000 individuals each year [1,2]. In Pakistan, estimated incidence rate of stroke is around 250/100,000 population [2]. Of them 7 to 20% die as per the estimates provided by various local studies [2,3]. Pathophysiologically, stroke results from the lumen blockage or laceration in the blood vessels, which gives rise to hemorrhagic stroke [3]. Hemorrhagic stroke has multifaceted etiology, of which hypertension is considered as the most common cause and has been reported among 64% of the stroke patients [3,4].

According to the standard proposed mechanism chronic hypertension damages small intracerebral arteries leading to blood leakage ultimately increasing the mortality risk [4]. Moreover, higher incidences of stroke are reported among males as compared to females at young age while the reversed case was seen among the patients aged 75 years or greater [5,6]. The reported prevalence risk is high among the people in developed countries as compared to those among the developing ones [7]. Though the patients among developing countries display high mortality index due to delayed presentation and inappropriate management [7}.

The brain associated hypertensive emergencies must be managed appropriately considering the pathophysiology of the brain damage i.e. each unique condition is specific with its features and consequences and should be tailored accordingly [8,9]. Basic life support strategies, prevention of hemorrhage, seizure and BP regulation are essential for the management of acute hemorrhagic stroke [9]. However, the optimal BP values among the patients with acute hemorrhagic stroke are not well - defined, but it is evident that high BP leads to rebleeding and hematoma expansion [10]. Based on the data provided by Intensive Blood Pressure Reduction in Acute Cerebral Hemorrhage Trial 2 (INTERACT 2), intensive BP control during the early treatment of hemorrhagic stroke has significantly lessen the absolute growth of hematomas [10]. The aim of the current study was to determine the frequency of early mortality among the acute hemorrhagic stroke patients with high BP. This will help to formulate a more comprehensive strategy to optimally control BP among these patients thus improving the survival chances.

MATERIALS AND METHODS

A prospective cohort study was conducted at the neurology department of Pakistan Institute of Medical Sciences, Islamabad from 31st January to 30th July 2015. Total 126 acute hemorrhagic stroke patients were registered through non-probability and sequential sampling method. Both male and female patients between 18 to 80 years of age with diagnosed acute hemorrhagic stroke and BP>140/90 were recruited. Patients with previous history of subarachnoid hemorrhage and any other co-morbidities like heart failure, renal failure, decompensated liver disease, chronic obstructive pulmonary disease, myocardial infarction, diabetes mellitus, malignancy and patients on anticoagulants or antiplatelets were excluded from the study. Recruited patients were then subjected for clinical examinations, detailed data including patient's age, gender, medical history and presenting blood pressure was recorded. Computed Tomography (CT) scan was performed upon arrival and the patients diagnosed with sudden hemorrhagic stroke were then enrolled. Early mortality was noted by recording in hospital death within 7 days of admission. The collected data was analyzed suing SPSS Version 17.0, all qualitative variables such as gender, early mortality and site of lesion were presented as frequency & percentages. While, age and BP were noted as mean and standard deviation. Chi-square test was used to estimate the effect of blood pressure among the stroke patients and the related risk of mortality where p - value ≤ 0.05 was considered significant.

The study was conducted after obtaining the ethical approval from the institutional review board and all ethical guidelines were followed. The study objectives were clearly demonstrated to the patients or caretakers, written informed consent were taken and the data confidentiality was maintained.

RESULTS

There were a total 126 stroke patients enrolled in the study with a mean age of 50.35 ± 28.72 years and majority of them were > 60 years of age (Table 1). Out of the total, 78 (62%) patients were males and 48 (38%) were female making the male to female ratio of 1.7:1. Out of the 126 patients, 53 died while 73 were discharged successfully. The mean systolic blood pressure (SBP) was significantly high among the dead stroke patients as compared to those discharged i.e. 200.28 ± 38.27 mmHg vs 184.43 ± 40.08mm Hg ($p<0.05$). Conversely, the diastolic blood pressure (DBP) was high among the discharged patients as compared to the dead patients (Table 2). Early mortality within the first 7 days was recorded among 53 (42%) cases, with increased mortality rate observed among males as compared to females. Out of the deceased patients, 51% died in 0 to 2 days, 23% died in 3 to 5 days and the rest of 26% survived longest i.e. 6 to 7 days. The mortality rate was highest among patients with basal ganglia, thalamus and cortical lesions (32%). It was 20% among patients with lesions in cortical and subcortical areas or with basal ganglia and thalamus lesions.

DISCUSSION

It is evident from previous literature that increased BP is an independent and powerful risk factor among patients with acute hemorrhagic stroke, and hence lowering BP is thought to decrease the recurrence rate [11]. The prognosis and management of acute hemorrhagic stroke varies on the basis of severity, site and the size of the hemorrhage [12]. Of the patients studied, 53 dead had high BP ($p=0.0003$) and majority of them were males 51.2% (Table 2 & 3). Similarly, a study by Celikand his colleagues reported mortality among 42.6% stroke patients but high incidence rate was observed among females as compared to males which was contradictory to our findings [12]. The presenting SBP was 205.28 ± 43.27 mm Hg among dead patients while it was 194.43 ± 42.08 mm Hg among those discharged ($p=0.022$) while DBP was 112.53 ± 24.82 mm Hg among deceased and 107.04 ± 21.50 mm Hg among discharged patients ($p=0.034$)[12]. Although in our study, the results were similar for SBP i.e. 200.28 ± 38.27 mm Hg (deceased) vs 184.43 ± 40.08 mm Hg (discharged) ($p=0.0003$). But in case of DBP, the findings were reversed and mean DBP was recorded as 118.53 ± 21.82 mm Hg among deceased and 105.04 ± 18.50 mm Hg among discharged patients ($p=0.0005$) (Table 2).

Additionally, it was observed that the SBP dropped on day 3 among the patients who initially had high BP at the time of admission, which again increased among the deceased patients within 7 days before mortality. Though no such variations were observed among the discharged and deceased patients in terms of DBP. Although, it is known that the BP reduction aids to reduce the stroke risk and limits the associated mortality but on the other hand lowering the BP after stroke is itself a risk [11]. Olivera-Fhilho and his colleagues concluded that lowering BP among the stroke patients within the first 24 hours of onset results in poor detrimental outcomes [11].

A study quoted hemorrhagic stroke indices were higher among older patients in connection to hypertension [13], i.e. also supported by our findings 48(38.3%) patients aged >60 years. Moreover, the overall in-hospital death rate was 42% which occurred in first 7 days (Table 2). Heuschman et al., reported 10 days in-hospital mortality among 4.9% stroke patients (of which 34% occurred in first 3 days and remaining 66% in first 7 days) [14]. Henon et al., showed that the overall mortality was 18% among hemorrhagic stroke patients, with mortality steadily increasing beyond the age at 60 years [15]. Which is consistent with our study reporting increased incidence and mortality among men beyond 60 years as compared to female's.

In local studies, the lobar hemorrhagic mortality was observed in 30% stroke patients, 40% with subcortical hemorrhage or lobar and basal ganglia / thalamic hemorrhage and 4% among patients with infratentorial hemorrhage [16,17]. In comparison, we found cortical lesion as contributing up to 17% of the mortality rate, 9.5% subcortical lesions and 20% both (Table 3). In hemorrhagic lesion, mortality was (i.e. basal ganglia & thalamus) 20% but it is 32% if the hemorrhage is both lobar and basal ganglia/thalamus.

The mean SBP was high among the deceased patients in the current study. However, this outcome proposed that the changes in SBP play more significant role in prevention of early mortality as compared to the DBP. Taking these findings into account, the diagnosis and management of BP among stroke patients will help preventing the associated morbidity and mortality risk. Hence, the limitations of this study must be considered i.e. the greatest limitation of this study was small sample size. Moreover, the atrial fibrillations (persistent or paroxysmal) were not assessed. This cohort included the acute stroke patients only and therefore, provides their preliminary data on early mortality.

CONCLUSION

The results indicated significant effect of high SBP on the outcomes of hemorrhagic stroke and associated mortality. Therefore, lowering the high BP effectively reduces the early & late deterioration, dependency and death. Early diagnosis and management of BP among acute hemorrhagic stroke patients is essential and new diagnostic and management strategies must be explored in order to prevent the associated mortality risk.

CONFLICTS OF INTEREST

None.

ACKNOWLEDGEMENT

The authors would like to acknowledge the Medical Affairs Department of Getz Pharma for providing their technical support during the preparation of this manuscript.

SOURCE OF FUNDING

None.

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Table 1: Demographic characteristics of the patients with acute hemorrhagic stroke (n=126)

Characteristics		n(%)
Gender	Male	78(62)
	Female	48(38)
Age Group (Year)	21-30	09(7.1)
	31-40	18(14.2)
	41-50	18(14.2)
	51-60	33(26.2)
	> 60	48(38.3)

Table 2: Comparison of Blood pressure by outcomes during the acute hemorrhagic stroke

Variables	Hemorrhagic Stroke (Mean ± SD)		p-value
	Discharged (n=73)	Dead (n=53)	
Systolic Blood Pressure (mmHg)	184.43 ± 40.08	200.28 ± 38.27	0.0003
Diastolic Blood Pressure (mmHg)	118.53 ± 21.82	105.04 ± 18.50	0.0005

Table 3: Stratification of mortality rate with gender, days distribution and site of lesion.

Mortality rate stratification		n(%)
Gender	Male	40(51.2)
	Female	13(27)
Days Distribution	Day 0-2	27(51)
	Day 3-5	12(23)
	Day 6-7	14(26)
Lesion Site	Basal ganglia + thalamus+ Cortical	17(32)
	Cortical + Subcortical	11(20)
	Basal ganglia + thalamus	11(20)
	Cortical	09(17)
	Subcortical	05(9.5)

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Effects of Mining Environments on Haematological Parameters of Some Vertebrates in Talcher, Odisha

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Received: 17 Feb 2020 Revised:19 Mar 2020 Accepted: 21 Apr 2020

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ABSTRACT

Indian mining sector plays a vital role in development of economy and improving livelihoods as well as providing space for environmental degradation. It also impacts on local air, water and natural resources that causes environmental instability. Talcher is one of the important mining areas of Odisha as well as India. This study is aimed to assess the effect of mining on haematological parameters of vertebrates. This included three species of vertebrates like fish (*Clarias batrachus*),hen (*Gallus gallus domesticus*) and goat(*Capra aegarus hircus*),which are exposed to mining environment. Various haematological parameters have shown a significant result in haemoglobin, red blood cells, white blood cells, packed cell volume, mean cell volume, mean cell haemoglobin and mean cell haemoglobin concentration are assessed. However mining has a major role in socio-economic development, but it creates mitigation with sustainability of environment. Proper management strategies in mining areas can help conservation of biodiversity.

Keywords: Mining,haematology, *Clarias batrachus*,*Gallus gallus domesticus* and *Capra aegarus hircus*.

INTRODUCTION

India is a land rich in variety of resources and dense forests. Odisha is one of the states of India, having wide range of variability with its biodiversity including floura and fauna. Several modern strategies now create disturbance among the faunal diversity. Development of socio-economic status of a country as well as the bio-physical characters of an environment are generally changed by the influence of mining at that particular region [1]. Environmental factor plays a major cause for incidence of diseases though there are development in the field of science and technology. Now climate change is wider aspect because of continuous release of several pollutants [2]. Mining has

its impact on air,soil, water, reduction of natural resources, rainfall and loss of agricultural fields despite the long term development [3]. Talcher is one of the important mining areas of Odisha as well as India. It is reported that there 87 minerals available in India and among these minerals coal fulfilling about 55% of energy requirements of the country[4].Due to mining activities, extensive manmade damage is done to the ecosystem. Heavy metals contamination creates impact on ecological balances and devastating effects on environment [4,5].

Traces metal like mercury present in soil, water and air causes' damage to kidney damage and digestive track [6]. The environmental pollution influence on haematological parameters of an organism due to exposure toxic pollutants and accumulation in the tissues [7].The haematological analysis is very important for monitoring the metabolism and health status of all vertebrates. Haematological analysis help us to obtain knowledge about the haematological parameters like red blood cells (RBC) count , white blood cells (WBC) count, concentration of haemoglobin (Hb), Packed cell volume (PCV), mean corpuscular haemoglobin(MCH), Mean cell volume(MCV) and Mean corpuscular haemoglobin concentration (MCHC). These parameters can indicate the health status of different vertebrates of mining area and disease caused due to their environmental conditions. The present investigation assesses the influence of mining environment on haematological parameters of vertebrates in the Talcher mining area, Odisha, India.

MATERIALS AND METHODOLOGY

The study was undertaken from the month of December 2019 to Februuary, 2020. The experimental analysis was done in the Laboratory of Department of Zoology and ATC laboratory of Centurion University of Technology and Management, Bhubaneswar campus, Odisha. The following vertebrates were taken such as fishes *Clarias batrachus*, goat *Capra aegagrus hircus* and hen (*Gallus gallus domesticus*) were collected for the experimental work.

Sample Collection

The blood samples were collected from control species that were not exposed to mining area and the selected mining area of Talcher, Odisha, India. Blood samples are collected by syringe 2.5 ml (needle size 0.55*25mm) and immediately transferred to the EDTA vial of 2 ml which acts as anticoagulant.

Haematological Examination

The haematological analyses were done for the examination of total RBC and WBC count in neubauer's chamber. Concentration of Hb was done with sahli's haemocytometer, PCV,MCH, MCV and MCHC), were analysed by standard protocol[8].

RESULTS AND DISCUSSION

Estimation of RBC

Red blood cell is also termed as erythrocytes. These are the most abundant blood cells in blood. Red blood cells help in gaseous exchange between the cells and lungs. Comparative study on vertebrate shows significant difference in their total number of RBC. The total RBC count in vertebrate ranges in selected normal area and mining area where some $2.92\pm0.1743\times10^6/mm^3$ to $1.86\pm0.1749\times10^6/mm^3$ in *Clarias batrachus,* $5.08\pm0.3215\times10^6/mm^3$ to $2.22\pm0.3411\times10^6/mm^3$ in *Capra aegagrus hircus* and $4\pm0.5822\times10^6/mm^3$ to $3.94\pm0.2925\times10^6/mm^3$ in *Gallus gallus domesticus* respectively. In mining area, the decrease in RBC counts of vertebrates as they are mostly exposed to the toxic pollutant. The pollutants have strong influence to the RBC of the vertebrate present the mining area due to the presence of fluoride. There was decrease in RBC count indicates immunological suppression [9].

Estimation of WBC

White blood cells are also known as leukocyte or white corpuscales. These are a part of the immune system that helps fight infection and different the body against other foreign materials. The results of total count of WBCs show the difference in normal areas as control and mining areas varies from 7.86±0.8189 to 3.68±0.4897×10^3/mm^3 in *Clarias batrachus,* 4.5 to 0.2144×10^3/mm^3 to 2.22 to 0.32×10^3/mm^3 and 1.8±0.3301 to 2.7±0.4427 in *Capra aegagrus hircus* and *Gallus gallus domesticus* respectively.

Estimation of Haemoglobin

Haemoglobin is a metalloprotein found in red blood cell. It is a protein that is made up of four chains, which are arranged in a tetrahedral structure. Haemoglobin has a critical role in the body, as it is the protein in red blood cell that carries oxygen. It imparts red color to blood, it buffers the blood Ph and maintain it to the tolerable limits. All vertebrates except fish have haemoglobin in the red blood cells as the oxygen carrier. The mean±SD of haemoglobin is 9.4±0.6095 to 7.96±0.3449, 8.68±0.3426 to 5.46±0.4118 and 9.22±0.8095 to 9.76±0.6697 in *Clarias batrachus, Capra aegagrus hircus and Gallus gallus domesticus* respectively. There was a significant decrease in the percentage of haemoglobin in *Clarias batrachus* and *Capra aegagrus hircus* as these are directly exposed to pollutants which indicates anaemic condition in vertebrates[10]. This may be due to a decrease in synthesis rate of Red blood cells or an increased loss of these cells [11]. The decrease in protein synthesis leads to decrease in haemoglobin [12] . The high count of haemoglobin concentration was highest in *Gallus gallus domesticus* which is not statistically significant at $P<0.05$. The haemoglobin value obtain in this study were far higher than the normal physiological value.

Estimation of PCV

Packed cell volume is the ratio of the volume occupies by packed red blood cell to that of the volume of whole blood as measured by the hematocrit [13]. The PCV value ranges from 32±1.4966 to 20.2±1.8547% in *Clarias batrachus*, 24.6±2.4819 to 21.4±1.5362% in *Capra aegagrus hircus* and 23±2.4494 to 20.6±2.9597% in *Gallus gallus domesticus*. In the above values due to the impact of mining, it causes significant decrement in count of PCV in all the three vertebrate which can lead to acute anaemia [14].

Estimation of MCV

The mean corpuscular volume is the estimation of the average volume of Red blood corpuscles. The MCV value ranges from 112.30±10.0936 to 98.04±20.5282 in *Clarias batrachus*, 49.11±5.6969 to 106.37±17.7124 in *Capra aegagrus hircus* and 62.66±9.9967 to 52.92±7.4224 in *Gallus gallus domesticus* due to an increase in the concentration of Fluoride (Guru et al,.2014)[15]. The increase in MCV in goat may be resulting in macrocytic anaemia due to high $PCO_2$and low PO_2 in the blood.

Estimation of MCH

MCH value refers to the average quantity of haemoglobin present in a single red blood cell. MCH is calculated by dividing the amount of haemoglobin in a given volume of blood by the number of red blood cell present. The MCH values of *Clarias batrachus, Capra aegagrus hircus and Gallus gallus domesticus* varies from selected areas to mining areas from 33.28±3.8764 to 44.45±4.6233, 17.30±1.0388 to 26.88±5.3371 and 24.68±3.1462 to 25.19±2.1707 respectively. The significant change in the MCH may be due to the reduction in cellular blood ion, resulting reduce oxygen carrying capacity of blood and eventually stimulating erythropoiesis [16].

CONCLUSION

This study has focused on exposure of mining environment on vertebrates and its influence on their haematological parameters. Assessment of these haematological indices determines the physiological status of an organism. Effect of this mining area might be pose threat to the biodiversity. The significant reduction in haematological parameters like erythrocytes, haemoglobin as well as increase in MCH,MCHC and MCV values showed the effect of mining environment. As mining activities are the integral part of development,how ever it has some mitigate with society. So,proper management strategie should be taken for the development of socio-economic status and sustainable ecosystem.

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Table 1.Comparative values of haematological indices of *Clarias batrachus* in Normal control area and Mining area

SL NO	Haematological parameters (Units)	Fish in Normal Area (X±SD) (n=10)	Fish in Mining Area (X±SD) (n= 10)	P Value
1	WBC ($\times 10^3/mm^3$)	7.86±0.818	3.68±0.459	0.0010
2	RBC ($\times 10^6/mm^3$)	2.92±0.174	1.86±0.174	0.0013
3	Haemoglobin (g/dl)	9.46±0.609	7.96±0.374	0.0346
4	PCV (%)	32.2±1.496	20.2±1.854	0.0005
5	MCV(fl)	112.30±10.093	98.04±20.528	0.2751
6	MCH (pg)	33.28±3.876	44.45±4.623	0.0506
7	MCHC (%)	29.60±2.291	67.50±28.783	0.1128

Table 2. Comparative values of haematological indices of *Capra aegagrus hircus* in Normal area and Mining area

SL NO	Haematological parameters (Units)	Goat in Normal Area (X±SD) (n=10)	Goat in Mining Area (X±SD) (n= 10)	P Value
1	WBC ($\times 10^3/mm^3$)	4.5±0.2144	2.22±0.32	0.00017
2	RBC ($\times 10^6/mm^3$)	5.08±0.3215	2.22±0.3411	0.00014
3	Haemoglobin (g/dl)	8.68±0.3426	5.46±0.4118	0.00015
4	PCV (%)	24.6±2.4819	21.4±1.5362	0.1524
5	MCV(fl)	49.11±5.6969	106.37±17.7124	0.0075
6	MCH (pg)	17.30±1.0388	26.88±5.3371	0.0579
7	MCHC (%)	36.34±2.7866	24.89±2.6428	0.0087

Table 3.Comparative values of haematological indices of *Gallus gallus domesticus* in Normal area and Mining area

SL NO	Haematological parameters (Units)	Hen in Normal Area (X±SD) (n=10)	Hen in Mining Area (X±SD) (n= 10)	P Value
1	WBC ($\times 10^3/mm^3$)	1.8±0.3301	2.7±0.4427	0.0709
2	RBC ($\times 10^6/mm^3$)	4±0.5822	3.94±0.2925	0.4644
3	Haemoglobin (g/dl)	9.22±0.8095	9.76±0.6697	0.3105
4	PCV (%)	23±2.4494	20.6±2.9597	0.2747
5	MCV(fl)	62.66±9.9967	52.92±7.4224	0.2283
6	MCH (pg)	24.68±3.1462	25.19±2.1707	0.4487
7	MCHC (%)	41.03±3.1631	52.03±8.5999	0.1321

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Optimization, Extraction and Proximate Composition Analysis of Gelatin from Fish (*Labeo rohita* and *Catla catla*) Skin and Scales

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Received: 18 Feb 2020 Revised:20 Mar 2020 Accepted: 22 Apr 2020

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ABSTRACT

The gelatine from skin / scales of two major carps namely *Labeo rohita* and *Catla catla* are extracted by alkali and acid treatment. The extraction result shows that fish gelatine obtained from these major carps were comparable from the fish previously reported. The extracted gelatine looks crystal like and creamish white in colour and light texture. The extraction was done by heating the scales in distilled water for 17 hour at 55°C. The results procured from the skin of *Labeo rohita* is observed to be higher as compared to *Catla catla* in terms of yield. The proximate composition of the gelatine obtained from both the fish varied significantly ($p<0.05$).Protein content was found to be maximum in gelatine of *Labeo rohita* skin (84.44%) where as maximum fat content was found in *Catla catla* skin gelatine *(1.06%).* Where as maximum moisture content was noted in *Labeo rohita* scale gelatine and maximum ash content was noted in rohu skin *gelatine (1.20%).* Basically the fish processing unit produced a huge quantity of waste which was reported to be 75% from scales and 35% from skin and bones. So the present study aims to utilize the scales / skin to extract gelatine from commonly available major carps *Labeo rohita* and *Catla catla*.

Keywords*: Labeo rohita, Catla catla*, Scale, Skin, Gelatin.

INTRODUCTION

Gelatin is a dull, insipid, crystalline food substance, generated from collagen obtained from animal body parts. Now it is a trend to use gelatine in various culinary area [1]. Because of the favourable properties such as nontoxicity, high water solubility, elasticity, high mechanical strength, gelatine are widely used as raw material in pharmaceuticals, cosmetic industries, photography, coating material for oral drugs, adsorbent for diluted chemicals and adhesive agents stabilizer of photosensitive reagents in photographic films. According to Zakaria and Bakar, 2015 [2] gelatine is a clear protein having rheological properties of thermo-reversible transformation between sol and gel. Gelatin has broad application in the food and packaging industries due to its stabilizing properties it is useful in preparing whipping cream, marshmallow, cream fillings. It is used to enhance the consistency, stability, and elasticity of food products. Generally the source of gelatine produced from bovine and pig skins. As bovine gelatine has a possible

threat of spreading Bovine Spongiform Encephalopathy (BSE) and some religious reasons [3], so the study and extraction of gelatine from fish scale, skin, and bone is of interest. The fish processing industries generates large amounts of waste every year and the cost of the fish waste disposal is also high which figures to be 75% of total fish weight and the remaining wastes which amounts to be 30% of the waste are from fish bones and skins [4]. Fish scales and bones are more recommended in the extraction of gelatine as it produces large quantity of gelatine due to the presence of enormous content of amino acids as compared to fish skin. The properties of gelatine obtained from pig skin and bones is almost same as the gelatine obtained from fish skin [5].

In several parts of the world, various fish species are being consumed daily in massive quantities, as a result, a huge number of by-products are obtained [6]. Inappropriate disposal of these by-products provokes serious environmental pollution with an obnoxious odour. Generally, these by-products have been chiefly applied as fertilizer [7] . Therefore, the maximized utilization of these by-products, especially for the production of value-added products is a better solution. To avoid the problem associated with undesirable flavour, fish scale can be used as the main source of gelatine with negligible offensive smell. So, the present study aims to extract the gelatine from fish scales of major carps which are of easy reach to the common people.

METHODS AND MATERIALS

Sample preparation

Labeo rohita and *Catla catla* fish was brought from Kalinganagar fish market, Bhubaneswar, and directly stored in the refrigerator. The scales were removed and washed separately and dirt was removed from the experimental sample using tap water and later sun dried [8] . The dry scales were then packed in clean dry zip pack bags and stored under room temperature until further use. The cleaned skins were washed and cut into small pieces, and the pieces were packed in polythene pack and stored at -20°C for gelatine extraction [9].

Gelatine extraction

The gelatine was extracted by following the standard procedure of Gudmudsson et al. 1997 [10] and Jakhar et al.2016 [11].The dry scales were treated with sodium hydroxide solution at 0.23M concentration for 2hour to remove the non-collagenous proteins from the scales. Alkali solution was replaced every 40 minute and repeated 3 times. After alkali treatment the scales were washed under running tap water until the pH of the scales was neutralized. Then it was soaked in 0.2% Sulphuric acid. After that, it was soaked in 0.7% citric acid. Each soaking and washing treatment was replicated 3 times for 2 hours. Then the scales were constrained to the final wash with distilled water. The final extraction was carried out in distilled water at 55°c for 18 hours. The ratio of skin and water used 1:3(W/V). The extract attained was filtered with Wattmann filter paper No.1, utilizing a Buchner funnel. After that the filtrate was kept in a glass petri dish and dried in hot air oven at 60°c for 18hr. The thin film of dried matter was obtained that was crushed and powdered, then weighed and packed for further use. The procedure for gelatine extraction from the skin of the both the fish species same as that of scales.

The yield of gelatine extracted can be calculated by using
% yield = weight of dried gelatine ÷ weight of fish skin or scale × 100

Proximate composition analysis
Protein

By using Lowry method (2015) [12] crude protein content was analysed.

Stock solution preparation method

Take 10ml of egg albumin added with 10ml of distilled water and mixed properly, then take 10ml from that mixture, then add 50ml of distilled water with that mixture, then stir the mixture well and kept undisturbed for 1day in order to settle it down.

Procedure for buffer solution

First 100ml of distilled water was taken in a beaker and in it 9.2 buffer tablet was added and mixed well. Then 6 cleaned test tubes were taken, in the first test tube 1ml of distilled water was added, in the second test tube along with 0.2ml stock solution, 0.8ml distilled water was added, in the third test tube 0.4ml stock solution + 0.6ml distilled water was added, in the forth test tube was added 0.6ml stock solution + 0.4mldistilled water, in the fifth test tube 0.8ml stock solution + 0.2ml distilled water was added, and the sixth tube was the experimental tube. Buffer solution of 5ml was added with gelatine powder 0.50gm and mixed well in the beaker and 1ml from the mixture was taken in the test tube. The component from the sixth tube is centrifuged at 8000RPM for 15min. Fehling A and Fehling B was added to make the reagent C in the proportion of 50:1. 5ml of this was added in each test tube then incubated at 37°c for 15min. Then reagent D was prepared by mixing Folin-ciocalteau phenol + distilled water. 0.5ml of this solution was also added in each test tube then incubated it for 30mins at 37°c. Finally the optimal density at wavelength 640nm was measured

Moisture

In gelatine the moisture content was determined by using AOAC, 2005 [13] official methods. First 1gm of gelatine was taken and spread on the clean plate and heated the sample at initial temp. of 100c and final temp. 180c until a stable weight was gained finally the moisture content can be calculated from the weight loss due to heating.

Crude fat

The crude fat content of gelatine was measured by using AOAC, 2005 [13] official methods. The difference in the initial and final weight of receiver flask was determined as fat content of sample and calculated on weight basis.

Ash

The ash content was determined by using AOAC, 2005 [13] methods. The sample was taken weight silica crucible. After that it was transferred to muffle furnace at 600^0c and kept for 6hr. After cooling the ash was weighed. Finally the percentage of ash was calculated from the weight difference.

RESULT AND DISCUSSION

Statistical analyses

In this study the statistical analyses of data obtained indicate the significant differences between the gelatine yields from different fish scale. Results of one way ANOVA, comparing the effect of fish species (Rohu and Catla) and fish scale and skin is given in Table 2.The effect of both the factors were highly significant having the $P \leq 0.005$(0.000 and 0.010).

Fish gelatine yield

Gelatine yield is one of the important parameter for the gelatine industry due to its potential economic importance. The yield of gelatine depends on the variables like soaking time, NaOH concentration, extraction time and extracting temperature. The gelatine yield in different fish scale and skin is given in Table 2. The gelatine yielded from the skin of Rohu (*Labeo rohita*) and Catla (*Catla catla*) was 9.72% to 10.92% and the gelatine yielded from the scales of both the fishes were 6.12% to 7.01%. The highest gelatine yield was obtained from the skin of Rohm that is 10.92% and the lowest yield was obtained from the scales of catla. The gelatine yield extracted from fish scale was less compared to that of fish skin and there is a clear difference in the yield of both (Table 2.) and skin of Rohu belong the fish with the higher gelatine yield certainly the fish which can produce more gelatine.

The gelatine extracted from the scales of black tilapia (*Oreochromis niloticus*) were found to be 16% (Zakaria and Bakar, 2015)[2]. The gelatine extracted from fish scales is less than mammalian gelatine which yield to 6% to 12% (expressed as grams of dry gelatine per 100g of clean fish skin and scale) (Karim and Bhatt, 2009)[14]. The low yield of gelatine from fish scales might be due to the loss of collagen during extraction and leaching during (Zakaria anda Bakar, 2015)[2]. In addition to that some heat stable proteases endogenous to the skin are involved in the degradation of gelatine molecules during the extraction process at particular temperature, which contribute low gelatine yield andlow bloom strength.

Rahman et al. (2008) [15], reported higher gelatine yield from yellow fin tuna skin (*Thunnus albacores*) as 18%, also shown that the yield of gelatine and quality of gelatine are not only influenced by the species or the tissue from which it is extracted but also depends on the extraction process. Gelatine extracted from Pollock skin is optimized and observed and the result showed that the gelatine yield varied from 3% to 19% and was very sensitive to pre-treatment temp. (Haug et.al, 2004) [16]. The yield value of gelatine obtained through acid / alkali treatment vary with the species, age of the fish, and the extraction techniques used (Songchotitunpan et.al, 2008) [17].

Proximate composition of fish scale gelatine

The proximate composition of gelatine obtained from different fish is given in Table 4. The moisture content of Catla and Rohu skin gelatine was 8.16% and 9.1% and the gelatine yielded from the scales of both fish was 8.61% to 8.58% it is seen that the highest moisture content was found in the gelatine yielded from the of the Rohu scale and minimum moisture content was found in Catla scale. The difference in moisture content in gelatine could be due to the variation in drying process. The edible gelatine contain less than 15% of moisture [18] . The moisture content of Pacu (*Piaractus brachypomus*) skin was observed as less than 6% (Sahoo et al., 2015)[9]. Haug et al., 2004[16] the moisture content of cod skin gelatine was 12.9% which was a litte bit higher. The moisture content of Ghol skin gelatine was 8.43% (Jakhar et al., 2012)[11].

The protein content in the sample when considered, the rohu scale gelatine was 65.9% and the Catla scale gelatine was 67.96%, the rohu skin gelatine was 84.44% and catla skin was 74.53. The maximum protein content was recorded in Rohu skin i.e. 84.44% and the minimum protein content was found in rohu scale gelatine i.e. 65.9%. The protein content of Pacu (*Piaractus brachypomus*) skin reported 84%-91% (Sahoo et al., 2015)[9]. In black kingfish skin gelatine the protein content reported 88.72% (Killekar et al., 2012)[19]. The protein content present in the gelatine extracted from the skin of tiger toothed croaker reported 86.45% and the extraction process done under 45°c (Koli et al., 2012) [20]. Sockalingam and Abdullah, 2014 [21]reported that the protein content of black tilapia skin scales gelatin was found to be 86.90% and the protein content of fish scales was found to be 23.06%.

The fat content when considered in this experiment, the fat content of Rohu scale gelatine was 0.86% and Catla scale gelatine was 0.64% and that of Catla skin and Rohu skin was 1.06% and 0.61% respectively. The maximum fat content was found in Catla skin gelatine and the lowest fat content was found in Rohu skin gelatine, in fat content

sector catla was a bit behind. According to [22] gelatines are fat free. The fat content of Pacu skin reported 0.92%-1.12% (Sahoo et al., 2015)[9].

Lastly, considering the ash parameter, the ash content of Catla scale gelatine was noted as.97%and Rohu scale was 1.12, the Catla and Rohu skin gelatine was 1.09% and 1.20%. Maximum ash content was found in Rohu skin gelatine i.e. 1.20% and minimum ash content found in catla scale gelatine i.e. 0.97 indicating that the Rohu exhibited comparatively more residue than the catla on combustion. The ash content of Pacu (*Piaractus brachypomus*) skin is reported as 0.92% (Sahoo et al., 2015)[9]. As the above experiment shows very low fat and ash content so the acid extraction process followed was appropriate for producing good quality gelatine. The results clearly showed that the extraction procedure of fish scale and skin was found very efficient as the gelatine contains high protein it is also good in nutritionally and it is also a better replacement for bovine and porcine gelatine as it gives good percentage of yield.

ACKNOWLEDGEMENT

The authors are grateful to the fish vendor of Kalinga Nagar market and Head of the department, Zoology of Centurion University of Technology and Management Dr. Siba Prasad Parida for extending his cooperation and timely help to complete this project.

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Table 1. Optimum values for the various independent variables for quality gelatine.

Extraction parameters	Optimum conc/time/temp
NaOH concentration	0.23M
Soaking time	47 minutes
Extraction temp.	55°C
Extraction time	18 hours

Table 2. Gelatine yield (%) from scale and skin of *Catla* and *Rohu*. All values are mean ± standard deviation of triplicate data. Different superscripts in the same row indicate significant differences (p<0.05).

Fish species	Fish skin gelatine yield (%)	Fish scale gelatine yield (%)
Catla catla	$9.72^{b} \pm 0.28$	$6.12^{b} \pm 0.10$
Labeo rohita	$10.92^{c} \pm 0.18$	$7.01^{b} \pm 0.11$

Table 3. Results of one-way analysis of variance (ANOVA) analysing the effect of sources (fish skin and fish scale) on gelatine yield (%). Significance level of the factors (fish scale and fish skin) p≤0.05.

		Sum of squares	df	Mean square	F	Sig.
Fish skin gelatine	Between groups	11.016	1	5.508	122.582	0.000
	Within groups	0.264	5	0.027		

	Total	11.28	6			
Fish scale gelatine	Between groups	1.054	2	0.527	12.407	0.010
	Within groups	0.116	4	0.023		
	Total	1.17	6			

Table 4. Proximate composition of gelatine obtained from different fish scale and skin (% on wet weight basis).

		Moisture	**Crude protein**	**Crude fat**	**Ash**
Fish scale gelatine	Catla	8.16 ± 0.166	67.96 ± 0.03	0.64 ± 0.023	0.97 ± 0.038
	Rohu	9.1 ± 0.057	65.9 ± 0.057	0.86 ± 0.031	1.12 ± 0.012
Fish skin gelatine	Catla	8.68 ± 0.26	74.53 ± 5.2	1.06 ± 0.38	1.09 ± 0.02
	Rohu	8.58 ± 0.16	84.44 ± 1.2	0.61 ± 0.02	1.20 ± 0.01

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Influences of Kitchen Waste Product to Restore Soil Fertility and Impact on Pulse Crop

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Received: 19 Feb 2020 Revised:21 Mar 2020 Accepted: 23 Apr 2020

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ABSTRACT

Kitchen waste are one of the strong waste which contribute fundamentally to ecological contamination by its smell, gives the ideal living space to pipes, harm the close by condition and furthermore makes some allergies individuals working close by territories when saved for longer time. Specialists have contemplated the utilization of different biomaterials for soil fruitfulness, However there is little report accessible on the utilization of egg shells and other waste. In the present investigation it was reported that the waste egg shells contain CaO, MnO, Fe_2O_3 ,Cl, Al_2O_3, SiO_2, and K_2O, which are the basic full scale and micronutrients for plants. Kitchen waste mixed with soil before crop cultivation. Both soil sample and kitchen waste were analyzed using XRF spectroscopy to determine pH, EC, water holding capacity and micronutrient content. As the kitchen waste fixes different beneficial micro or macronutrients in soil that small amount of different metals enhance the plant growth. Waste egg shells can be utilized as plant manure because of essence of 95% of calcium carbonate which is an intense wellspring of lime to reduce the pH of acidic soil. This waste powder applied on different types of pulse seed in field condition. It was also found that pulse crops seeds were grown better in waste product treated soil that is 10 mm larger than the plant grown in control. From this research, it can be concluded that waste egg shells can fulfill the mineral requirement for the growth of pulse as it enhanced the nutrient level in soil.

Key words: kitchen waste, macro and micro nutrient, pulse.

INTRODUCTION

Agriculture is one of the significant jobs in different nations. Because of the utilization of concoction pesticides and composts, the nature of the soil changes (such pH, collection of overwhelming metals and so on). Subsequently there is a need to utilize biomaterials in farming that may improve the nature of harvests as well as the nature of the land. Utilization of waste biomaterials may help diminish the expense too. Natural contamination at various destinations are incredibly impacted by the utilization example and propensities for squander the executives (Vaccariet. *et al.*

2019). Usage of waste progressive system ought to be expected to deal with the waste measurements to arrive at the roundabout economy (Ana and Martinho 2019). The aggregate sum of waste produced all through the world is around 3.2 billion tons, out of which 70% of waste isn't reused or reused (Tisserant *et al.* 2017). Then again utilization of bio compost is condition well-disposed and furthermore assists with expanding the dirt ripeness for better creation and yield of harvest. As eggshell powder is a balancing out materials which improves the properties of soil (Ok *et al.*2011) these can be utilized as bio-fertilizer in the harvest field to diminish the plant infections and improve the profitability of soil.

Kitchen waste contain major quantity of nutrients in this way satisfies the measure of growth in plant. So the utilization of waste egg shells as an elective hotspot for rectifying soil ripeness as a bio-manure and furthermore can lessen the natural effect supported by the removal of this result. In this way the present research work was directed to examine the response of kitchen waste to soil richness and its effects on the development of pulse seedling. The primary targets of this present work are waste product can supply supplements to the pulse plant and furthermore keeps up the pH parity of the soil. It being a waste bio-material includes less expense and makes less issue to nature.

MATERIALS AND METHODS

Current study was under taken at the university farm, which is red soil zone under Centurion University and Technology and Management, in the Gajapati district of Odisha is situated at 18.80° N, latitude, 84.14° E longitude, with an altitude at 10.9 m above the mean sea level. The climate at this region is sub-humid tropic. The selected area represents new alluvial agro-climatic zone and consists dominantly with illiates clay as mixed clay mineral. The experiment was conducted during 2018 – 2019. Waste material were collected from different fast food shops and vending zone near Centurion University of Technology and Management, Parlakhemundi, Odisha, India. After collection remove bacterial and viral contact, firstly waste items was washed in luke warm water. Then items were treated with boil water for 5 min. This was followed by the sunlight drying for around 72 hrs (14 days peak sunshine hours). Applied dry egg cell powder with soil and mixed it properly (Chakraborty 2016). Soil samples were collected and brought to the laboratory spread out on paper. Leaves, Coarse concretions, stones, pieces of roots, and other un-decomposed organic residues were removed. Big lumps of moist soil were broken by hand. Samples were air dried at 20-25^{o} C and 20% to 60% relative humidity inside the laboratory under shade. Samples were mixed during drying to expose bottom layer to top. After air drying soil samples were crushed gently with help of a wooden hammer and sieved through a 2 mm sieve. The material larger than 2 mm was discarded This mixture were stored with proper labeling in polythene bottles for analysis of physical, chemical & other properties (Basak 2000). The physico-chemical parameters of soil like pH, electrical conductivity (E.C.) were measured by electro-potential method, soil moisture percent (%), water holding capacity were measured by gravimetric method and elemental content(CaO, MnO, Fe_2O_3 , Cl, Al_2O_3, SiO_2, and K_2O etc.) of both soil and waste products were analyzed using XRF spectroscopy(Arzoo *et al*. 2017)

Uniform sized healthy pulse seeds were sterilized with 0.1% $HgCl_2$ for about five minutes and then washed several times with tap water followed by distilled water. The sterilized seeds were germinated in different concentration of kitchen waste treated soil along with control soil. Seeds were allowed to germinate at room temperature (28 ± 2^{o}C) in darkness for five days.

RESULTS AND DISCUSSION

The physico-chemical parameters like pH, electrical conductivity (E.C.), soil moisture content (%), water holding limit and various components/compound substances of a nursery soil and nursery soil treated with waste egg shells were estimated and recorded (Table 1). Result of study revealed that pH of the nursery soil was expanded from 6.54 to 7.38 which is incredibly noteworthy change after expansion of waste egg shells powder. Electrical conductivity of

the soil example viable was seen as 0.82 dS/m. The electrical conductivity of soil treated with squander was seen as 0.85 dS/m which portrayed the little increment from the soil without kitchen waste. After expansion of waste product, Soil moisture was seen as expanded from 11.00 % to 11.50% which was not altogether changed. The measure of water that soil can hold is significant for plant development. The water holding limit of nursery soil and kitchen waste blended nursery soil were seen as 157.54 ml/kg and 159.32 ml/kg individually, little addition was found in squander treated soil which is extremely valuable for plant development.

Different elemental content of the soil was also analyzed in current study. It was reported that the distinctive basic substance like, nitrogen, hydrogen, carbon , Al_2O_3, K_2O, CaO, MnO, Fe_2O_3, CuO, Cl, ZnO, value expanded with expansion of kitchen waste which could build the soil richness and help in better plant development. From table 2, it was observed that SiO_2 has significant contribution on pH, electrical conductivity, water holding capacity and percentage of moisture content. However, for electrical conductivity Al_2O_3, SiO_2 and Fe_2O_3 were found to have significant effect. So it can be concluded that the waste material can neutralize the pH of soil, which is more beneficial for plants rather than normal soil. Same soil test result found in Adhikary *et al* (2020).

Pulse seed germination is the most important stage of plant growth and it can be used as a reference for plant development and an indication for early response of plants. The percentage of germination was found to be increased by increasing the weight of waste products. Effect of kitchen waste on the germination and development parameters of pulse seed germination is the most significant phase of plant development and it very well may be utilized as a source of perspective for plant advancement and a sign for early reaction of plants. The level of germination was seen as expanded by expanding the heaviness of waste items. The level of germination of pulse seeds from control to 1000 mg/L were seen as expanded from 84.76 to 94.02 (Fig.1). The root and shoot lengths of developed seeds were additionally found in an expanding pattern with expanding grouping of kitchen waste (Fig. 2). As kitchen waste upgrades the soil supplement content, so it was indicated better outcome as far as germination and development of seedling. Result of previous studies revealed that the waste egg shell contains high measure of calcium carbonate (Martin *et al.* 2007), which acts as an immobilizing agent to fix the metals in the soil (Ok *et al.* 2011).

Various minerals like iron, copper and zinc are essential for plants and animals. The availability of minerals such as copper, zinc, iron, manganese, molybdenum and nickel varies from region to region and such metals are essential micronutrients for plant growths (Arzoo *et al*. 2017). According to Ok *et al.* (2011) egg shell was used for fixing of lead (Pb) and cadmium (Cd) in acidic soils, which ultimately increased the pH of soil towards its neutrality. As the egg shell fixes different beneficial micro or macronutrients in soil that small amount of different metals enhance the plant growth cobalt in *Vigna mungo* L., cadmium in *Triticum aestivum* (Kalita *et al*., 1993), Chromium in *Salvia sclera* (Corradi *et al*., 1993), food waste in peanut crops (Daud *et al*., 2016), cobalt and zinc in *Penisetum americanum* and *Parkinsonia acculata* (Terry 1981) which argue the confirmation of the result that found in this research that kitchen waste enhance growth of seedling due to presence of different micro or macro nutrient.

CONCLUSION

By experiment with different types of kitchen waste can be used as manure due to presence of 95% of calcium carbonate and can regulate the amount the pH of acidic soil also it regulates many metabolic process and biochemical function. From this research, it comes to know that waste egg shells powder can fulfill the mineral requirement as macro and micro nutrients for the growth of pulse crop as it enhanced the nutrient level in soil. So from this research it can be concluded waste egg shells can fulfill the mineral requirement for the plant's growth. Moreover, it is excellent organic manure for better growth and development of pulse crops in field conditions.

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Table 1. Comparison between different physico-chemical parameters of normal soil and kitchen waste mixed soil

Parameter	pH	Ec	WHC (%)	Moisture content (%)	OC (%)
Normal Soil	6.542±0.052	0.820±0.003	157.542±0.582	11±0.572	0.45±0.02
Kitchen waste + Soil	7.384±0.012	0.854±0.004	159.321±0.562	11.502±0.564	0.61±0.03
P value	**0.007**	**0.004**	**0.23**	**0.949**	**0.002**

Values of four replicates ± SEM

Table 2. Comparison between the amount of compounds present in normal soil and kitchen waste mixed soil

Elements (%)	Normal soil	Kitchen waste mixed with soil	P value
N	1.05±0.012	1.39±0.005	1.054
C	11.9±0.026	12.6±0.062	1.760
H	0.25±0.002	0.26±0.003	0.014
SiO_2	66.184±0.062	61.18±0.006	7.250
Al_2O_3	15.712±0.063	18.714±0.003	3.220
P_2O_5	1.034±0.002	1.011±0.001	0.078
K_2O	1.446±0.005	1.964±0.005	1.300
CaO	0.138±0.003	4.336±0.002	1.632
MnO	0.145±0.003	0.210±0.003	2.730
Fe_2O_3	6.213±0.031	9.216±0.009	1.010
SO_3	0.663±0.002	0.365±0.008	5.635
Cl	0.193±0.002	0.195±0.006	0.126
CuO	0.010±0.002	0.010±0.001	0.404
ZnO	0.014±0.003	0.018±0.001	0.008

Values of four replicates ± SEM

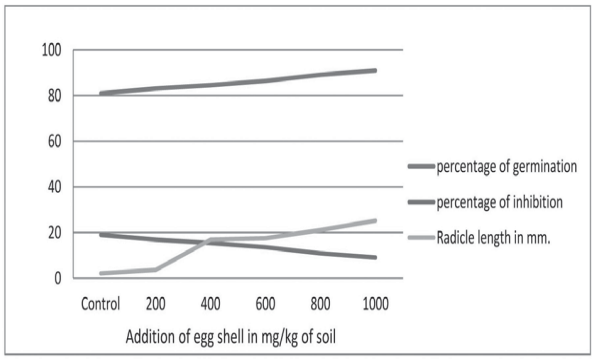

Fig. 1. Impact of kitchen waste on germination and length of pulse seeds

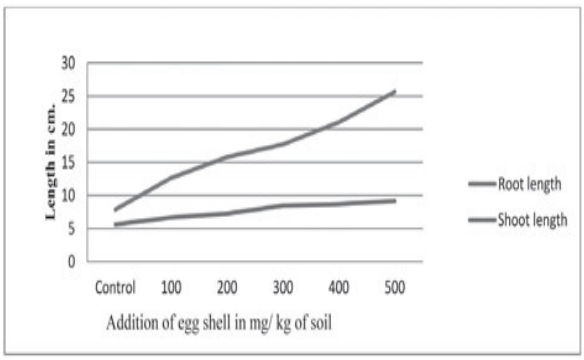

Fig.2. Impact of waste product on growth of the pulse seedlings after 15 days of treatment

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

A Baseline Studies on Vertebrate Diversity, Status, Threats and Conservation Measures to Rukhi Reserve Forest, Part of Eastern Ghats, Odisha, India

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Received: 17 Feb 2020 Revised:20 Mar 2020 Accepted: 23 Apr 2020

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ABSTRACT

A preliminary study was conducted in different pockets of Rukhi hill ranges, Nayagarh from June 2018 to March 2019 regularly in 4-5 days in every week. The standard methodologies were followed in the assessment of vertebrate fauna. The key parameters for identification of vertebrate species are good photographs, visual observation and vocal sounds. After spontaneous field study we observed that 12 species of amphibians, 22 species of reptiles, 46 species of avifauna both migratory and residential and 22 species of mammals presently inhabit in the study area. All the finding animals were marked on WPA Status, CITES Status and IUCN Red list category. Further their status, threats were studied and conservation measures were also proposed. The current scenario of study area, there is need of awareness and moderate use of natural resources for conservation and sustainable development.

Keywords: Biodiversity conservation, Vertebrate assessment, Conservation measures, IUCN, Sustainable development.

INTRODUCTION

Biodiversity refers to variety of flora, fauna and microorganisms in a particular area. It is the collection of genes, species and ecosystems in a locality (WRI; IUCN; UNEP, 1992). More the biodiversity more will be an effective surroundings. Assemblage of trees, shrubs, vines, grasses and other herbaceous (non-woody) plants, mosses, algae, fungi, insects, mammals, birds, reptiles, amphibians, and microorganisms living on the plants and animals and in the soil interact with one another and with the non-living part of the environment - including the soil, water, and minerals, to make up what we know as a forest (ICNH, 2006).Currently, total forest cover of India is 7, 08,273km^2 which forms 21.54% of the geographical area of the country.Odisha occupy 51,345km^2 of total forest cover area which

is 32.98% of total geographic area of the state. (FSI, 2017). Macro fauna in the forest is generally vertebrates. The dominant groups of animals on the earth are vertebrates. They are placed at the top in every matter likely abundance, large body sizes and food-chain (Das *et al.*, 2016). The vertebrates like wild boar, porcupine, pangolin, sambar deer, barking deer, different birds and various herpetofaunas currently inhabit in this area. The major important fauna inhabits in protected areas; but many other faunas, who habit in different forests are neglecting. Although the number of such animals is huge, they shall be conserving through various strategies. Otherwise total ecology may disrupt. Assessment is the main parameter for conservation and useful for ecological balance and sustainable development (IUCN, 2010).Main functions of the Intergovernmental Panel on Biodiversity and Ecosystem Services is to"perform regular and timely assessments of knowledge on biodiversity" (IPBES, 2013). So, it is very important to assess the biodiversity of a local area whether the area is small and large.

Worldwide, populations of wild flora and fauna are being depleted due to anthropogenic disturbances (Barnosky*et al.*, 2011; Dirzo*et al.*, 2014). Loss of diversity is a terrible trade worldwide.Themain causes for declination of biodiversity in the study area are deforestation, global climate change, forest fire, collection of fire wood, illegal expansion of urban areas etc. Chivian and Bernstein, (2010) predict that by 2050, climate change alone is expected to threaten 25% or more of all species on land with extinction. Natural habitats and species are declining by between 0.5 and 1.5% per year; As a result of our activities, 32% amphibians, 12% birds and 25% mammals are threatened with extinction in the next century (UNEP FI, 2008).

Nayagarh is bounded by districts of Angul and Cuttack in North West, Kandhamal in West, Ganjam in South and Khordha in the East. Rukhi hill ranges (Fig.12) are placed in the southern side of Nayagarh town which is located in 20^0 06′ 56″ – 20^0 07′ 40″ N latitude and 85^0 04′ 52″ – 85^0 06′ 15″ E longitude. The Rukhi Hill Forest comprises of various types of flora and fauna. The forest mostly seen in the hill are South Indian moist mixed deciduous forest, deciduous forest, mixed tropical green forest, Miscellaneous forest, tropical moist deciduous forest etc. The Rukhi Hill also contain hill rocks, as it is placed in the eastern Indian states, the primary forest is tropical moist deciduous forest. The main soils are seen in this forest are (Forest & Hill soils) tropical brown forest soil, laterite soil and red loam etc. The climate of Nayagarh district is characterized by hot summer and high humidity all the year around and good seasonal rainfall. Three prominent seasons are observed in a year. These are hot and dry summers, hot and humid rainy season and moderate winter season. It is a semi tropical region hill forest. The floral diversity of this hill mainly composed of rich in Sal forest (*Shorea robusta*), Sisoo (*Dalbergia sissoo*), Teak (*Tectona grandis*) and Eucalyptus (*Eucalyptus globulus*) etc.

MATERIALS AND METHODS

The study has been conducted from June 2018 to March 2019 regularly in 4-5 days in every week. During morning 03 hours (06:00- 09:00) and evening 2.5 hours (16:00- 18:30) were devoted for the field study. The standard methodologies were followed which are given in the "Handbook of Biodiversity Methods Survey, Evolution and Monitoring" (Hill *et al.*, 2005), "Practical methods in Ecology" (Henderson, 2003). Besides these, the book of "The Conservation Handbook: Research, Management and Policy" (Sutherland, 2004) also followed for better study. The key parameters for identification of vertebrate species are good photographs, visual observation and vocal sounds (Daniels 2002; Prater 2005 andManikadan*et al.*, 2012).

RESULTS AND DISCUSSION

After spontaneous field study we observed that 12 species of amphibians, 22 species of reptiles, 46 species of avifauna both migratory and residential and 22 species of mammals presently inhabit in the study area (Table-1).

Threats to Vertebrates

Some anthropogenic activities were seen during study. These are as follows:

Habitat destruction

The harvesting and utilization of the natural resources by human beings is the leading cause of habitat destruction. The expansion of agricultural fields in the close vicinity of Rukhihills causing much severe structural threats to biodiversity especially by creating disturbance to Vertebrate fauna. The illegal expansion of urban area and agriculture development are the main cause of habitat destruction in Rukhi hill forest. Collection of firewood is the daily practice of local people.

Poaching

Unlawful hunting by local community creates interruption in feeding and breeding of various mammals such as Sambar Deer, Barking Deer, Indian Hare, Indian Porcupine and Indian Pangolin. These herbivore animals have been killed for food or commercial uses. Haunting causes a severe disturbance in the biodiversity and also affects the ecosystem.

Grazing

The main human induced factors include grazing of livestock, hunting, agriculture and encroachment of land near the study area. Livestock grazing has been an important issue for the conservation of biodiversity. Free roaming of livestock in the study area was a great threat for the survival of faunal species.

Conservation Strategies

- Preservation of endangered species through strict protection against poaching of animals and deforestation.
- Providing adequate forest cover to different wild animals within their habitat is necessary for their shelter and protection from weather, predators and enemies.
- To safeguard the natural habitat of the forest with its immensely rich biodiversity, people in general and the youth in particular is to be made aware of the status, problems and conservation concerning wildlife and its habitat.
- Strict enforcement of laws according to the Wildlife (Protection) Act, 1972will provide the safety and wellbeing of wild animals.

Vertebrates are one of the well-studied groups of animals found in Rukhi hill forest. Various anthropogenic activities are the main causes of declination of biodiversity in this hill. Illiterate and lack of awareness among people is also the main cause for declination. There is an urgent need to safeguard vertebrate diversity by protecting natural habitat of the Hill Forest. Otherwise each and every species may become history in the study area.

ACKNOWLEDGEMENT

I am thankful to all the faculties of the Department of Zoology, Centurion University of Technology and Management, Bhubaneswar.

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Table 1. Checklist of all Vertebrate findings at Rukhi hill with IUCN Status

Sl. No.	Scientific Name	Common Name	IUCN Status
AMPHIBIANS			
1	*Haplobatrachus tigerinus*	Indian Bull Frog	LC
2	*Euphlyctis hexadactylus*	Indian Pond Frog	EN
3	*Haplobatrachus crassus*	Jerdon's Bull Frog	LC
4	*Euphlyctis cyanophlyctis*	Indian Skipper Frog	LC
5	*Sphaerotheca breviceps*	Indian Burrowing frog	LC
6	*Fejervarya limnocharis*	Asian grass Frog	LC
7	*Polypedates maculates*	Indian Tree Frog	LC
8	*Duttaphrynus melanostictus*	Common Toad	LC
9	*Duttaphrynus stomaticus*	Indian Marbled Toad	LC
10	*Microhyla ornate*	Ornate narrow mouthed Toad	LC
11	*Ramanella variegate*	Termite Nest frog	LC
12	*Kaloula taprobanica*	Painted Frog	LC
REPTILES			
1	*Chamaeleo zeylanicus*	Indian Chamelion	LC
2	*Eutropis multifasciata*	Golden Skink	LC
3	*Eutropis macularia*	Bronze Grass Skink	LC

4	*Lygosoma punctata*	Common Dotted Garden Skink	LC
5	*Calotes versicolor*	Garden Lizard	LC
6	*Monilesaurus rouxii*	Roux's Forest Lizard	LC
7	*Hemidactylus leschenaultia*	Leschenault's Gecko	LC
8	*Varanus bengalensis*	Common Indian Monitor	EN
9	*Ptyas mucosa*	Indian Rat Snake	LC
10	*Amphiesma stolatum*	Buff Striped Keelback	LC
11	*Xenochrophis piscator*	Checkered Keelback	LC
12	*Dendrelaphis tristis*	Bronze back tree Snake	LC
13	*Lycodon jara*	Twin spotted wolf Snake	LC
14	*Ahaetulla nasuta*	Green Vine Tree snake	LC
15	*Macropisthodon plumbicolor*	Green keelback	LC
16	*Bungarus fasciatus*	Banded Krait	LC
17	*Bungarus caeruleus*	Common Krait	LC
18	*Naja kauthia*	Monocellate Cobra	LC
19	*Naja naja*	Spectacled Cobra	LC
20	*Eryx johnii*	Indian Sand Boa	LC
21	*Python molurus*	Indian Rock Python	LC
22	*Daboia russelii*	Russell's Viper	LC
	AVES		
1	*Corvus splendens*	Indian Crow	LC
2	*Corvusle vaillantii*	Indian Jungle Crow	LC
3	*Gracula religiosa*	Hill Myna	LC
4	*Acridotheres fuscus fuscus*	Jungle Myna	LC
5	*Acridotheres tristis*	Common Myna	LC
6	*Gracupica contra*	Pied Starling (Pied Myna)	LC
7	*Leptocoma zeylonica*	Purple rumped sunbird	LC
8	*Passer domesticus*	House Sparrow	LC
9	*Amandava amandava*	Red Avadavat	LC
10	*Geokichla citrina*	Orange Headed Ground Thrush	LC
11	*Dicrurus macrocerus*	Black Drongo	LC
12	*Pycnonotus jocosus*	Red Whiskered Bulbul	LC
13	*Pycnonotus cafer*	Red vented Bulbul	LC
14	*Copsychus saularis*	Oriental Magpie Robin	LC
15	*Turdoides striata*	Jungle babbler	LC
16	*Oriolus kundoo*	Indian Golden Oriole	LC
17	*Ploceus philippinus*	Baya Weaver	LC
18	*Hirundo rustica*	Common Swallow	LC
19	*Hirundo smithii*	Wire tailed Swallow	LC
20	*Anthus trivialis*	Tree Pipit	LC
21	*Columba livia*	Rock Pigeon	LC
22	*Spilopelia chinensis*	Spotted Dove	LC
23	*Streptopelia decaocto*	Collared Dove	LC
24	*Ardeola grayii*	Indian Pond Heron	LC
25	*Bubulcus ibis*	Cattle Egret	LC
26	*Egretta garzetta*	Little Egret	LC
27	*Ardea intermedia*	Median Egret	LC

28	*Microcarbo niger*	Little Cormorant	LC
29	*Centropus bengalensis*	Lesser Coucal	LC
30	*Eudynamys scolopaceus*	Asian Koel	LC
31	*Centropus sinensis*	Greater Coucal	LC
32	*Coracias benghalensis indicus*	Indian Roller	LC
33	*Halcyon smyrnensis*	White throated Kingfisher	LC
34	*Merops orientalis*	Little Green Bee-eater	LC
35	*Merops philippinus*	Blue tailed Bee-eater	LC
36	*Anastomus oscitans*	Asian open billed Stork	LC
37	*Elanuis caeruleus*	Black Winged Kite	LC
38	*Pavo cristatus*	Indian Peafowl	LC
39	*Gallus gallus*	Red Jungle fowl	LC
40	*Vanellus indicus*	Red Wattled Lapwing	LC
41	*Tringa ochropus*	Green Sandpiper	LC
42	*Ocyceros birostris*	Indian Grey hornbill	LC
43	*Psittacula krameri*	Rose ringed Parakeet	LC
44	*Psittacula eupatria*	Alexandrine Parakeet	NT
45	*Aerodramus unicolor*	Indian Swift	LC
46	*Dinopium javanense*	Flameback Woodpecker	LC
MAMMALS			
1	*Rusa unicolor*	Sambar Deer	VU
2	*Muntiacus muntjak*	Indian Muntjac	LC
3	*Sus scrofa*	Indian Wild boar	LC
4	*Funambulus palmarum*	Three striped palm Squirrel	LC
5	*Golunda ellioti*	Indian Bush rat	LC
6	*Rattus rattus*	Black rat	LC
7	*Hystrix indica*	Indian Porcupine	LC
8	*Semnopithecus entellus*	Hanuman Langur	EN
9	*Macaca mulatta*	Rhesus Macaque	LC
10	*Viverricula indica*	Small Indian Civet	LC
11	*Canis aureus*	Indian Jackal	LC
12	*Vulpes bengalensis*	Indian Fox	LC
13	*Canis lupus*	Indian Wolf	LC
14	*Herpestes edwardsi*	Indian Grey Mangoose	LC
15	*Felis chaus*	Jungle Cat	LC
16	*Hyaena hyaena*	Striped Hyena	NT
17	*Corynorhinus townsendii*	Micro Bat	LC
18	*Pteropus giganteus*	Indian Flying Fox	LC
19	*Lepus nigricollis*	Indian Hare	LC
20	*Manis crassicaudata*	Indian Pangolin	EN
21	*Suncus murinus*	Asian House Shrew	LC
22	*Prionailurus bengalensis*	Leopard Cat	LC

Some Photographs of animals finding at Rukhi Hill

Fig. 1. Indian Tree Frog

Fig. 2. Spectacled Cobra

Fig. 3. Indian Chameleon

Fig. 4. Bronze back Tree snake

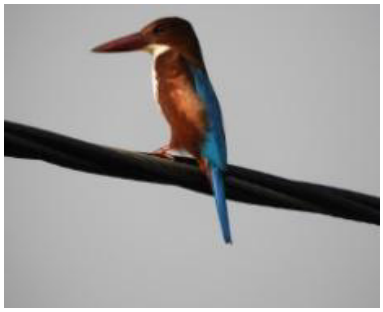

Fig. 5. White throated Kingfisher

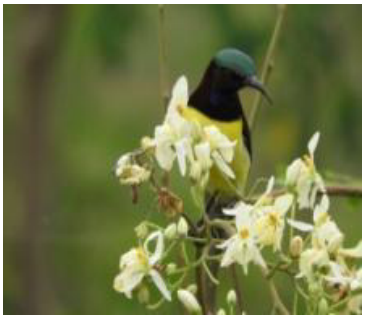

Fig. 6. Purple Rumped Sun Bird (Male)

Fig. 7. Indian Roller

Fig. 8. Indian Wild Boar

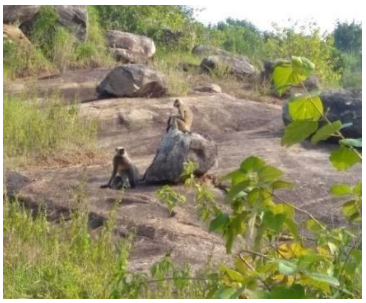

Fig. 9. Hanuman Langur

Fig. 10. Green Vine Snake

Fig. 11. Common Krait

Fig. 12. Satellite view of Rukhi R.F

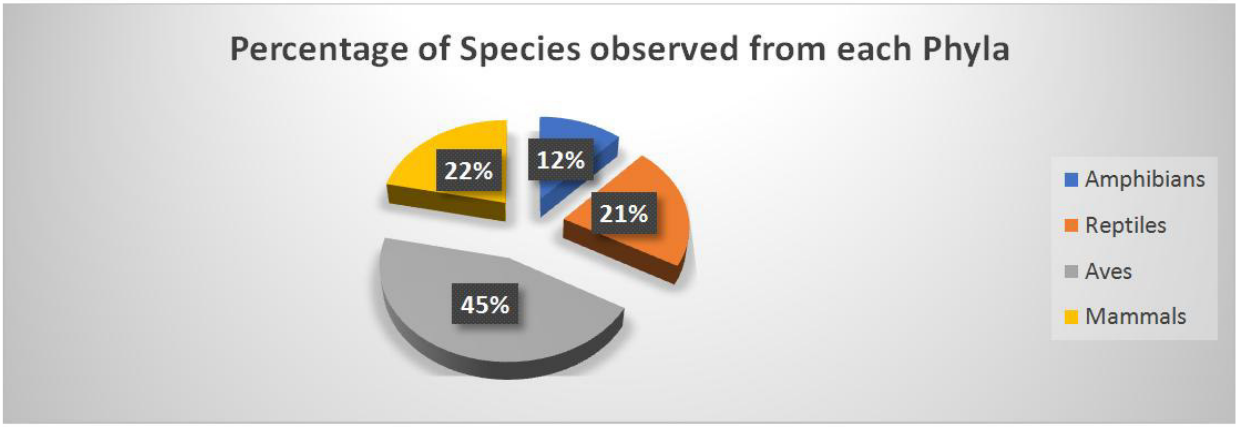

Fig. 13. Chart showing percentage of species observed from each phylum.

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Studies on Myelin Formation and Particulate Growth in Mixed Surfactant System

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Received: 16 Feb 2020 Revised:18 Mar 2020 Accepted: 23 Apr 2020

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ABSTRACT

Surfactant behavior on dissolution in water is an interesting phenomenon resulting in the formation of various non equilibrium microstructures. Our study is based on one of these non equilibrium structures well known as myelin. Myelins are formed when sparingly water soluble surfactants come in contact with water. In this report the myelin formation & structure in mixed surfactant system has been investigated with the help of digital video microscopy. It is observed that myelin growth is slower in mixed surfactant system as compared to the single surfactant system. We have also reported here on the fabrication of calcium phosphate particles using myelin structure formed by mixed surfactant system as a model system

Keywords: Surfactant, Myelin, Microstructures, Calcium phosphate

INTRODUCTION

The dynamic aspects of non equilibrium microstructures have drawn considerable attention in the recent past . Several research groups have investigated the interfacial instabilities extensively. These studies could be useful for potential applications in the field of nanotechnology & biomedical science these unusual phenomenons was first observed by Dr Rudolf Virchow in 1854 & since then it has been an area of active interest [1]. Myelins are highly viscous, gel-like, microstructures consisting of elongated tubules composed of concentrically stacked multi lamella formed with water and insoluble surfactants [2]. These are named myelins because of the structural similarities with myelin sheath of nerve cells. Surfactants (surface active agent) designate a substance which exhibits some superficial or interfacial activity. They are also called ampiphiles which means they have dual affinity. A typical amphiphilic molecule consists of two parts: on the one hand a polar group which contents heteroatom such as O, S, P or N on the other hand, an essentially apolar group which is in general a hydrocarbon chain. The polar portion exhibits a strong

affinity for polar solvents, particularly water, and it is often called hydrophilic part or hydrophile**.** The apolar part is called hydrophobe or lipophile. Based on their dissociation in water they are classified into anionic, cationic or non ionic [3,4].

Sodium bis(2-ethylhexyl) sulfosuuccinate ($C_{20}H_{37}NaO_7S$), often known as Aerosol OT or AOT, is an excellent anionic surfactant used in several experiments related to surfactant chemistry[5-7] It is a model surfactant as it has a nearly balanced hydrophilic & lyophilic properties. Physically it appears to be a white wax like substance having a characteristic odour .It essentially stabilizes monodisperse, spherical water – in – oil microemulsion droplets in alkanes. Phosphatidylcholine is one of the most commonly used surfactants. They belong to a class of phospholipids having choline as a head group. They are mainly found in biological membranes and can be easily obtained from a variety of sources such as egg yolk or soybeans from which they are mechanically extracted or chemically extracted. They are also a member of the lecithin group of yellow-brownish fatty substances occurring in animal and plant tissues. Myelins of this system have been studied in detail by many groups. [8-9]

Mixed surfactant systems are now days widely used in nearly all practical applications of surfactants. This is due to the fact that it is very difficult to prepare chemically pure surfactants. The most important fact is there is an advantage of perfrormance or synergism when we deliberately mix different surfactant as compared to their single counterparts [3]. When surfactants are added together in water, physicochemical properties are altered, which may be because of the net interaction between the amphiphiles, or in other words we can say that there is non ideal mixing. [4]. Surfactants assemble to form structures like micelles, micro emulsions and many supramolecular aggregates. These structures are otherwise known as equilibrium structure. These structures are formed when the surfactant molecules are at a higher concentration from that of a particular concentration called the critical micellar concentration. The aggregation behavior of the surfactant strongly depends on the concentration, chemical structure and nature of the medium.

The myelins are classical examples of non equilibrium structures. These kinds of growth are observed when complex fluids which are not in equilibrium state progresses towards equilibrium causing instabilities. Such instabilities are exhibited only when the lamellar phase is nearly insoluble in excess solvent and has a large miscibility gap with a phase comprising almost pure water. [10-13] . When water permeates into the lamellar phase swelling occurs and the back flow of surfactant leads to the formation of myelins. They consist of multilamellar tubules of concentric alternating amphiphile & water layers with core axis of water [14]. They exist in multiple shape and sizes. Some previous studies report that the growth of myelin is governed by diffusion process i.e. diffusion of the surfactant in solution [13-16] . Myelins can be also model for chemical reactions have been observed in many experiments. Mostly some precipitation reactions have resulted in the synthesis of particles [17-20].

MATERIALS AND METHODS

Materials used

The surfactants AOT [sodium bis (2-ethylhexyl) sulfosuccinate] was purchased from Sigma, with 99% purity, Chloroform AR grade from Merck (India), calcium chloride from Merck India Ltd, sodium phosphate AR grade from S.D Fine, India. All the above mentioned chemicals were used without any further purification. Throughout all the experiments doubly distilled water was used.

Myelin structure formation

Three sets of stock solutions were prepared. The first one was of AOT in chloroform, the second one was PC in chloroform & the third stock was made by mixing equimolar concentration of both AOT & PC.A drop of this

solution was taken on the glass slide. After the evaporation of solvent, a round cover slip was gently pressed onto the dry surfactant droplet & myelin growth was observed when surfactant phase was contacted with a drop of water. Myelin growth kinetics was observed using an optical microscope. The concentration of the stock solutions was varied between 0.2M to 0.5M.

Particle synthesis in myelin structure

For particle synthesis in myelin structure, stock solutions were prepared by mixing different concentrations to the AOT/$CHCl_3$ with aqueous calcium chloride solution. A glass slide was taken and the above micro emulsion comprising of the $CaCl_2$ solution was placed on it and was left undisturbed until the solvents, chloroform and water, get evaporated from its surface leaving behind a dry droplet of AOT containing $CaCl_2$. A cover slip was gently pressed onto the droplet and was fitted over the sample holder of the microscope. The schematic representation of both the experiments is represented below:- As discussed earlier the surfactant phase was then contacted with water containing Na_3PO_4, by incorporating a small drop of the Na_3PO_4 solution.This results in a precipitation reaction by which calcium phosphate particles are formed by capillary action.

RESULTS AND DISCUSSION

Optical microscope

The Olympus BX51 microscope, an advanced optical system with a CCD camera has been used in this experiment. The camera is alternatively connected to a video television set up for real time video capture. The whole system is connected to a personal computer. The image analysis software has been used for capturing time acquired images at different time intervals. The specimen under observation is positioned approximately perpendicular to the objective lens which is then illuminated leading to reflection of some light back to the lens. The specimen image is optimized at a magnification of 10X in all cases. The quality of the image depends not only on its position & illumination but on its characteristics as well.

Myelin growth kinetics

The varying growth & structures of myelin have been reported by different groups and it has been established that these growths are an outcome of back flow of water and dissolution process (Buchnan et.al 2000). A similar mechanism is observed in our case also. After an initial delay, formation of simple cylindrical rod like structures were observed which gradually elongated with increasing time. The growth was then quantitatively analyzed by measuring distance between the myelin roots and myelin fonts at different time intervals. Fig.3 to Fig. 8 show the growth of myelin at different times. To get a better precision several lengths were averaged to get a mean growth of myelin. The square of the average length (L^2) has been plotted with corresponding time of all the systems examined.

From the figures it is clear that the curve is approximately linear in both the cases and a best fit straight line is obtained. It indicates that the myelin growth in mixed system follows approximately the same order as that of individual system. A striking dissimilarity is observed in the duration of initial delay before the appearance of myelin. While it is about 4 to 5 seconds in the former, in the later it is about 30 seconds.

Effect of surfactant concentration on myelin growth

We have also studied the effect of surfactant concentration on the growth behavior of myelin. We have varied the AOT concentration & the mixed surfactant concentration from 0.2M to 0.5M. Fig 9 to 12 shows the growth pattern at different concentrations. We have observed that concentration of the surfactant is affecting myelin growth. With

increase in concentration rate is decreasing. When compared to single system, the myelin growth is slower in mixed system and the period of initial delay is also high ranging from 10-25 seconds.

Particle formation

Along with the structural aspects we have also studied the precipitation chemical reactions in lamellar phase. The chemical reaction can be represented by :

$$3CaCl_2 + 2\ Na_3(PO_4) \longrightarrow Ca_3(PO_4)_2 + 6NaCl$$

The Fig. 13 represents the formation of calcium phosphate particles in myelin.
The particles were formed at the root of the myelin and gradually aggregated in the form of a chain.

CONCLUSION

We have studied the formation and growth of myelin using AOT/PC/$CHCl_3$ mixed surfactant system. It is found that, in comparison to individual system the growth of myelin is slower in mixed system. The growth curve of myelin of mixed system is approximately linear like that of the individual system. Formation of calcium phosphate particles indicate organized water flow on the length scales of several hundred microns.

ACKNOWLEDGEMENTS

Authors thank the administration and management of Centurion University of Technology and Management, Odisha, India.

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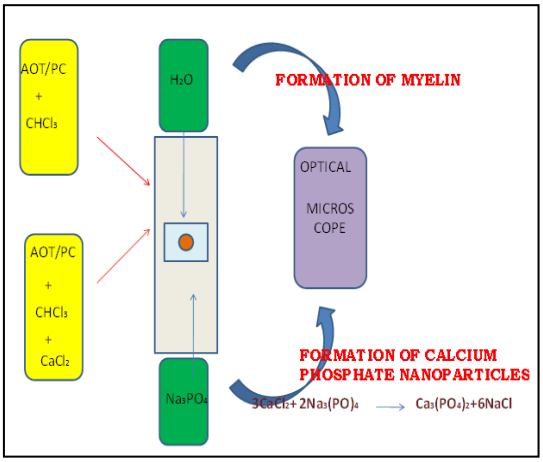

Fig. 1 Schematic representation of both the experiments is represented below

Fig.2 Olympus BX51 Optical microscope

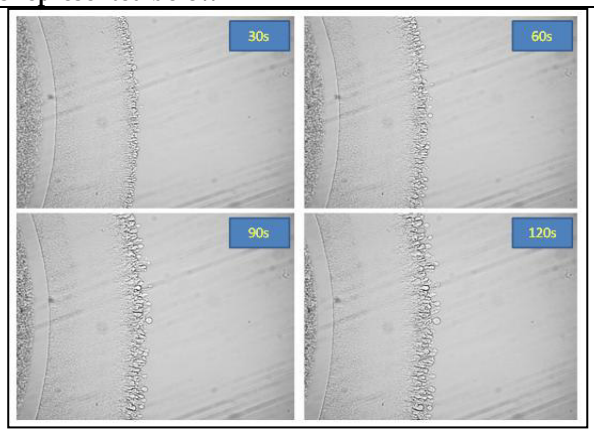

Fig. 3 Myelin growth w.r.t time in 0.3M AOT

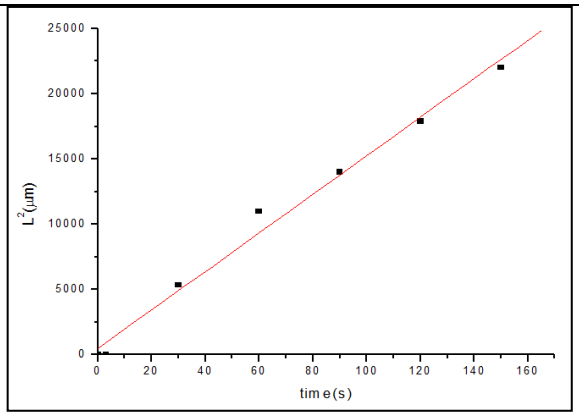

Fig.4. Plot between L^2 vs time of AOT/H_2O (0.3M)

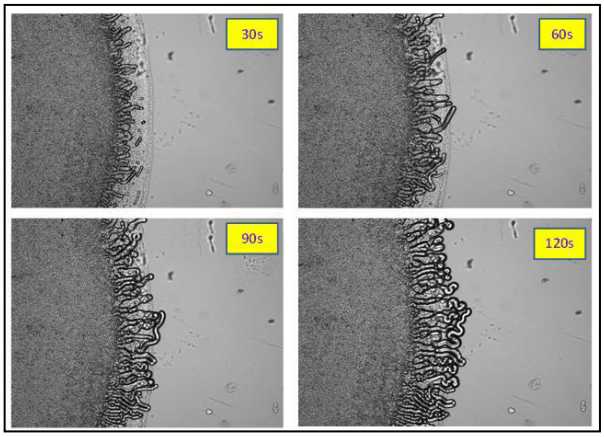

Fig. 5 Myelin growth w.r.t time in 0.3M PC

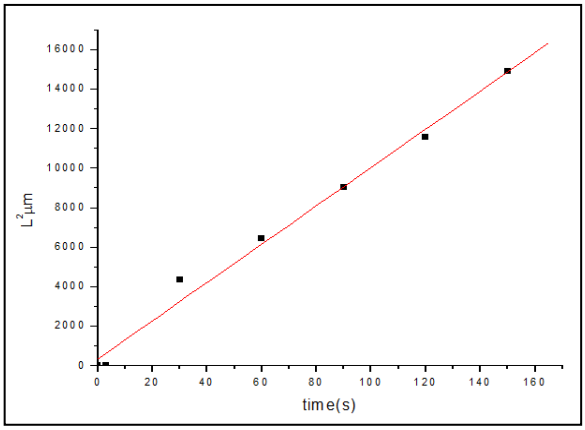

Fig.6 Plot of L^2 vs time of PC/H_20(0.3M)

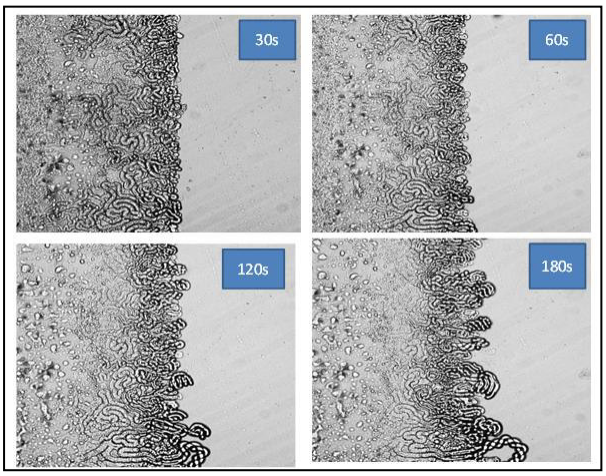

Fig 7. Myelin growth w.r.t time in 0.3M mixed (AOT & PC)

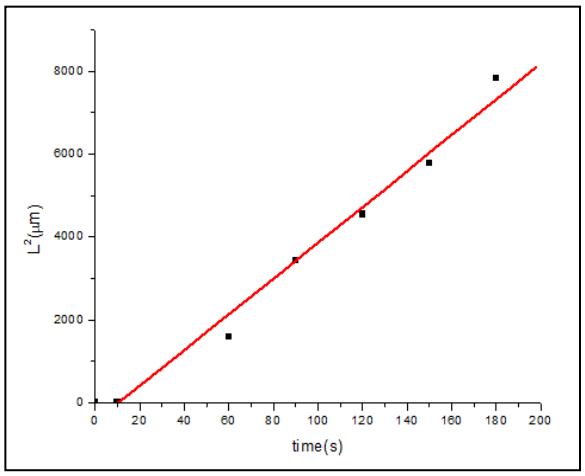

Fig.8 Plot of L^2 vs. time of mixed (AOT/PC)/H_2O 0.3M

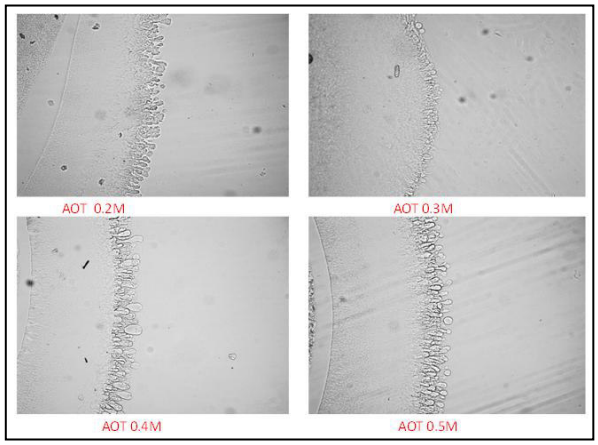

Fig. 9 Myelin growth after 60s at different conc. of AOT water system

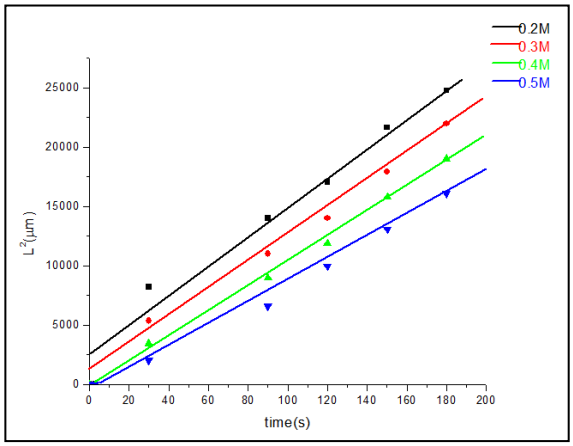

Fig.10 Plot of L^2 vs time for different concentration of AOT

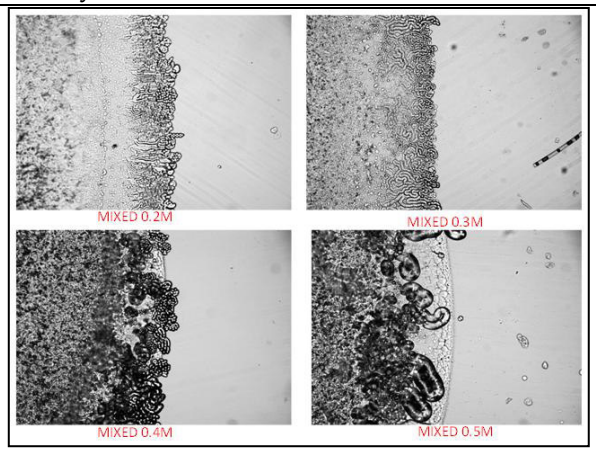

Fig.11. myelin growth after 60 at different conc. of mixed system

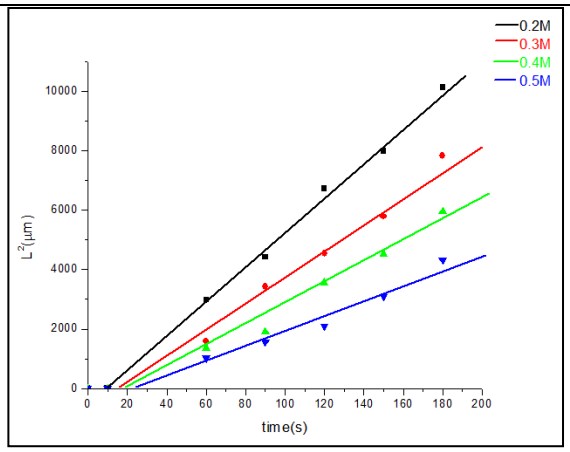

Fig. 12. Plot of L^2 vs time for different concentration of AOT/PC

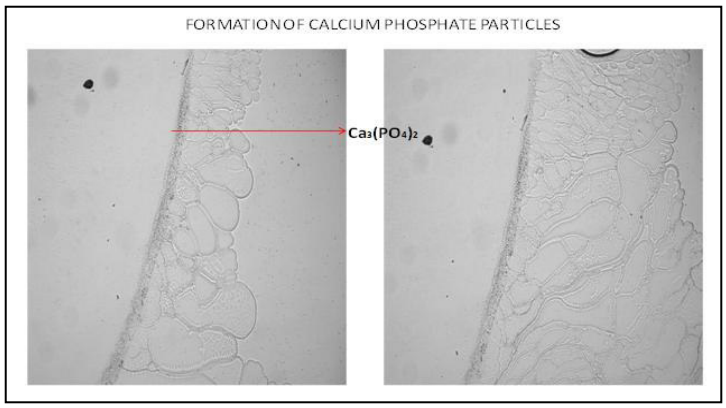

Fig. 13. formation of calcium phosphate particles at myelin roots

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Effects of ESL Teachers' Self-Efficacy on Reading Comprehension Skills of Students: A Case of Secondary Government Schools in Hyderabad, India

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Received: 10 Mar 2020 Revised:13 Apr 2020 Accepted: 15 May 2020

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ABSTRACT

The purpose of this phenomenological, qualitative research study was to explored and probe the effects of ESL teachers'self-efficacyon reading comprehension skills of students, as comprehension is the primary purpose of reading, and good readers are engaged in this process. This process can help students understand better and remember what they have read. Good instructions can help a student in effective verbal and written communication with others. Though students' reading must be purposeful and active, this depends on their reading skills. Mostly students struggle in developing their reading skills. Teachers are one of the important contributors in learning reading skills. Therefore, teaching reading skills is a very challenging job, Considering the importance of teachers in developing students reading comprehension skills, the research was conducted in Hyderabad. Focus of the study was to analyzed the effects of ESL (English as a second language) teachers' self-efficacy. Data was collected through a semi-structured interview of four ESL teachers in the Hyderabad region (Government Secondary School) of India, and analyzed through NVivo software. Results shows that an efficacious teacher can enhance students' learning by improving the learning skills, and the levelsof teachers 'efficacy positively effects on the reading comprehension skills of the students and motivate them to read. Hence, results of this study provide a clear picture that how important is to be efficacious for the teachers in improving the reading comprehension skills of government secondary school students in Hyderabad, India.

Keywords: Teacher self-efficacy, English as second language ESL, Efficacious teaching, Reading comprehension skills.

INTRODUCTION

Background of the Study

The present schooling system in India has incurred tremendous efforts to ensure quality and value-based education system in multiple disciplines. Because India is a highly populated county so it has to incur more efforts to create opportunities for country's development (Chaudhri, 2016). The literacy rate has been increased to heighten the living standard of society and to overcome poverty, unemployment, and inequality (NCEE, 2005). Schooling provides foundation for individual life-long learning, national development and teachers are main contributors in this development(Shazadi, Khatoon, Aziz, & Hassan, 2011). Furthermore, aprosperouscountrywide development and a civilization truly prosperous with knowledge all begins from its teachers (Ashton & Webb, 1986). Emerging quality and effective future teachers is a keystone of all teacher education programs. Exploration is constantlyinspecting diverse ways in increasing and indulgent effective teaching and teachers (Akman,2016).

Moreover, an overwhelming main stream of teachers rely on completing the prescribe course material is the main and only professional concern. Thus, reading comprehension skills stayed passive in school curriculum (Silva & Cain, 2015). Reading to build or derive meaning as a means of language acquisition and communication and sharing of information and ideas often gets neglected and the child fails to become a skilled reader. The Central Board of Secondary Education (CBSE) has emphasized reading as a "focus area for language education". School text books and syllabi are burdened with information absorbing, content heavy and memorizing tasks, so much that the pleasure of reading for its own sake is missed out. The Yashpal Committee in its report on *"Learning without BURDEN (1993) had highlighted the meaningless and joyless nature of school based learning in India, strongly raised the issue of non-comprehension in the classroom".*

Furthermore, English reading comprehension is the "essence of reading" (Durkin, 1993) reading comprehension is a complex thinking process that require the reader to construct meaning from the text. Student need explicit instruction in English reading comprehension. Good readers have strong comprehension skills. Comprehension develops through reading texts read (Habibian & Roslan 2014).

MATERIALS AND METHODS

Research Objectives

1. To explore the Effects of ESL teacher's Self-efficacy on reading comprehension skills of students
2. To probe into the levels of ESL teacher's Self-efficacy on reading comprehension skills of students

Effects of Teachers' self-efficacy

Teacher efficacy has demonstrated and robustlyconnected to numeroussignificantscholasticconsequences such as teachers' determination, passion, assurance and instructional performance, as well as student results such as accomplishment, motivation, and self-efficacy beliefs (Ghaith & Shaaban 1999). However, determined amount of complications have over whelmed individuals who have pursued to study teacher efficacy (Tschannen-Moran & Hoy, 2001). In addition, teacher efficacy also narrates to their performance in the classroom. According to Baltaoglu, Sucuoglu, Yurdabakan, 2015, Efficacy effects the teacher's speculation in instruction, the goals they set, and their level of ambition. Chiefly, teachers with a resilient sense of efficacy tend to reveal better levels of preparation and association (Allinder, 1994). Therefore, teachers with a high level of self-efficacy observe their effort as an experiment and flourish in managing more efficiently with difficulties. However, teachers with low self-efficacy involvement

more emotions of anxiety, concern and self-doubt harmful their capacity to manage meritoriously with encounters (Schwarzer etal., 2005). In relation to this, while probing the teachers' self-efficacy in urban city of Hyderabad, the study discovered, as indicated in the figure the following sub sub-themes Social Self-Efficacy, Self-regulatory self-efficacy and Academic Self-Efficacy as dimensions to the effects of teachers' self-efficacy.

Social Self-Efficacy

In line with the scholars, perspectives, (Skaalvik & Skaalvik, 2010) the informants interviewed expressed their views on the effects of teachers' self-efficacy as the ability to form and maintain relationships in addition to be engaged in leisurely activities (Coladarci, 2010). This perception was therefore coded as social self-efficacy. According to informant 4,"The impact of teacher self-efficacy in school can be seen in the way students uphold rapport as well as how they take part in all the school activities". Buttressing this observation, informant 2 disclosed thus, "The effects of teacher self-efficacy on teaching reading comprehension skills in my school is noticeable in the ability of our students to be self-confident in all events set out for them". And this will help the student to enhanced their reading comprehension skills.

Self-regulatory self-efficacy

Emerged from the findings was what the informants identified as self-regulatory self-efficacy which is the ability to resist peer pressure as well as avoid high risk activities. This sub sub-theme described the effects of Teachers' self-efficacy. For instance, Informant 2 noted that, the effect of teachers' self-efficacy which 'I know in relation to my school is that students have learnt to repel all unnecessary force and things that could be problematic in that case.
Furthermore, explaining self-regulatory as an effect of teachers' self-efficacy, Informant 1 stressed that, "In my school our students have developed the capability to understand the different kinds of stress to be avoided andshould be knowledgeable about what is good and what is risky". While corroborating this perception, informant 4 in his opinion concisely explained that, "Presently, reading comprehension skills in my school is very adequate, standard and encouraging because it is the result of teacher self-efficacy".

Academic Self-Efficacy

In relation to the effect of teachers' self-efficacy, academic self-efficacy also emerged. According to the secondary teachers in urban city of Hyderabad, it refers to the ability to do course work, regulate learning activities and meet expectations. For instance, informant 3 explained this thus, "One of the effect of teachers' self-efficacy that can be identified is the fact in my school most of the students have developed good skill in reading comprehension". This standpoint was also echoed in the perception of informant 1 who said, "As for me, the self-efficacy effect in my school is identified as my students' reading abilities to perform various reading tasks, such as grasping the main idea". Similarly, Informant 3 justified his perception thus, "Teacher self-efficacy plays a key role in the language learning and motivates our students effectively in their course work" (Gaylon et.al.,).

Levels of Teachers' self-efficacy

Teachers' philosophies about teaching and learning have been regularly found to play avital role in teachers' effectiveness and their high-quality of teaching practices (van de Schaaf, Stokking & Verloop 2008; Wilkins, 2008). As part of this study's aim to know the levels of teacher self-efficacy in school, the findings generated two sub sub-themes: low self-efficacy and high self-efficacy.

Low Self-efficacy

Asignificant factor in the strength of a teacher's sense of efficacy is, not astonishingly, experience, or what Bandura (1977), a groundbreaker in the development of self-efficacy theory, calls performance endeavors. This study identified low self-efficacy among some of the teachers. For instance, Informant 2 narrated his experience, "In my school, new teachers show expressively lower levels of self-efficacy more than experienced teachers". Explaining further, Informant 4 described the level of teachers' self-efficacy: "I discovered that some teachers who are newly posted to my school usually have a lower level of self-efficacy in the first three months of their resumption". Looking at it from another dimension, Informant 1 noted that, "As for me, sometimes the levels of teacher self-efficacy in my school is mostly low each time there is a change in the course of teaching".

High Self-efficacy

Teachers with a high sense of efficacy and perform on it are supplementaryprospective to have students who acquire learning(Shaughnessy, 2004). Contrary to previous perception, some informants agreed that, "Sometimes we notice high self-efficacy in our teachers whenever a teacher observes other teacher spending mostly effective practice and thus sense more self-confident that, determined its use, she could be more efficacious in attainment her students". Raising the salient point on the efficacy of teachers, Informant 2 said, "The level of self-efficacy of teachers in my school varies. Sometimes a teacher returned from further studies where he or she had gone to acquire more knowledge, they always show high self-efficacy". Buttressing this observation, Informant 3 narrated the levels of teacher self-efficacy in his school: "I can say that teachers with a higher sense of self-efficacy who provide more support for student learning and create a more positive classroom environment are those who are highly efficacious, in my school".

RESULTS

The secondary school teachers tried their best to be honest and to reveal the full picture what they believe they make a difference in terms of student achievement in reading comprehension skill. Many of the teachers had enlighten the reasons that has affected them negatively as well as positively. Theexploration of study sought to as certain additional evidence about teachers' self-efficacy as experienced by secondary school teachers who displayed high self-efficacy and low as well, in Hyderabad government schools. The study revealed that the teachers of the government secondary school believe that they can exercise a positive influence on students, by maintaining positive relationships with students and continue teaching well even when disrupted by a difficult student.

CONCLUSION

The researchers try to explored the effects of ESL teacher self- efficacy on their teaching and the levels of their self-efficacy. The goal of this study was to investigate the teacher's view and their experiences of self-efficacy in the teaching profession and how it effects on students reading comprehension skills. Teachers self-efficacy plays a vital role on student achievement reading for academics or reading for pleasure/entertainment, teacher is the excel of wheel where all the students learn their skills.The results of the study revealed the positive effects and shows the levels of efficacious teachers on teaching reading comprehension skills. Furthermore, methodology used in classroom does not lead to development of skills, English is taught as a subject with focus on imparting information and not on development of language skills (Khan, 2011). In Indian curriculum of schools writing and reading remains to be narrowed to textbooks to a large amount, regardless of the giving effect of "National Curriculum Framework 2005" under RTE Act, 2009.

Undoubtedly, teacher efficacy is a teachers' belief's in his/heraptitude to monitor their learners to accomplish in their academic (Azam & Kingdon, 2015). This study suggests that teachers with a resilient sense of self-efficacy is expected tobewell planners, more toughover failure, and extraprogressive and compassionatewith their students.

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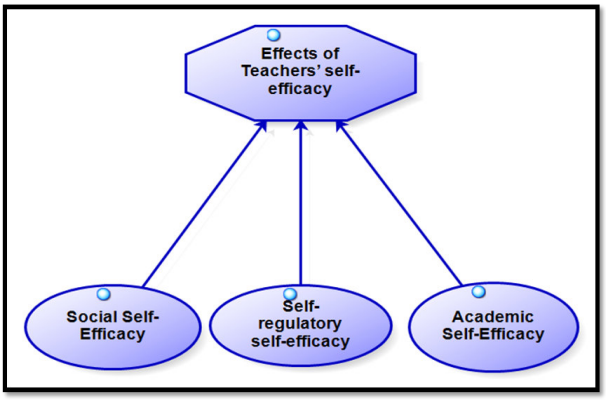

Fig.1. Effects of Teachers' self-efficacy

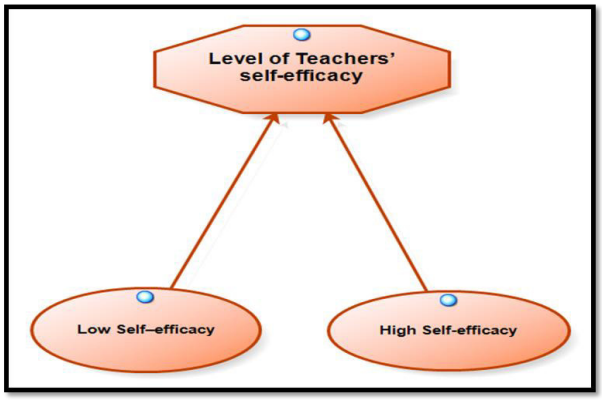

Fig.2.Levels of Teachers' self-efficacy

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Studies on Effect of Size of Fishes (*Clarias Batrachus*) on Bioaccumulation of Heavy Metals of Daya River, Bhubaneswar

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Received: 18 Feb 2020 Revised:20 Mar 2020 Accepted: 23 Apr 2020

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ABSTRACT

Daya River and Chilika Lake are the Reverine system of Odisha due to its suitable ecological environment as well as Daya River is also known as the main source for economic purpose. It provides sufficient amount of fish to human beings in Khurda for feeding purpose. But the suitable environment of these two River are getting affected due to polluted water outfall from Gangua Nallah into it. Gangua Nallah is the collecting source of all industrial as well as some domestic wastage of Khurda which falls into Daya River as well as Chilika Lake. Due to the presence of Industrial wastage the heavy metals presence detection of Gangua Nallah water was done in XRF- Spectrophotometer instrument. It was a differential measurement between two different sizes of fishes (*Clarias batrachus*) to know if the heavy metals accumulation capability varies on the basis of size. Besides this the instrument clearly detected the heavy metal bioaccumulation in fishes which was affected by size, as the smaller fish accumulated more heavy metals than the larger fish. The experiment was done using the flesh of the fish. As being a common source of water pollution, heavy metals can be accumulated by fishes and the presence of heavy metals can be decreased without affecting fish life in water. Although it is a biological method to prevent the heavy metals related pollution in the water sources which can cause different type of health effect while being used. Necessary steps should be taken to handle such pollution and to decrease the environmental risk of pollution.

Keywords: Bioaccumulation, Heavy metals, *C. batrachus,* Daya River, Gangua Nallah

INTRODUCTION

Water in its purest form is odorless, nearly colorless and tasteless. Water is the only substance that occurs naturally as solid (ice), a liquid and a gas(water vapour). It covers about 70% of the earth for a total of approximately 332.5 million cubic miles. From a poem, "The Rime of the Ancient Mariner" there is a line saying " Water, water, everywhere ,nor any drop to drink" which represents that most of the water (97% of it) is undrinkable because its salt water . Only 3% of the world's water supply is freshwater (Shrestha et al., 2017), and 77% of that is frozen, only a half a percent is available to supply every plant, animal and person on Earth. If water is supplied with any inadequate form the its distribution is worth not using. The water necessity can be sacrificed due to presence of different anthropogenic sources for water pollution (Rao et al., 2018).

Gangua nallah, a stream originated from the western upland area of Bhubaneswar city (Chandaka Reserve Forest area) and flows along the south eastern side of the city almost parallel to Daya river along city-side and ultimately outfalls into Daya river near village Vadimula of Khurdha district in the state of Odisha. The total length of Gangua nallah from its origin to the confluence point is approximately 45 Km. After traversing along the city side, Gangua nallah confluences with Daya river approximately 9 km towards south direction from the city limit. This flows along the city side of Capital city of Bhubaneswar. The city does not have any organised sewage treatment system and the sewage is discharged through storm water drains. There are about ten numbers of storm water drains in the city that carry untreated waste water and outfall into Gangua nallah at different locations. Thus Gangua nallah receives untreated sewage of the capital city and hence is polluted by the discharge of municipal waste water of the city only through several storm water drains. After traversing a distance of 13 Km from its origin, Gangua nallah starts receiving wastewater of the city through storm water drains at different points. Hence, the polluted stretch of Gangua nallah covers a distance of about 32 Km. There is an average flow of 1.17 meter/ second throughout the stretch. After receiving the wastewater of the city of Bhubaneswar, through storm-water drains, Gangua nallah has become a waste water carrying nallah and there are no beneficial uses of its water till its confluence with Daya River.

There is an article by JOSE K JOSEPH, a reporter on twitter saying the situation of water pollution in Bhubaneswar which is reflecting Daya river and Chilika lake as well. He particularly uploaded the news with caption ," Gangua nala today transports the city's garbage to river Daya and also end up polluting chilika lake". This issue really has been a big problem when it started affecting the aquatic organisms as well and the problem couldn't be denied. In chemistry, these are the metals that have relatively high densities, atomic weights or atomic number. 99 out of 118 known elements belong to heavy metals. Commonly, a density of atleast $5gcm^3$ is used to differentiate it from other light metals. These occur in earth's crust in very low concentration and somehow chemically bound in carbonate, sulfate, oxide, or silicate rocks and also in their elemental form.(K. Martin, S. Hosam M;" Introductory chapter: Introducing heavy metals" ; June 2018) There are a lot of varieties in heavy metals such as gold, silver, platinum are precious among the heavy metals; Iron, Copper, Phosphorous, silicon are abundant along with some less known heavy metals including Gallium, Thallium etc. Over last few decades detection of heavy metals among water sources has been increased due to its direct affect on food supplies and daily uses (Velusamy et al., 2014; Fatima et al., 2015; Arulkumar et al., 2017; Pal and Maiti, 2018; Kumari et al., 2018).

On the basis of IUCN red list *Clarias batrachus* can be found in stagnant, muddy and swampy water of high turbidity (Pandey M., et al., 2014). They are tropical species which can survive in temperature upto $9.8^{\circ}C$ as they have moderate tolerance to cold water. They mostly feed on insect larvae, earthworms, shells, shrimps, debris and aquatic plants. (GSMFC, 2006, Fishbase, 2009,Ros, 2004c). The fish can be helpful in bioaccumulation due to its pollution tolerance ability. The fish has a big tolerance to harsh living conditions, and it can leave the water to wiggle to a better location as long as it stays moist. It can be seen not only in SouthAsia but different part of the world including India, Pakistan, Philippines with different local names and their feeding habits. They have been black listed in several countries including USA because of their aggressive nature.

There are many factors of fish that affect the rate of heavy metals bioaccumulation in their organs. The factors may include: age, size, weight, feeding habits, body physiology and external environment includes concentration and bioavailability of metals in the water column, physiochemical properties of water and other climatic factors. The heavy metals accumulation in tissues is generally different depending on the structure and functions of the tissues. Generally metabolically active tissues such as gills, liver and kidneys have higher accumulation of heavy metals than other tissues such as skin and muscles (G. John R. et al., "sources and sinks of heavy metals", Reston, Virginia, 1995).

MATERIALS AND METHODS

Description of study area

The study area is Gangua Nallah situated in the location of Chakeisiani, Bhubaneswar, Odisha. The place Chakeisiani is directly connected with Mancheswar Industrial Estate. The waste canal present there, contains all the industial disposal remaining falling from the industries of Mancheswar. The selection of the area was based on the presence of different anthropogenic source of heavy metals present near the location, Mancheswar Industrial Estate.The waste product falling out of the industry directly gets in contact with Gangua Nallah. And the Gangua Nallah that is the main drainage system in Bhubaneswar city gets mixed with Daya River in an area known as Kesura which is in Bhubaneswar. It has been reported that Gangua Nallah is also the ultimate reason of pollution in Chilika lake (fig :- 1). So if the treatment gets started from the main pollution site then the Gangua Nallah may become less polluted while getting mixed Daya River or Chilika Lake.

Analysis of water
pH analysis of water

The pH meter was first calibrated. A 100 ml of beaker was rinsed with the sample water for 3 times. The electrode was rinsed with distilled water and made blot dry. 50 ml of sample water was poured in 100 ml beaker. The electrode part of the beaker was put into the sample water. It was once stirred with the electrode. The electrode was not allowed to touch the bottom or side of the beaker. It was kept there until the pH meter shows the constant number of pH. Once the reading showed a constant value the electrode was removed from the beaker and the result was noted.

Conductivity analysis of water

The conductivity meter was first calibrated to neglect the resultant errors. Electrode of the Conductivity meter was sterilized with distilled water and was made blot dry with blotting paper. A 100 ml beaker was sterilized with distilled water. After drying with blotting paper it was rinsed with the sample water for 3 times. The beaker was then filled with 100 ml of sample water. Result was not obtained until the meter show a constant reading of conductivity. The constant result was seen nearly after 10 mins of wait. The result of the water conductivity was noted.

Total Solid Count

Total solid = Total suspended solid + Total dissolved solid

Total suspended solid

100 ml of beaker was rinsed with distilled water and dried with a blotting paper for sterilization process. A sterilized conical flask and another beaker were taken. Weight of a filter paper was measured in weighing machine. The

conical flask was kept above the vacant beaker and a folded filter paper was settled on that conical flask. 100 ml of sample water was taken in the other beaker and was poured through the filter paper of conical flask. After the filtration was completed the suspended particles were visible on the filter paper. Let the filter paper dry naturally. Weights of the filter paper after being dried along with the suspended particles on it were measured on the weighing machine. The result was calculated by subtracting the weight of the empty filter paper from the weight of the filter paper that contained visible suspended particles.

Total dissolved solid

The beaker in which the sample water was filtered for the count of total suspended solids was previously weighed before the filtration was done. Then after the filtration the beaker contained only water and total dissolved solids. The sample water was then evaporated from the beaker in evaporator. Then after the evaporation process the beaker was weighed again in weighing machine. Then the TDS was calculated by subtracting the initial weight of the beaker from the final weight of the beaker.

Fish Sample Collection

Two different sizes of live *Clarias batrachus* were bought from local fish market. It was kept in a fish aquarium in sample water for continuously 21 days without proving any food or oxygen. The initial water level was noted and if the water level was seen to be lower again the level was maintained with sample water. The water in aquarium was used to change in every 7 days with the sample water only. Since, the fishes were bottom feeders they didn't want heavy water amount. After 21 days the fishes were sacrificed to detect the presence of heavy metals in muscle tissues of the fish. The weight and length of both the fishes were observed
Fish A- length - 18.9 cms, weight – 43. 6 gms
Fish B - length - 14.8 cms, weight – 23.7 gms

Sample Preparation for Heavy Metal Analysis

Water samples collected were acidified with Nitric acid and were detected presence of heavy metals in XRF – Spectrophotometer. The fishes were dissected removing the skin and the flesh of the fishes were removed with the help of scalpel in sterilized petridishes. Then 25ml of 69% Nitric acid was prepared. The flesh of the fishes were then dried in the hot air oven in a minimum temperature of 100°C for about 32 hours. After getting dried the sample was digested in the prepared Nitric acid solution for about 30 mins. Then the dried flesh along with the Nitric acid was grinded in Mortar and Pestle. After making it a fine paste, it was then kept in hot air oven for about 20 mins in 180°C. After taking out of the oven it was seen that the paste was completely converted into liquid form. Then the liquid samples were analyzed for the presence of heavy metals in XRF Spectrophotometer machine.

RESULTS

Result of water analysis

1. pH of water – 6.45
2. Conductivity – 1.213 MHOS/Cm
3. Total suspended solid = 200mg / L
4. Total Dissolved solid = 600 mg / L
5. Total solid = (600 + 200) mg/ L = 800 mg/L

The heavy metals that were detected in water sample are P> S > Cl > Ca > Fe > Sn > Eu. Phosphorous is the mostly detected heavy metal which was present in a significance amount.
Normalisation factor – 1.579

Heavy metals	Range (ppm)
Phosphorous (P)	572.6
Sulphur (S)	348.3
Chlorine (Cl)	132
Calcium (Ca)	243.3
Iron (Fe)	58.4
Tin (Sn)	40.5

Heavy Metals Analysis in Fishes

Fish A – length – 18.9 cm and weight – 43. 6 gms
Normalisation factor – 1.640

Heavy metals	Range (ppm)
Phosphorous (P)	854.6
Sulfur (S)	602.3
Chlorine (Cl)	354.9
Calcium (Ca)	272.6
Iron (Fe)	24.9
Tin (Sn)	41.1

Fish B – length – 14.8 cm and weight – 23.7 gms
Normalisation factor – 1.595

Heavy metals	Range (ppm)
Phosphorous (P)	913.3
Sulfur (S)	673.1
Chlorine (Cl)	280.1
Calcium (Ca)	279.4
Iron (Fe)	18.0
Tin (Sn)	40.0

DISCUSSION

Presence of heavy metals in the environment can cause various harmful effects. It depends on the source it is coming from. The increased amount of industrialization which has been imposed to improve the urbanization and economical availability for the local people can cause some effects to the people living near the place. From many various experiments it had been seen that the fish had been a very useful living organism in terms of accumulating heavy metals in their body. So, this process can be used in a positive way to decrease the industrial water pollution in some places. Although, consumption of those fishes that accumulated heavy metals can be dangerous sometimes as it can affect the food chain of the environment. But if the water pollution is in its initial stages that do not contain very harmful heavy metals like Mercury, lead, Cadmium etc that can be treated with fishes. The heavy metals that will be accumulated may not cause very harmful effect if it is present in between its minimum and maximum range that has been calculated from various experiments. If that lower range of heavy metals get possessed from fishes it won't worsen the situation by time. The harmful heavy metals do not pollute any environmental sources until or unless it directly gets mixed with it. If there is a lots of metals that can give rise to any complex forms then it will take time and may form any complex compound or element that will make the source really harmful for use of human

being in later stages, so it is convenient to make the environmental factor pollute free from its initial sources. The heavy metal analysis from fish has already shown that the accumulated amount of heavy metals is more than the heavy metals present in fish. The possibilities from above observation can be that fishes had already accumulated some heavy metals from its real habitat or the place where it belonged from before the experiment so that it show the range of heavy metal higher than the water sample. From the fish market where the fishes were bought it had been confirmed that the fish belonged from Daya River. The fisherman had collected the fish from Daya River and sold it to market. This can also say that Daya River already had some amount of heavy metals that was due to the outfall of Gangua Nallah. Both of the fishes that accumulated heavy metals inside their body was represented graphically to show the difference between them. This shows the decreasing appearance of heavy metals from P > S > Cl > Ca > Fe > Sn. Although the presence of such heavy metals does not cause a much harmful effect on human beings until they are present in their average range. For now, the presence of elements is in their normal range. So if the treatment is done beforehand it won't cause heavy effect in later stages.

CONCLUSION

The main purpose of this investigation is to decrease the water pollution in Daya River and Chilika Lake by decreasing the heavy metals amount from Gangua Nallah, Chakeisiani, Bhubaneswar. Being the important pollution creating source for river it contains the industrial wastage effluents so the treatment must be done from this place only so that when it gets mixed up with Daya River will cause less toxic effect on the water. The objective was to detect the presence of heavy metals in fish muscle to detect the increased amount of heavy metals in fishes from the water sample. The value detected were not more than maximum permissible limit. The unit used here is Parts per Million (PPM) that represents presence of less amount of heavy metals in Gangua Nallah. The graph showed that the fish with larger size accumulated less amount of heavy metals than the fish with smaller size. The difference may have seen due to the metabolic process. Usually the metabolic process in young organisms is more frequent and viable than the larger or old organisms. Although, the age of the fishes were not detected, but it had been seen that the smaller fish had no signs of aging. So, younger fishes can be used for the treatment. It is necessary to regulate the quality of water that is used by the rest of the population. This process of pollution control treatment does not need any huge amount of money. And if the heavy metals present is in convenient amount except the harmful or red listed heavy metals then the fish consumption from this area won't affect food chain vigorously. The heavy metals contained iron and calcium in a permissible amount so it won't affect the human source food chain lot. It can be concluded that for now consumption of fishes from Daya River is not much harmful for now.

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TOXIC GANGUA NALA POLLUTING CHILIKA LAKE

Once a fresh water stream, Gangua Nala today transports the city's plastic bottles, cardboard boxes and garbage into River Daya to also end up and pollute Chilka Lake. Official agencies fail to act, look the other way

JOSE K JOSEPH, OP

BHUBANESWAR, July 22: While several agencies have been given the task to ensure free flow of water through drainage lines, their attention has not reached up to Gangua Nala. What was once a freshwater stream is now the main ejection mechanism for exit of waste water from capital Bhubaneswar -- a city that finds a place in the central list of Smart Cities.

Currently, about 10 drainage channels run west to east criss-crossing the city for discharge of waste water. Nine out of 10 channels finally reach Gangua Nala and discharge, on an average, more than 120 million litres of untreated liquid waste to this system on a daily basis.

"We have turned Gangua Nala from a freshwater stream into a drainage system. Currently, this stream bears the burden of domestic and industrial waste from the Capital. We need to stop this dumping of untreated waste to the Nala and protect the stream and its ecosystem," said Ranjan K Panda, also known as the Waterman of Orissa.

Gangua Nala becomes shallower due to deposition of silt

Flow of water in Gangua being obstructed by weeds OP PHOTOS

Historic link

Gangua Nala held the distinction of being the lifeline of capital Bhubaneswar. This stream was known as Gandhabati in ancient times and was used as a moat (deep, wide ditch) around the historic Sisupal Garh (fort). Historians say it was probably the fort of Emperor Kharavela. But, urbanisation has turned this stream into a drainage system for Bhubaneswar.

According to sources, the 35.7km long Gangua Nala discharges approximately 652 cusecs of water into River Daya. Scientists confirmed that discharge of waste into Gangua is affecting the quality of the river. "We have served notice on the state government in this respect," said BN Bhol, environmental scientist with the Orissa state pollution control board.

In fact, in 2015, the National Environmental Engineering Research Institute (NEERI), a Central government entity for treatment of waste water, was asked by the state government to prepare a detailed project report (DPR) to treat water of Gangua Nala using the "phytorid" method -- a low-cost technology that involves a constructed wetland exclusively designed for treatment of municipal, urban, agricultural and industrial wastewater. NEERI also was known to have worked out a plan to give training to officials of the Orissa Water Supply and Sewerage Board (OWSSB) for implementation of the project. But, top officials of OWSSB are not aware of this. "We have not handled any project related to Gangua," confirmed MR Dash, member secretary, OWSSB, MR Dash.

Top officials of the Water Resources Department (WRD) claimed that some proposals have been made to clean Gangua. But, nothing has been finalised. They are under consideration," said P Nayak, engineer-in-chief, WRD.

Environmentalists opined that a delay in coming up with a concrete project to save the river would affect even Chilika Lake. "We have to come up with a project to save Gangua Nala from pollution. The issue is being kept dragging. Any further delay could affect even Chilika since the polluted water from Gangua Nala will reach Daya and through Daya it will end up in Chilika," said Sunder Narayan Patro, eminent environmentalist and president of Orissa Environmental Society.

Heaps of garbage dumped near the natural stream on the outskirts of Capital city

Fig 1: A report regarding toxicity of Gangua Nallah. (photo source: internet)

Fig:- 2 The water sample collected area

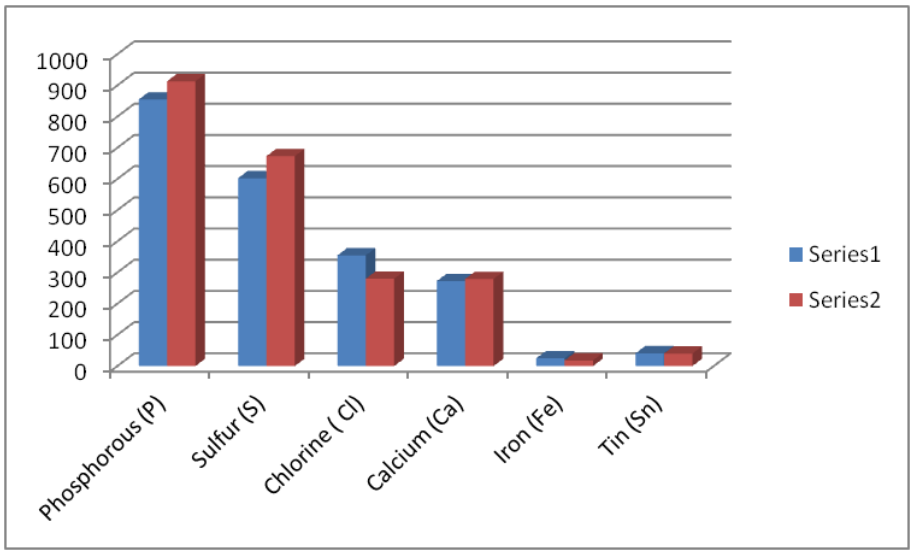

Fig 3 : A graphical representation of heavy metals accumulated by both the fishes

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Assessment of Avifauna Diversity and their Seasonal Fluctuation in an Urban Park, Bhubaneswar, Odisha, India

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Received: 19 Feb 2020 Revised:22 Mar 2020 Accepted: 24 Apr 2020

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ABSTRACT

Shape complexity or habitat fragmentation substantially reduces the availability of suitable habitat for avifauna. As urbanization continues, urban parks are major attraction for avifauna because of diverse microhabitat structure. A preliminary study was carried out in Ekamra Kanan Botanic Garden (20.3039^0 N, 85.8057^0 E), Bhubaneswar, Odisha between November 2016 to October 2017 with major objective to assess avifaunal species diversity, their ecological status, habitat distribution, feeding habit and migrating nature. This study resulted in a total number of 146 species of birds belongs to 56 families and 18 orders. Out of total species, 67 (45.89%) bird species were residing in both forest patches and park and garden. Among total species, 64 (43.83%) species were commonly found, 40 (27.39%) were uncommon and 42 (28.76%) were rarely sighted. Insectivorous birds 45 (30.82%) were dominantly found over omnivorous 42 (28.76%), carnivorous 31 (21.23%), herbivorous 3 (2.05%), grainivorous 13 (8.6%), piscivorous 5 (3.42%), frugivorous 8 (5.47%) and nectorivorous 3 (2.05%). Out of total bird species recorded, 57 species found in all season, 64 species found in S_1 (rainy), 138 species found in S_2 (winter) and 87 species found in S_3 (summer). 52 (35.61%) species are winter migrant, 8 (5.47%) species are summer migrant, 29 (19.86%) species are local migrant and 57 (39.04%) species are resident. Data were analysed through shanon-winer diversity index which showed S_2 had maximum diversity (Hmax=4.9262) followed by S_3 (Hmax=4.465) and then S_1 (Hmax=4.1592). This paper provides some basic information about birds found in this urban park which may helpful for future conservation planning to sustain both migratory and resident avifauna.

Keywords: Avifauna, species, habitat, diversity, urban park

INTRODUCTION

Birds are one of the most beautiful, widely admired, entertaining and most studied group of animal on earth since they are conspicuous and significant component of an ecosystem. Thorough observation of the habitat leads to diverse understanding of avifauna and its relationship with the ecosystem as avifaunal diversity is one of the important ecological indicator of a healthy habitat. Despite their most enviable migrating nature, birds remain threatened by all the environmental evils like pollution, high land conversion rate for urban use and increasing anthropogenic pressure on biodiversity due to rapid growth of human population. But the major factor is continuous natural habitat fragmentation by cutting the trees for commercial use of wood and land which ultimately narrow down the breeding and nesting habitat of avifauna [1]. Thus avifauna may be forced to reside in urban green spaces. Parks and other green spaces present within cityscape have been regarded as protection centre as urbanisation is actively associated with loss, fragmentation and disturbance of natural habitat [2]. In an urban area, ecological functions and the ecosystem services of biodiversity mostly influence by the environment present in and around an urban green space [3] [4]. However During the past decade research on urban biodiversity has become crucial and pivotal - not only because of the increasing impact of urbanization on natural ecosystems, but also because of the growing recognition of urban areas as hosts for innovative ways to conserve and promote biodiversity [5]. Researchers have stated that urban parks, due to their often high levels of habitat diversity and microhabitat heterogeneity, can constitute particularly important hotspots for biodiversity in the cityscape, though their primary role is recreational [6]. Birds are mostly attracted by the green areas in an urban cityscape [7] [8].

Urban woodlands host a large number of resident as well as migratory bird species because they offer higher diversity and higher quality than other urban habitats [8] [9] [10]. The green coverage rate of an urban park has most important ecological and environmental effect on urban climate, though climate plays foremost role in migration of avifauna [11]. With the accelerating conversion of land to human-dominated uses, conservation and management of native bird populations will require a more comprehensive understanding of how urbanization impacts both resident and migrating birds [12]. Moreover recreational activity of human may greatly influence by avifauna diversity of an urban park [12]. Therefore to take proper management decision by urban planners, better knowledge about factors affecting avifauna diversity is much needed.

On the basis of general survey we found that in and around Bhubaneswar area, urban parks and wetlands have potential to attract local as well as migratory bird species. So we select Ekamra Kanan Botanical Garden for the survey because it is adjoining to Chandaka wildlife sanctuary and have a well protected wetland. For scientific management, thorough observation and monitoring the bird population are necessary since their diversity, distribution and activities indicate the environmental quality and ecological functionality. Bird's ecology might be studied directly in terms of its feeding habit, foraging behaviour or population dynamics and important knowledge of habitats can be gleaned from good census studies [14]. Population of avifauna often fluctuates greatly through migration which mostly influenced by seasonal food supply, natural disaster, pollution and habitat destruction. Our study is an attempt to prepare a baseline data on avifaunal species diversity, their ecological status, habitat distribution, feeding habit and migrating nature in Ekamra Kanan campus despite of intense human activity. As proper knowledge about avifauna diversity, their ecological behaviour and distribution is a fundamental tool for their future management and conservation. The study will also help to increase the local awareness towards biodiversity issues and prove to be helpful in conservation efforts.

MATERIALS AND METHODS

Study area

Ekamra Kanan Botanical Garden (20.3039^0 N, 85.8057^0 E) spreading over 500 acres as an urban park adjoining Chandaka wildlife sanctuary has been known for its avifaunal diversity. It is situated west of Bhubaneswar, 2

kilometres away from NH-5. It is a part of Regional Plant Resource Centre (RPRC) established mainly for economic, education, research and conservation of flora and fauna. Dynamic floral diversity along with a protected wetland (small lake) makes this urban park suitable habitat for both migratory as well as resident avifauna.

Methods

A preliminary survey was carried out regularly from November 2016 to October 2017. Birds were observed and counted by using binocular (Olympus, 10*50 X) and a SLR camera (Nikon p900) for later identification and documentation. Field guide books were referred for identification of avifauna up to species level [15] [16] [17]. The present study was based on direct observational methods. Three different methods were considered according to habitat types in order to cover entire study area for observation and counting of avifauna. [1] Transect method - It was adopted for dividing whole area along with area surrounding the lake into small transects. Avifauna were observed and identified while walking on those transects. [2] Point count method - Birds were observed and counted in and around 18 well established observation point or site. [3] Direct observation – Birds were seen directly by ocular method. Census methods used to count birds for seasonal fluctuation analysis.

Key for occurance

Birds were enlisted along with their habitat, abundance, feeding habit, seasonal variation, residential status and protection status according to IUCN and CITES. Habitat types: On the basis of habitat in which they live are categorised as wetland (WL), forest patch (FP), and park and garden (PG). Abundance: On the basis of frequency of sighting, birds were categorised as common (CN), sighted more than 15 times out of 30 visits. Uncommon (UCN), sighted 5-15 times out of 30 visits. And rare (R), sighted less than 5 times out of 30 visits. Feeding habit: Birds were observed at different times of the day to study their feeding habit. A small survey had been conducted to identify and estimate major food components available in study area and on this basis birds were categorised as omnivorous (O), herbivorous (H), carnivorous (C), insectivorous (I), grainivorous (G), frugivorous (F), piscivorous (P), nectorivorous (N). Season types: On the basis of seasonal variation, the period of survey can be classified into three seasons. [S_1] mid-June to mid-October (Rainy), [S_2] mid-October to mid-February (winter) and [S_3] mid-February to mid-June (summer). Residential status: On the basis of migrating nature, birds are categorised as resident (RS), which do not perform any annual or seasonal migration. Winter migratory (WM), which visits only in winter season (S_2). Local migratory (LM), which migrate to other local regions for some period of time. And summer migratory (SM) which visits only during summer season (S_3). Protection status: According to IUCN threatened category: Least Concern (LC), Near Threatened (NT), Vulnerable (VU), Endangered (EN), Critically Endanger (CR), Extinct in Wild (EW), Extinct (EX). And according to Convention on International Trade in Endangered Species of Wild Fauna and Flora: Appendix-I (APP-I), Appendix-II (APP-II), Appendix-III (APP-III), NY-Not yet studied.

Data analysis

The data recorded were analysed by using Shannon–Wiener general diversity index formula; species diversity (H′), maximum diversity (H_{max}), and equitability or evenness (J).

$$H' = -\sum_{i=1}^{s} Pi \log Pi$$

$$H_{max} = \log s \qquad J = H'/ H_{max}$$

Where "s" is the number of species present in the habitat and Pi is the proportion of species in a sample (habitat) of "s" species.

RESULTS AND DISCUSSION

Our study resulted in a total number of 146 species of birds belongs to 56 families and 18 orders (Table-1). Highest number of bird species have been recorded under order Passeriformes (n=73), and family Muscicapidae (n=10). Lowest number of bird recorded under order Podicipediformes, Ciconiiformes and Bucerotiformes both having (n=1) and family Podicipedidae, Ciconiidae, Threskiornithidae, Anhingidae, Phalacrocoracidae, Burhinidae, Jacanidae, Laridae, Caprimulgidae, Apodidae, Upupidae, Tytonidae, Strigidae, Coraciidae, Pittidae, Aegithinidae, Rhipiduridae, Chloropseidae, Ploceidae, Pelloneidae, Timallidae, Liethrichidae, Sylviidae, Zosteropidae and Phylloscopidae having each (n=1) species (figure-2) . Among the total number of species, 36 numbers of birds were recorded from only wetland, 29 birds are recorded from only forest patch and 10 birds were from only park & garden. 4 number of birds found in both wetland and park & garden. 67 numbers of birds are found to be inhibited in both forest patch and park & garden (Figure-3).

It was noted that many species used two or many habitats. Higher population of avifauna in park and forest was due to the regional connectivity between Chandaka forest and park which increases the local migration. The distribution and occurrence of avifauna in different habitat appeared to be associated with the vegetation pattern of the area which is of great significance [18] [11]. Many exotic as well as native plant species maintained for research and conservation purpose play significant role in distribution of avifauna in this urban park. The wetland also hosted higher residential as well as migratory bird population as it can sustain diverse water bird community during both the breeding and winter season and apparently provide functional habitat for a variety of seasonal needs [19]. Among total species, 64 (43.83%) species are commonly found, 40 (27.39%) species are uncommon and 42 (28.76%) are rarely sighted as depicted in figure-4. Out of 146 species observed 45 (30.82%) are Insectivorous, 42 (28.76%) are omnivorous, 31 (21.23%) are carnivorous, 3 (2.05%) are herbivorous, 13 (8.6%) are grainivorous, 5 (3.42%) are piscivorous, 8 (5.47%) are frugivorous, 3 (2.05%) are nectorivorous (Figure-6). Insectivorous birds are help in controlling of some harmful insects such as caterpillars, weevils, beetles, flies etc. RPRC campus was considered to be suitable feeding habitat for most of the bird population as it hosts many fruit and flowering trees like *Syzygium cumini, Diospyros melanoxylon, Ziziphus spp, Carissa spinarum, Trema orientalis* etc. Variety of aquatic weeds, aquatic plants and fish population made the protected lake a favorable feeding habitat for the waterfowls. But siltation, pollution and shrinkage were major threats to water dependant avifauna [20].

Omnivorous birds mostly feed upon crustaceans, mollusks, insects, grains, aquatic weeds etc. Some birds are also feed on grains and seeds as sown in table-1. Seasonal fluctuation of avifauna species is considered as adaptive phenomena to derive maximum benefits from favorable environment conditions [21]. Out of total bird species recorded, 57 species found in all season, 64 species found in S_1 (rainy), 138 species found in S_2 (winter) and 87 species found in S_3 (summer) as depicted in figure-5. The resident birds are frequently observed during the survey period but most of the migratory birds are observed during winter months. The higher occurrence of bird population during winter season suggests that the area provides a favorable condition for foraging, breeding and nesting. Our study reveals that the richness of migratory avifauna during winter along with residential birds in this urban park is due to a distinct seasonal variation pattern. The prior reasons which may affect seasonal variation of avifauna are habitat quality, change in weather condition and fluctuation in food availability [22] [23]. Among total 146 species recorded 52 (35.61%) are winter migrant, 8 (5.47%) species are summer migrant, 29 (19.86%) species are local migrant and 57 (39.04%) species are resident as depicted in figure-7. According to International Union for Conservation of Nature and Natural resources (IUCN) protection status Pale-capped Pigeon (*Columba punicea*) and Indian Skimmer (*Rynchops albicollis*) comes under Vulnerable (VU) category. Black-headed Ibis (*Threskiornis melanocephalus*), Oriental Darter (*Anhinga melanogaster*) and Alexandrine Parakeet (*Psittacula eupatria*) comes under Near Threatened (NT)

category. Remaining all avifauna comes under Least Concern (LC) category [24]. According to Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) Indian Peafowl (*Pavo cristatus*) comes under APP-III, Black-winged Kite (*Elanus caeruleus*), Oriental Honey Buzzard (*Pernis ptilorhynchus*), Crested Serpent Eagle (*Spilornis cheela*), Shikra (*Accipiter badius*), Black Kite (*Milvus migrans*), Common Barn Owl (*Tyto alba*), Spotted Owlet (*Athene brama*) and Alexandrine Parakeet (*Psittacula eupatria*) comes under APP-II [25].

By analyzing the recorded data of table-2, it was found that S2 (winter) showed maximum diversity (H_{max}=4.9262) followed by S_3 (H_{max}=4.465) and then S_1 (H_{max}=4.1592). It was also found that S_3 (summer) showed higher diversity and equitability (H'=3.101, j=0.6945) than other two season due to individual population number were proportionally greater than any other season even winter season recorded higher number of species [26]. Diversity index is dependent on species richness and evenness. And three most important factors which determine species richness is climate, food availability and predation pressure [27].

CONCLUSION

Basic information on bird diversity was unavailable in many forest areas in Bhubaneswar region. Such lacks of information deter the conservation planning. So a preliminary study was carried out to document the occurrence of avifaunal diversity and their relation to this particular urban environment. Therefore our study will be helpful to forest department, environmentalist, researcher and students for study and conservation purpose. Our study resulted in 146 numbers of bird species during study period which is higher than other regions of the cityscape. Due to anthropogenic disturbances like boating in the wetland and recreation of this park for aesthetic purpose causes threat to avifaunal population. Hence proper habitat conservation of this urban park is most important for sustaining the migratory and other resident birds. And the impact of the tourism should be minimized through suitable tourism management planning. Habitat management through increasing patch size, reducing anthropogenic pressure and increasing vegetation complexity through plantation of all season fruit and flowering trees will lead to healthy urban environment and enhancement in avifaunal diversity. Conservation can be obtained through awareness. Education and training is to be imparted to optimize people's enjoyment. However, better targeted research along with monitoring and conservation is needed to understand the impact and threat of urbanization upon avifauna.

ACKNOWLEDGEMENT

For their much valued suggestions, we would like to thank: Dr. Rajesh Kumar Mohapatra, Bibhu Prasad Panda, Asutosh Mallick and Digant Sovan Chand.

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Table-1: Checklist of bird species recorded in Ekamra Kanan Botanic Garden during studied year 2016-2017.

COMMON NAME	SCIENTIFIC NAME	HABITAT	ABUNDANCE	FEEDING HABIT	SEASON	STATUS	PROTECTION STATUS		AVERAGE No. PER SEASON		
							IUCN	CITES	S_1	S_2	S_3
ORDER: 1. Anseriformes, FAMILY: 1. Anatidae											
Lesser Whistling Duck	*Dendrocygna javanica*	WL	CN	H	ALL	RS	LC	NY	3100	4500	700
Northern Pintail	*Anas acuta*	WL	R	H	S_2	WM	LC	NY	0	1	0
Common Teal	*Anas crecca*	WL	R	H	S_2	WM	LC	NY	0	1	0
Cotton Pygmy Goose	*Nettapus coromandelianus*	WL	UCN	O	S_2	WM	LC	NY	0	2	0
ORDER: 2. Galliformes, FAMILY: 2. Phasianidae											
Indian Peafowl	*Pavo cristatus*	FP,PG	CN	O	ALL	RS	LC	APP-III	18	22	16
Grey Francolin	*Francolinus pondicerianus*	FP,PG	UCN	O	ALL	RS	LC	NY	6	11	8
Red Junglefowl	*Gallus gallus*	FP	R	O	ALL	RS	LC	NY	1	3	1
Red Spurfowl	*Galloperdix spadicea*	FP,PG	R	O	ALL	RS	LC	NY	1	2	1
ORDER: 3. Podicipediformes, FAMILY: 3. Podicipedidae											
Little Grebe	*Tachybaptus ruficollis*	WL	CN	C	ALL	RS	LC	NY	2	2	2
ORDER: 4. Columbiformes, FAMILY: 4. Columbidae											
Rock pigeon	*Columba livia*	PG	CN	G	ALL	RS	LC	NY	37	39	46
Pale-capped Pigeon	*Columba punicea*	FP,PG	UCN	F	S_1 & S_2	LM	VU	NY	4	6	0
Oriental Turtle Dove	*Streptopelia orientalis*	FP,PG	R	G	S_2 & S_3	LM	LC	NY	0	2	2
Eurasian Collared Dove	*Streptopelia decaocto*	PG	R	G	S_1 & S_3	LM	LC	NY	1	0	1
Laughing Dove	*Streptopelia senegalensis*	FP,PG	R	G	S_3	SM	LC	NY	0	0	2
Orange-breasted Green Pigeon	*Treron bicinctus*	FP,PG	UCN	F	S_2	LM	LC	NY	0	15	0
Emerald Dove	*Chalcophaps indica*	FP	R	F	S_2 & S_3	LM	LC	NY	0	2	1

COMMON NAME	SCIENTIFIC NAME	HABITAT	ABUNDANCE	FEEDING HABIT	SEASON	STATUS	PROTECTION STATUS		AVERAGE No. PER SEASON		
							IUCN	CITES	S_1	S_2	S_3
Spotted Dove	*Spilopelia chinensis*	FP,PG	CN	G	ALL	RS	LC	NY	32	41	33
ORDER: 5. Caprimulgiformes, FAMILY: 5. Caprimulgidae											
Indian Nightjar	*Caprimulgus asiaticus*	FP	R	I	S_3	SM	LC	NY	0	0	1
ORDER: 5. Caprimulgiformes, FAMILY: 6. Apodidae											
Asian Palm Swift	*Cypsiurus balasiensis*	PG	UCN	I	S_2	WM	LC	NY	0	27	0
ORDER: 6. Cuculiformes, FAMILY: 7. Cuculidae											
Greater Coucal	*Centropus sinensis*	FP,PG	CN	C	ALL	RS	LC	NY	14	19	11
Lesser Coucal	*Centropus bengalensis*	FP,PG	UCN	C	ALL	RS	LC	NY	1	3	2
Blue-faced Malkoha	*Phaenicophaeus viridirostris*	FP	R	I	S_2	WM	LC	NY	0	1	0
Jacobin Cuckoo	*Clamator jacobinus*	FP,PG	R	I	S_1	SM	LC	NY	3	0	0
Common Hawk Cuckoo	*Hierococcyx varius*	FP,PG	CN	I	ALL	RS	LC	NY	5	6	5
Grey-bellied Cuckoo	*Cacomantis passerinus*	FP,PG	UCN	I	S_2 & S_3	SM	LC	NY	0	1	4
Plaintive Cuckoo	*Cacomantis merulinus*	PG	R	I	S_2	WM	LC	NY	0	1	0
Asian koel	*Eudynamys scolopaceus*	FP,PG	CN	O	ALL	RS	LC	NY	19	29	23
ORDER: 7. Gruiformes, FAMILY: 8. Rallidae											
White-breasted Waterhen	*Amaurornis phoenicurus*	WL	CN	O	ALL	RS	LC	NY	8	13	7
Common Moorhen	*Gallinula chloropus*	WL	R	O	S_2	WM	LC	NY	0	1	0
ORDER: 8. Ciconiiformes, FAMILY: 9. Ciconiidae											
Asian Openbill Stork	*Anastomus oscitans*	WL	CN	C	ALL	RS	LC	NY	21	18	13
ORDER: 9. Pelecaniformes, FAMILY: 10. Ardeidae											
Black-crowned Night Heron	*Nycticorax nycticorax*	WL	CN	C	ALL	RS	LC	NY	14	17	13
Striated Heron	*Butorides striata*	WL	R	C	S_2 & S_3	LM	LC	NY	0	1	1
Indian Pond	*Ardeola grayii*	WL	CN	C	ALL	RS	LC	NY	24	31	17

COMMON NAME	SCIENTIFIC NAME	HABITAT	ABUNDANCE	FEEDING HABIT	SEASON	STATUS	PROTECTION STATUS		AVERAGE No. PER SEASON		
							IUCN	CITES	S_1	S_2	S_3
Heron											
Grey Heron	*Ardea cinerea*	WL	R	C	S_2	WM	LC	NY	0	1	0
Purple Heron	*Ardea purpurea*	WL	UCN	C	S_2	WM	LC	NY	0	2	0
Little Egret	*Egretta garzetta*	WL	CN	C	ALL	RS	LC	NY	6	9	7
Cattle Egret	*Bubulcus ibis*	WL,PG	CN	C	ALL	RS	LC	NY	168	176	141
Intermediate Egret	*Ardea intermedia*	WL	UCN	C	S_2 & S_3	LM	LC	NY	0	4	3
Great Egret	*Ardea alba*	WL	R	C	S_2 & S_3	LM	LC	NY	0	2	1
ORDER: 9. Pelecaniformes, FAMILY: 11. Threskiornithidae											
Black-headed Ibis	*Threskiornis melanocephalus*	WL	R	O	S_2	WM	NT	NY	0	4	0
ORDER: 10. Suliformes, FAMILY: 12. Phalacrocoracidae											
Little Cormorant	*Microcarbo niger*	WL	CN	P	ALL	RS	LC	NY	11	14	14
ORDER: 10. Suliformes, FAMILY: 13. Anhingidae											
Oriental Darter	*Anhinga melanogaster*	WL	CN	P	ALL	RS	NT	NY	2	2	2
ORDER: 11. Charadriiformes, FAMILY: 14. Burhinidae											
Thick-knee/Stone Curlew	*Burhinus oedicnemus*	PG	R	C	S_3	SM	LC	NY	0	0	1
ORDER: 11. Charadriiformes, FAMILY: 15. Charadriidae											
Little Ringed Plover	*Charadrius dubius*	WL	UCN	C	S_2 & S_3	LM	LC	NY	0	4	6
Yellow-wattled Lapwing	*Vanellus malabaricus*	WL	CN	C	S_2 & S_3	LM	LC	NY	0	13	7
Red-wattled Lapwing	*Vanellus indicus*	WL	CN	C	ALL	RS	LC	NY	11	14	19
ORDER: 11. Charadriiformes, FAMILY: 16. Jacanidae											
Bronze-winged Jacana	*Metopidius indicus*	WL	CN	O	ALL	RS	LC	NY	16	21	9
ORDER: 11. Charadriiformes, FAMILY: 17. Scolopacidae											
Wood Sandpiper	*Tringa glareola*	WL	UCN	C	S_2	WM	LC	NY	0	9	0
Common Sandpiper	*Actitis hypoleucos*	WL	R	C	S_2	WM	LC	NY	0	3	0

COMMON NAME	SCIENTIFIC NAME	HABITAT	ABUNDANCE	FEEDING HABIT	SEASON	STATUS	PROTECTION STATUS		AVERAGE No. PER SEASON		
							IUCN	CITES	S_1	S_2	S_3
Green Sandpiper	*Tringa ochropus*	WL	UCN	C	S_2	WM	LC	NY	0	6	0
ORDER: 11. Charadriiformes, FAMILY: 18. Laridae											
Indian Skimmer	*Rynchops albicollis*	WL	R	P	S_2	WM	VU	NY	0	1	0
ORDER: 12. Accipitriformes, FAMILY: 19. Accipitridae											
Black-winged Kite	*Elanus caeruleus*	FP,PG	UCN	C	S_2 & S_3	LM	LC	APP-II	0	3	3
Oriental Honey Buzzard	*Pernis ptilorhynchus*	FP	R	O	S_3	SM	LC	APP-II	0	0	1
Crested Serpent Eagle	*Spilornis cheela*	FP	UCN	C	S_2 & S_3	LM	LC	APP-II	0	1	1
Shikra	*Accipiter badius*	FP,PG	CN	C	ALL	RS	LC	APP-II	6	5	7
Black Kite	*Milvus migrans*	FP,PG	CN	C	ALL	RS	LC	APP-II	12	18	19
ORDER: 13. Strigiformes, FAMILY: 20. Tytonidae											
Common Barn Owl	*Tyto alba*	PG	UCN	C	ALL	RS	LC	APP-II	1	2	1
ORDER: 13. Strigiformes, FAMILY: 21.Strigidae											
Spotted Owlet	*Athene brama*	FP,PG	CN	C	ALL	RS	LC	APP-II	2	5	2
ORDER: 14. Bucerotiformes, FAMILY: 22. Upupidae											
Common Hoopoe	*Upupa epops*	FP,PG	UCN	I	S_2	WM	LC	NY	0	8	0
ORDER: 15. Piciformes, FAMILY: 23. Picidae											
Black-rumped Woodpecker	*Dinopium benghalense*	FP,PG	CN	I	ALL	RS	LC	NY	6	7	5
Rufous Woodpecker	*Micropternus brachyurus*	FP,PG	R	C	S_2 & S_3	LM	LC	NY	0	2	1
ORDER: 15. Piciformes, FAMILY: 24. Megalaimidae											
Brown-headed Barbet	*Psilopogon zeylanicus*	FP,PG	CN	O	ALL	RS	LC	NY	24	35	33
Coppersmith Barbet	*Psilopogon haemacephalus*	FP,PG	CN	O	ALL	RS	LC	NY	13	19	15
ORDER: 16. Coraciiformes, FAMILY: 25. Meropidae											
Green Bee-eater	*Merops orientalis*	FP,PG	CN	I	ALL	RS	LC	NY	26	37	22
Chestnut-headed Bee-eater	*Merops leschenaulti*	FP,PG	CN	I	ALL	RS	LC	NY	16	18	12

COMMON NAME	SCIENTIFIC NAME	HABITAT	ABUNDANCE	FEEDING HABIT	SEASON	STATUS	PROTECTION STATUS		AVERAGE No. PER SEASON		
							IUCN	CITES	S_1	S_2	S_3
Blue-tailed Bee-eater	*Merops philippinus*	WL,PG	CN	I	S_2 & S_3	LM	LC	NY	0	8	14
ORDER: 16. Coraciiformes, FAMILY: 26. Coraciidae											
Indian Roller	*Coracias benghalensis*	FP,PG	UCN	C	ALL	RS	LC	NY	3	5	5
ORDER: 16. Coraciiformes, FAMILY: 27. Alcedinidae											
Common Kingfisher	*Alcedo atthis*	WL	UCN	P	S_1 & S_2	LM	LC	NY	3	5	0
Pied Kingfisher	*Ceryle rudis*	WL	UCN	P	S_1 & S_2	LM	LC	NY	2	2	0
White-throated Kingfisher	*Halcyon smyrnensis*	WL,PG	CN	C	ALL	RS	LC	NY	15	18	17
ORDER: 17. Psittaciformes, FAMILY: 28. Psittacidae											
Plum-headed Parakeet	*Psittacula cyanocephala*	FP	R	F	S_2	LM	LC	NY	0	6	0
Alexandrine Parakeet	*Psittacula eupatria*	FP	UCN	F	S_2 & S_3	LM	NT	APP-II	0	6	3
Rose-ringed Parakeet	*Psittacula krameri*	FP,PG	CN	F	ALL	RS	LC	NY	19	24	18
ORDER: 18. Passeriformes, FAMILY: 29. Pittidae											
Indian Pitta	*Pitta brachyura*	FP	R	I	S_3	SM	LC	NY	0	0	1
ORDER: 18. Passeriformes, FAMILY: 30. Campephagidae											
Small Minivet	*Pericrocotus cinnamomeus*	FP	UCN	I	S_2	WM	LC	NY	0	3	0
Large Cuckooshrike	*Coracina javensis*	FP,PG	R	O	S_2	WM	LC	NY	0	1	0
Black-winged Cuckooshrike	*Lalage melaschistos*	PG	R	O	S_2	WM	LC	NY	0	1	0
Black-headed Cuckooshrike	*Lalage melanoptera*	FP,PG	R	O	S_2	WM	LC	NY	0	2	0
ORDER: 18. Passeriformes, FAMILY: 31. Oriolidae											
Black-hooded Oriole	*Oriolus xanthornus*	FP,PG	CN	O	ALL	RS	LC	NY	7	8	11
Indian Golden Oriole	*Oriolus kundoo*	FP,PG	CN	O	S_2	WM	LC	NY	0	11	0
Black-naped Oriole	*Oriolus chinensis*	FP,PG	CN	O	S_2	WM	LC	NY	0	2	0

COMMON NAME	SCIENTIFIC NAME	HABITAT	ABUNDANCE	FEEDING HABIT	SEASON	STATUS	PROTECTION STATUS		AVERAGE No. PER SEASON		
							IUCN	CITES	S_1	S_2	S_3
ORDER: 18. Passeriformes, FAMILY: 32. Aegithinidae											
Common Iora	*Aegithina tiphia*	FP,PG	UCN	I	S_2 & S_3	LM	LC	NY	0	3	5
ORDER: 18. Passeriformes, FAMILY: 33. Dicruridae											
Black Drongo	*Dicrurus macrocercus*	FP,PG	CN	I	ALL	RS	LC	NY	37	42	38
Ashy Drongo	*Dicrurus leucophaeus*	FP	UCN	I	S_2	WM	LC	NY	0	5	0
White-bellied Drongo	*Dicrurus caerulescens*	FP	R	O	S_2	WM	LC	NY	0	2	0
Spangled Drongo	*Dicrurus hottentottus*	FP	UCN	O	S_2	WM	LC	NY	0	8	0
ORDER: 18. Passeriformes, FAMILY: 34. Rhipiduridae											
White-throated Fantail	*Rhipidura albicollis*	FP	UCN	I	S_2 & S_3	LM	LC	NY	0	2	7
ORDER: 18. Passeriformes, FAMILY: 35. Laniidae											
Brown Shrike	*Lanius cristatus*	FP,PG	CN	C	S_2	WM	LC	NY	0	2	0
Long-tailed Shrike	*Lanius schach*	FP,PG	UCN	C	S_2	WM	LC	NY	0	1	0
ORDER: 18. Passeriformes, FAMILY: 36. Corvidae											
Rufous Treepie	*Dendrocitta vagabunda*	FP,PG	CN	O	ALL	RS	LC	NY	15	27	21
House Crow	*Corvus splendens*	FP,PG	CN	O	ALL	RS	LC	NY	36	38	33
jungle crow	*Corvus macrorhynchos*	FP,PG	CN	O	ALL	RS	LC	NY	23	27	31
ORDER: 18. Passeriformes, FAMILY: 37. Monarchidae											
Black-naped Monarch	*Hypothymis azurea*	FP,PG	UCN	I	S_2	WM	LC	NY	0	3	0
Indian Paradise-flycatcher	*Terpsiphone paradisi*	FP,PG	CN	I	S_2 & S_3	LM	LC	NY	0	2	5
ORDER: 18. Passeriformes, FAMILY: 38. Dicaeidae											
Thick-billed Flowerpecker	*Dicaeum agile*	FP	R	F	S_2 & S_3	LM	LC	NY	0	3	2
Pale-billed Flowerpecker	*Dicaeum erythrorhynchos*	FP	R	F	S_2 & S_3	LM	LC	NY	0	2	1

COMMON NAME	SCIENTIFIC NAME	HABITAT	ABUNDANCE	FEEDING HABIT	SEASON	STATUS	PROTECTION STATUS		AVERAGE No. PER SEASON		
							IUCN	CITES	S_1	S_2	S_3
ORDER: 18. Passeriformes, FAMILY: 39. Nectariniidae											
Purple-rumped Sunbird	*Leptocoma zeylonica*	FP,PG	CN	N	ALL	RS	LC	NY	34	53	39
Purple Sunbird	*Cinnyris asiaticus*	FP,PG	CN	N	ALL	RS	LC	NY	8	13	4
Loten's Sunbird	*Cinnyris lotenius*	PG	R	N	S_2	WM	LC	NY	0	2	0
ORDER: 18. Passeriformes, FAMILY: 40. Chloropseidae											
Jerdon's Leafbird	*Chloropsis jerdoni*	FP	UCN	O	S_2 & S_3	LM	LC	NY	0	8	6
ORDER: 18. Passeriformes, FAMILY: 41. Ploceidae											
Baya Weaver	*Ploceus philippinus*	FP	CN	G	ALL	RS	LC	NY	18	16	26
ORDER: 18. Passeriformes, FAMILY: 42. Estrildidae											
Indian Silverbill	*Euodice malabarica*	FP	R	G	S_3	SM	LC	NY	0	0	8
White-rumped Munia	*Lonchura striata*	FP	R	G	S_2 & S_3	LM	LC	NY	0	1	2
Scaly-breasted Munia	*Lonchura punctulata*	FP,PG	CN	G	ALL	RS	LC	NY	18	22	28
ORDER: 18. Passeriformes, FAMILY: 43. Motacillidae											
Forest wagtail	*Dendronanthus indicus*	FP,PG	R	I	S_2 & S_3	LM	LC	NY	0	2	2
Olive-backed Pipit	*Anthus hodgsoni*	FP	R	I	S_2	WM	LC	NY	0	7	0
Yellow Wagtail	*Motacilla flava*	WL	CN	I	S_2	WM	LC	NY	0	11	0
Grey Wagtail	*Motacilla cinerea*	WL	UCN	I	S_2	WM	LC	NY	0	5	0
Citrine Wagtail	*Motacilla citreola*	WL	UCN	I	S_2	WM	LC	NY	0	9	0
White-browed Wagtail	*Motacilla maderaspatensis*	WL	CN	I	ALL	RS	LC	NY	2	5	2
White Wagtail	*Motacilla alba*	WL,PG	UCN	I	S_2	WM	LC	NY	0	7	0
ORDER: 18. Passeriformes, FAMILY: 44. Cisticolidae											
Grey-breasted Prinia	*Prinia hodgsonii*	FP,PG	UCN	I	S_2	WM	LC	NY	0	2	0
Ashy Prinia	*Prinia socialis*	FP,PG	UCN	I	S_2 & S_3	LM	LC	NY	0	9	4

COMMON NAME	SCIENTIFIC NAME	HABITAT	ABUNDANCE	FEEDING HABIT	SEASON	STATUS	PROTECTION STATUS		AVERAGE No. PER SEASON		
							IUCN	CITES	S_1	S_2	S_3
Common Tailorbird	*Orthotomus sutorius*	FP,PG	CN	I	ALL	RS	LC	NY	16	21	29
ORDER: 18. Passeriformes, FAMILY: 45. Acrocephalidae											
Clamorous Reed Warbler	*Acrocephalus stentoreus*	WL	UCN	I	S_1 & S_2	LM	LC	NY	1	1	0
Blyth's Reed Warbler	*Acrocephalus dumetorum*	FP	R	I	S_2	WM	LC	NY	0	2	0
ORDER: 18. Passeriformes, FAMILY: 46. Hirundinidae											
Barn Swallow	*Hirundo rustica*	WL	CN	I	S_2	WM	LC	NY	0	78	0
Wire-tailed Swallow	*Hirundo smithii*	WL	R	I	S_2	WM	LC	NY	0	2	0
Red-rumped Swallow	*Cecropis daurica*	FP,PG	CN	I	S_2	WM	LC	NY	0	21	0
ORDER: 18. Passeriformes, FAMILY: 47. Pycnonotidae											
Red-whiskered Bulbul	*Pycnonotus jocosus*	FP,PG	CN	O	ALL	RS	LC	NY	29	37	31
Red-vented Bulbul	*Pycnonotus cafer*	FP,PG	CN	O	ALL	RS	LC	NY	34	45	39
White-browed Bulbul	*Pycnonotus luteolus*	FP,PG	CN	O	ALL	RS	LC	NY	21	27	18
ORDER: 18. Passeriformes, FAMILY: 48.Sylviidae											
Yellow-eyed Babbler	*Chrysomma sinense*	FP,PG	UCN	O	S_2	WM	LC	NY	0	4	0
ORDER: 18. Passeriformes, FAMILY: 49. Zosteropidae											
Oriental White-eye	*Zosterops palpebrosus*	FP	UCN	O	ALL	RS	LC	NY	2	5	4
ORDER: 18. Passeriformes, FAMILY: 50. Timaliidae											
Tawny-bellied Babbler	*Dumetia hyperythra*	FP	R	O	S_2	WM	LC	NY	0	2	0
ORDER: 18. Passeriformes, FAMILY: 51. Pellorneidae											
Puff-throated Babbler	*Pellorneum ruficeps*	FP	CN	O	ALL	RS	LC	NY	13	16	18
ORDER: 18. Passeriformes, FAMILY: 52. Leiothrichidae											
Jungle Babbler	*Turdoides striata*	FP,PG	CN	O	ALL	RS	LC	NY	39	47	33

COMMON NAME	SCIENTIFIC NAME	HABITAT	ABUNDANCE	FEEDING HABIT	SEASON	STATUS	PROTECTION STATUS		AVERAGE No. PER SEASON		
							IUCN	CITES	S_1	S_2	S_3
ORDER: 18. Passeriformes, FAMILY: 53. Sturnidae											
Rosy Starling	*Pastor roseus*	FP,PG	R	O	S_2	WM	LC	NY	0	39	0
Asian Pied Starling	*Gracupica contra*	FP,PG	CN	O	ALL	RS	LC	NY	43	49	38
Brahminy Starling	*Sturnia pagodarum*	FP,PG	UCN	O	S_1 & S_2	LM	LC	NY	1	2	0
Chestnut-tailed Starling	*Sturnia malabarica*	FP,PG	CN	O	ALL	RS	LC	NY	24	28	32
Common Myna	*Acridotheres tristis*	FP,PG	CN	O	ALL	RS	LC	NY	38	44	52
Jungle Myna	*Acridotheres fuscus*	FP,PG	CN	O	ALL	RS	LC	NY	14	19	11
ORDER: 18. Passeriformes, FAMILY: 54. Muscicapidae											
Indian Robin	*Saxicoloides fulicatus*	FP	UCN	I	S_2	WM	LC	NY	0	2	0
Oriental Magpie Robin	*Copsychus saularis*	FP,PG	CN	I	ALL	RS	LC	NY	18	23	16
White-rumped Shama	*Kittacincla malabarica*	FP	CN	I	S_2	WM	LC	NY	0	6	0
Asian Brown Flycatcher	*Muscicapa dauurica*	FP,PG	CN	I	S_2	WM	LC	NY	0	7	0
Tickell's Blue Flycatcher	*Cyornis tickelliae*	FP,PG	UCN	I	S_2	WM	LC	NY	0	1	0
Blue-throated Blue Flycatcher	*Cyornis rubeculoides*	PG	R	I	S_2 & S_3	WM	LC	NY	0	1	1
Asian Verditer Flycatcher	*Eumyias thalassinus*	FP,PG	UCN	I	S_2	WM	LC	NY	0	11	0
Red-breasted Flycatcher	*Ficedula parva*	FP,PG	CN	I	S_2	WM	LC	NY	0	6	0
Taiga Flycatcher	*Ficedula albicilla*	FP,PG	CN	I	S_2	WM	LC	NY	0	7	0
Black Redstart	*Phoenicurus ochruros*	PG	R	I	S_2	WM	LC	NY	0	2	0
ORDER: 18. Passeriformes, FAMILY: 55. Turdidae											
Orange-headed Thrush	*Geokichla citrina*	FP,PG	CN	O	ALL	RS	LC	NY	6	8	9

COMMON NAME	SCIENTIFIC NAME	HABITAT	ABUNDANCE	FEEDING HABIT	SEASON	STATUS	PROTECTION STATUS		AVERAGE No. PER SEASON		
							IUCN	CITES	S_1	S_2	S_3
Scaly Thrush	*Zoothera dauma*	FP	R	O	S_2	WM	LC	NY	0	1	0
ORDER: 18. Passeriformes, FAMILY: 56. Phylloscopidae											
Greenish Leaf Warbler	*Seicercus trochiloides*	FP	CN	O	S_2	WM	LC	NY	0	18	0

Table 2: Comparison of the shanon-winer index for different seasons of survey period:

	S_1	S_2	S_3
H	1.499	1.803	3.101
H_{max}	4.1592	4.9262	4.465
J	0.3604	0.366	0.6945

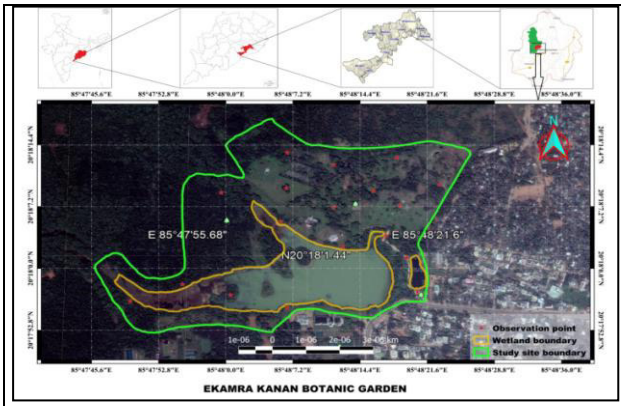


Figure 1: Geographical location of the study site; Ekamra Kanan Botanic Garden, Bhubaneswar, Odisha, India.

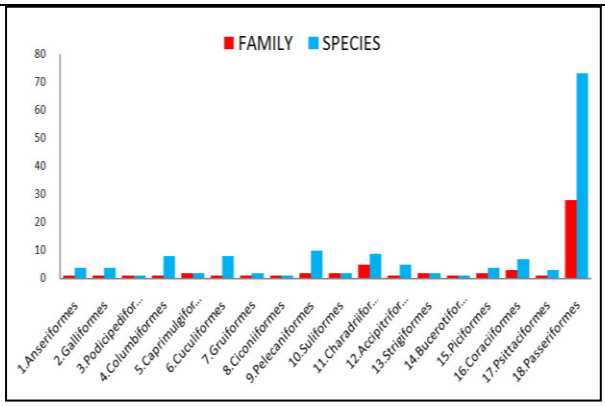


Fig. 2: Occurance of family and species per order during studied year

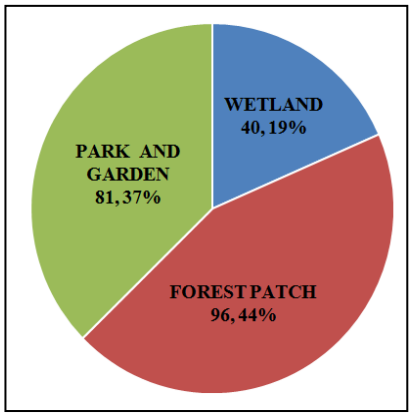


Fig. 3: Habitat distribution of avifauna during studied year

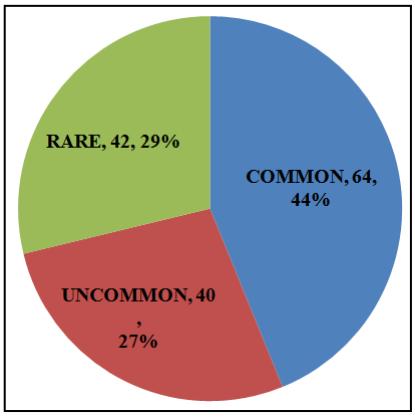


Fig. 4: Abundance status or frequency of sighting of birds during study year.

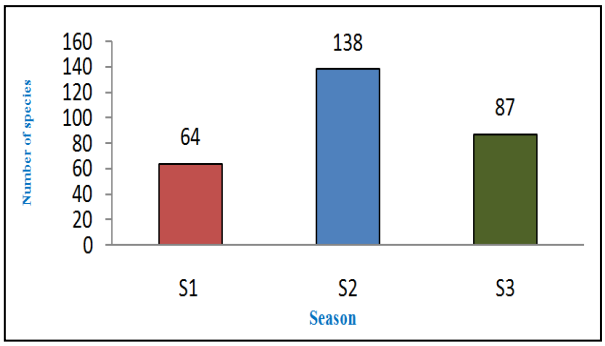


Fig. 5: Seasonal variation of bird species during studied year.

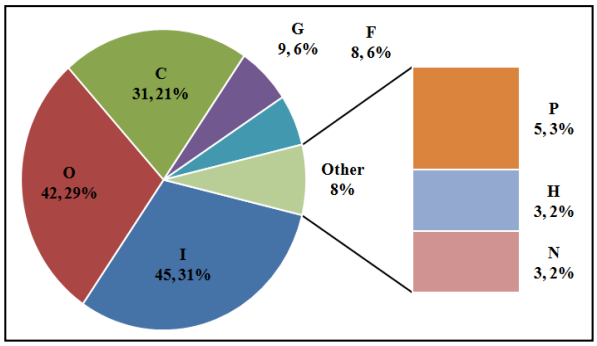


Fig. 6: Feeding habit of avifauna population during studied year

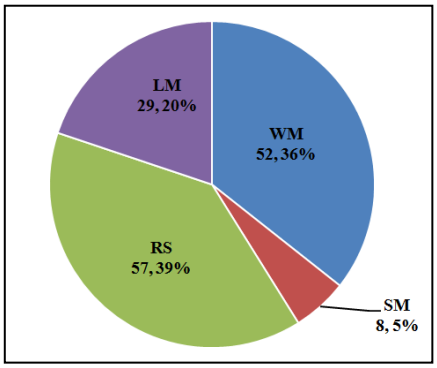
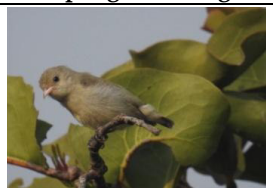
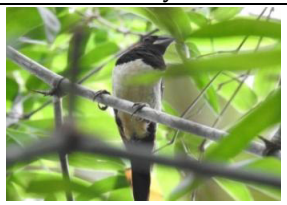
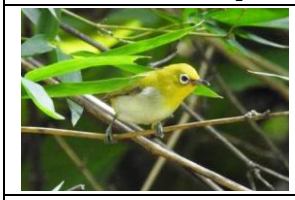
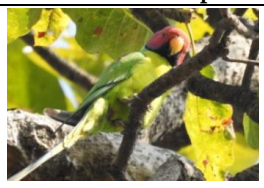
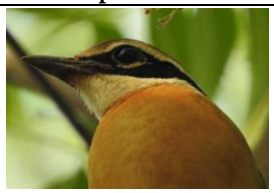
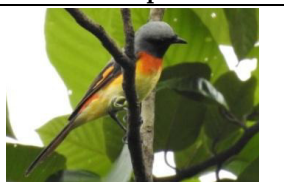


Fig. 7: Migration pattern of avifauna during studied year

Plate 1: Image of few birds recorded during study period (2016-2017) in Ekamra Kanan Botanic Garden, Bhubaneswar, Odisha, India.

			
Lesser Whistling duck	**Common Teal**	**Pale-capped Pigeon**	**Oriental Turtle Dove**
			
Orange B. Green Pigeon	**Indian Nightjar**	**Blue-Faced Malkoha**	**Jacobin Cuckoo**

			
Oriental Darter	**Crested Serpent Eagle**	**White Ibis**	**Indian Skimmer**
			
White Bellied Drongo	**Spangled Drongo**	**White-Throated Fantail**	**Indian P. Flycatcher**
			
Thick Billed Flower pecker	**Pale B. Flower pecer**	**Purple Sunbird**	**White Rumped Munia**
			
Oriental White Eye	**Plum-Headed Parakeet**	**Indian Pitta**	**Small Minivet**
			
Large Cuckooshrike	**Black H. Cuckooshrike**	**Black Redstart**	**Brown Shrike**

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Implementation of Mealy and Moore State Machine using Sequence Detector in VHDL Environment

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Received: 16 Feb 2020 Revised:20 Mar 2020 Accepted: 24 Apr 2020

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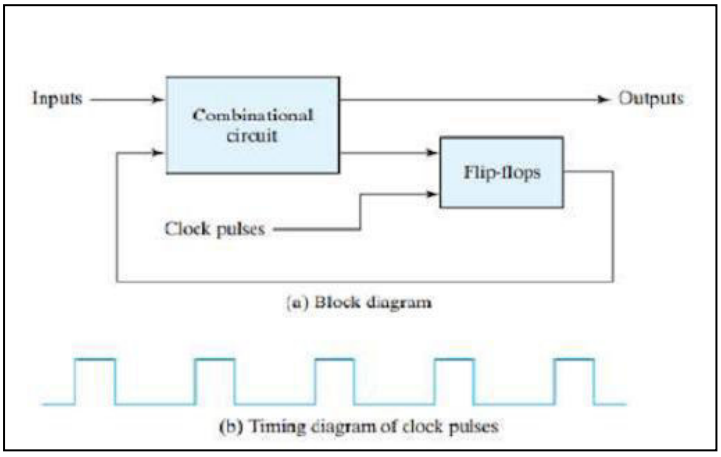
ABSTRACT

Field Programmable Gate Array (FPGA) provides Excellent performance capacity and system integration while optimizing to enhance FPGA devices based on CAD tools in the very high speed integrated circuit Hardware Description Language (VHDL), which demonstrate the logic, function and behaviours of system hardware. State machines are used mostly as the backbone of FPGA. By selecting the Appropriate architecture and implementation methods will ensure that an optimal solution can be obtained. For a designer, the best way to identify these works and sequences is to use a state machine. State machines are logical constructs that allows transition among a finite number of states. It will however, move between states depending upon a number of stimulus applied. This paper mainly focuses on the design of Moore and Mealy state machine in FPGA. RTL logic has been used for designing purposes. The minimum time period, less number of flip flops and registers etc. have been taken into consideration to design the proposed Moore and Mealy state machines more effectively.

Keywords: Finite state machine, Moore state machine, Mealy state machine, State transition, Sequential systems, Time delay, Test bench, Xilinx ISE simulator.

INTRODUCTION

This paper introduces the concept of two types of Finite State Machines (FSMs); Mealy and Moore, and the modelling designs to develop such machines. FSM are sequential circuit used in many digital systems to control the behaviour of systems and dataflow paths. Sequential circuits are realized using combinational logic and one or more clocked memory elements. The general structure of such a circuit is shown in Fig. 1



The circuit has primary inputs, and produces outputs. The values of the outputs of the flip-flops are referred to as the state of the circuit. Under control of the clock signal, the flip-flop outputs change their state which is determined by the combinational logic that feeds the inputs of these flip-flops. Thus the circuit moves from one state to another. To ensure that only single transition takes place during one clock cycle, the flip-flops are to be of the edge-triggered type. The sequence detector is considered which output is equal to one when the sequence. The output is not determined only by the present state of input, so it exists different states in the sequential circuit. Sequential circuits are also called finite state machines (FSMs) because the functionality of the circuits can be represented using a finite number of states. There are two basic ways to design clocked sequential circuits. They are: Moore machine and Mealy machines. This paper consists of three parts: one for designs of state diagrams, state tables, logic expression and logic diagrams for Mealy and Moore state machines, two for programming of these designs using VHDL codes and other for verifying the processes by using Xilinx ISE tool.

State Machine

Different memory elements such as Latches as well as latches with control signals, Flip-flops, Registers are used to hold the running state of the machine. The state of the machine can be used to perform sequential operations.synchronous sequential circuits change affect their states for every positive or negative or negative transition of the clock signal based on the input. So, this behavior of synchronous sequential circuits can be represented in the graphical form and it is known as state diagram.

A synchronous sequential circuit is also called as Finite State Machine (FSM), if it has finite number of states. There are two types of FSMs.

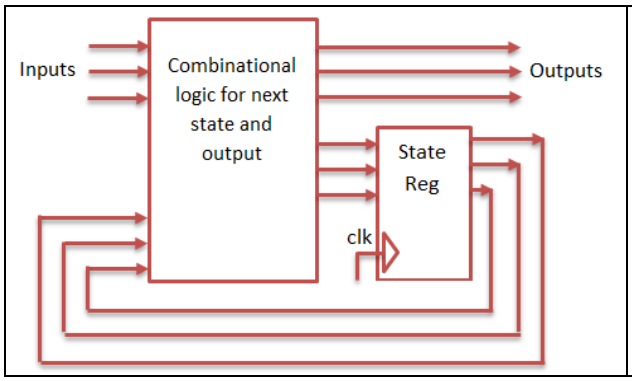
- Mealy State Machine
- Moore State Machine

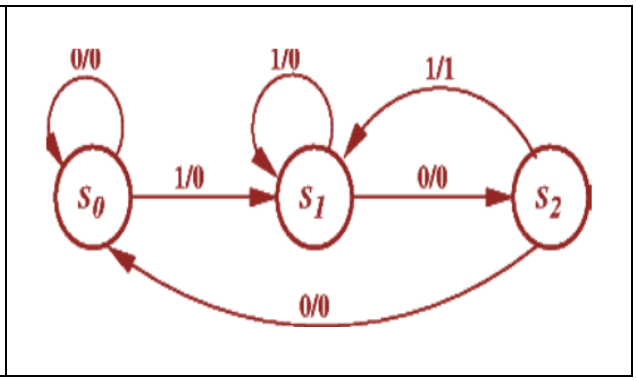
Mealy state machine

A mealy state machine is one in which the output changes on the translations of the device. In other the output of the next state are determined by the current state and the current inputs. This type of state machine is in contrast to the Moore state machine in which each value of an output is associated with a specific current state.in a mealy machine, the during a particular state can be different at different times, depending on how the machine entered the current state.

In Mealy state diagram the output of the system is set to 1,whenever the system is in the state 'zero' and the value of the input signal level is 1 i.s. the output depends upon both the state and the input.

The output of the state machine depends on both present state and current input. when the input changes, the output of the state machine updated without waiting for change in clock input.
Construction of the sequence detector for the sequence 101 using mealy state machine which is given below.
Mealy state machine require only three states st0,st1,st2 to detect the 101 sequence.

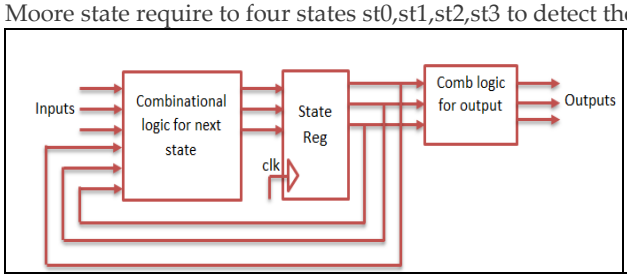


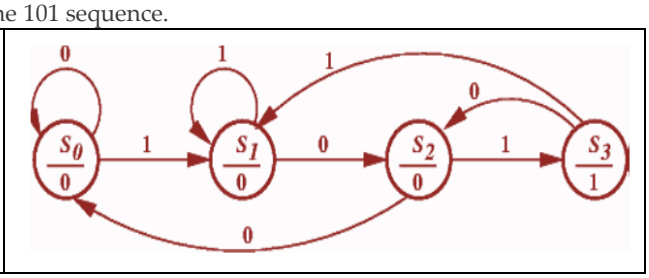


Moore state Machine

Moore state machine is a reading and writing memory device. The state machine begins in the IDLE state after a reset. It remains in the IDLE state until it receives a read or a write signal. It goes to the write state when receiving a write signal, assigning the write enable and the acknowledged signal for one cycle. When the write operation is completed in a single cycle then it backs to the IDLE state. It goes to the READ1 state when receiving a read signal, assigning the output enable. It waits for the ready signal from the memory device, signalling that valid data is being output. If the ready signal is not asserted, the machine stays in the READ state.

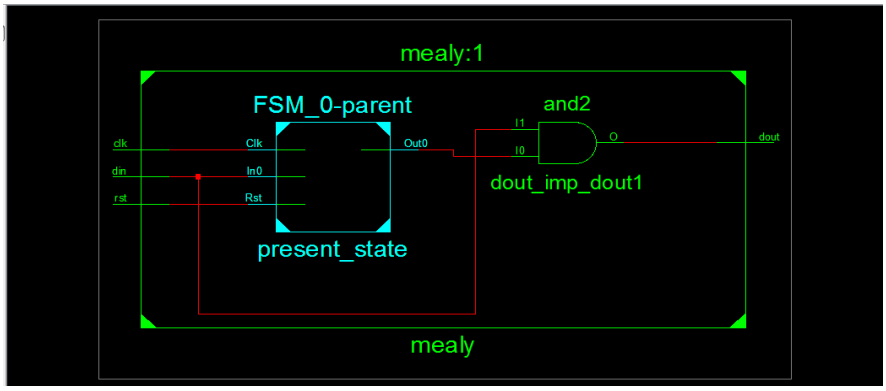
The Output of the State machine depends only on present state. The output of state machine are only updated at the clock edge.
Construction of the sequence detector for the sequence 101 using moore state machine which is given below.
Moore state require to four states st0,st1,st2,st3 to detect the 101 sequence.



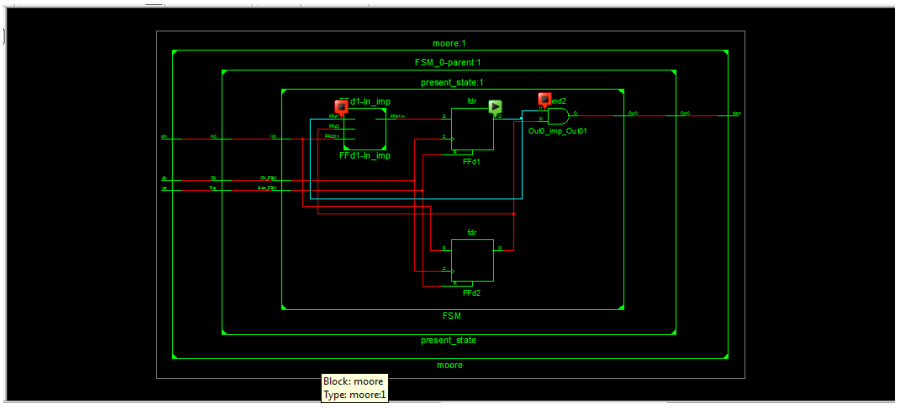


EXPERIMENTAL RESULTS

RTL Schematic of Mealy state machine using sequence detector

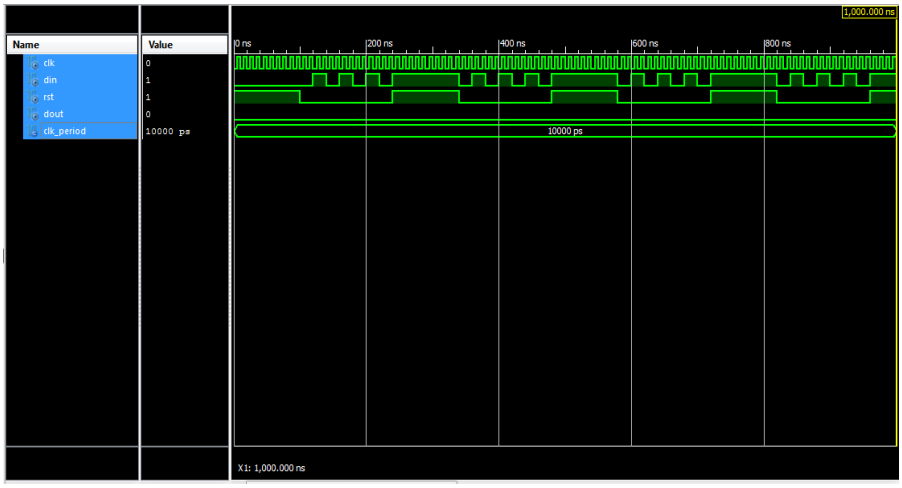


RTL Schematic of Moore state machine using sequence detector

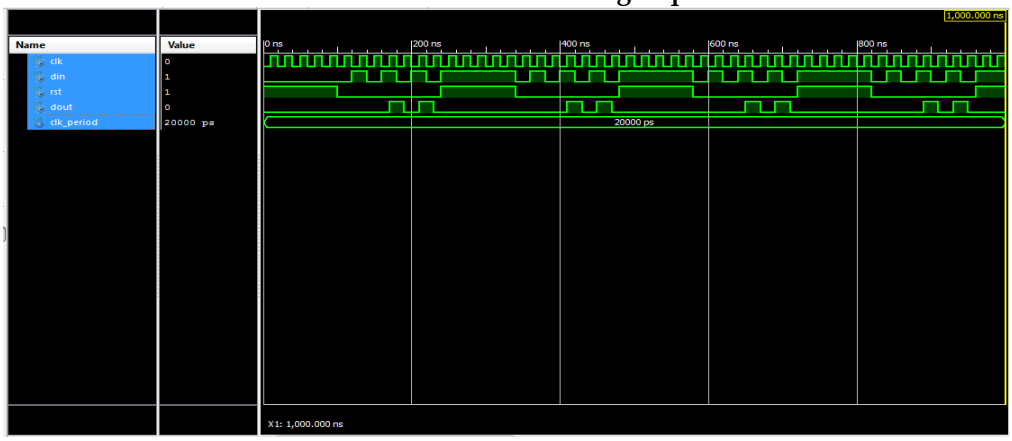


SIMULATION RESULTS

Test bench waveform of Mealy state machine using sequence detector



Test bench waveform of Moore state machine using sequence detector



Device utilization summary of mealy state machine using sequence detector:

mealy Project Status (04/21/2020 - 16:24:45)			
Project File:	mealyfinal1.xise	**Parser Errors:**	No Errors
Module Name:	mealy	**Implementation State:**	Placed and Routed
Target Device:	xc3s100e-5cp132	**•Errors:**	No Errors
Product Version:	ISE 14.4	**•Warnings:**	No Warnings
Design Goal:	Balanced	**•Routing Results:**	All Signals Completely Routed
Design Strategy:	Xilinx Default (unlocked)	**•Timing Constraints:**	All Constraints Met
Environment:	System Settings	**•Final Timing Score:**	0 (Timing Report)

Device Utilization Summary [-]				
Logic Utilization	**Used**	**Available**	**Utilization**	**Note(s)**
Number of Slice Flip Flops	2	1,920	1%	
Number of 4 input LUTs	2	1,920	1%	
Number of occupied Slices	1	960	1%	
Number of Slices containing only related logic	1	1	100%	
Number of Slices containing unrelated logic	0	1	0%	
Total Number of 4 input LUTs	2	1,920	1%	
Number of bonded IOBs	4	83	4%	
Number of BUFGMUXs	1	24	4%	
Average Fanout of Non-Clock Nets	1.60			

Device utilization summary of moore state machine using sequence detector

moore Project Status			
Project File:	moorefinal.xise	**Parser Errors:**	No Errors
Module Name:	moore	**Implementation State:**	Synthesized
Target Device:	xc3s100e-5cp132	**•Errors:**	No Errors
Product Version:	ISE 14.4	**•Warnings:**	No Warnings
Design Goal:	Balanced	**•Routing Results:**	
Design Strategy:	Xilinx Default (unlocked)	**•Timing Constraints:**	
Environment:	System Settings	**•Final Timing Score:**	

Device Utilization Summary (estimated values) [-]			
Logic Utilization	**Used**	**Available**	**Utilization**
Number of Slices	1	960	0%
Number of Slice Flip Flops	2	1920	0%
Number of 4 input LUTs	2	1920	0%
Number of bonded IOBs	4	83	4%
Number of GCLKs	1	24	4%

CONCLUSION

In this paper using VHDL language Mealy and Moore sequence detector for a particular sequence is designed and simulated using Xilinx ISE 14.4 . It presents the relationship between FSM and VHDL code with models.A Moore machine produces a unique output for every state irrespective of inputs. Accordingly the state diagram of the Moore machine associates the output with the state in the form state-notation/output-value. Since a Mealy machine associates outputs with transitions, an output sequence can be generated in fewer states using Mealy machine. A Moore FSM has a simplified design at the cost of requiring more states. The Moore FSM has outputs that change synchronously with the clock. Often a Mealy FSM requires fewer states. That can reduce the number of bits requires to store the state number. It is possible to design either a Moore or a Mealy FSM to exhibit identical functionality.
In further extension, the circuit can be analysed in terms of Power(both static and dynamic) as well as the timing analysis can also be estimated using FPGA development board.

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

The Relationship Between Workplace Spirituality and Organizational Citizenship Behaviour: An Empirical Investigation

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Received: 19 Feb 2020 Revised:21 Mar 2020 Accepted: 24 Apr 2020

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ABSTRACT

The purpose of the paper is to understand the relation between workplace spirituality and organizational citizenship behaviour among various working professionals and also to check the mediating role of organizational commitment on that relationship. An internet-based survey has been designed to collect data from employees working in different private manufacturing and service based industries operating in India. The relationships are tested by using Partial Least Squares Structural Equation Modelling (PLS-SEM) and for mediation Preacher and Hayes (2008) procedure has been used. The PLS-SEM and Preacher and Hayes (2008) mediation results reveals that workplace spirituality has a substantial effect on organizational citizenship behaviour. There is also a substantial effect of workplace spirituality on organizational commitment and organizational commitment on organizational citizenship behaviour. Organizational commitment is found to be mediating fully between workplace spirituality and organizational citizenship behaviour.

Keywords: Workplace Spirituality, Organizational Commitment, Organizational Citizenship Behaviour, Employees, PLS-SEM

INTRODUCTION

Workplace spirituality has become one of the emergent research domains in organizational behaviour, which intrigues many organizational researchers to explore spirituality as a field of inquiry (Duchon&Plowman, 2005; Fry, 2003). According to Giacalone & Jurkiewicz (2003, p.13), "It is a frame work of organizational values evidence in the

culture that promotes employees' experience of transcendence through the work process, facilitating their sense of being connected to others in ways that provides feelings of completeness and joy". Spirituality at workplace mainly focuses in "meaningful work", "transcendence" and "sense of community". Most definitions of workplace spirituality include the "notions of meaning" and "purpose and being connected to others" (Ashmos&Duchon, 2000; Conger, 1994). Organizational commitment is the bond employees experience with their organization. It is an individual's psychological attachment to the organization (Charles O'Reilly lll& Jennifer Chatman, 1986). Organizational citizenship behaviour can be defined as "Individual behaviour that is discretionary not directly or explicitly recognized by the formal reward system and in the aggregate promotes the effective functioning of the organization" (Organ, 1988, p.4).

Objectives of the Study

To understand the relationship between workplace spirituality and organizational citizenship behaviour and also to understand the mediating role of organizational commitment on this relationship.

Literature Review and Hypothesis Development

Workplace Spirituality

Ashmos & Duchon (2000) explored various perspectives of workplace spirituality and tried to validate them in their study. Fry (2003) in his seminal paper highlighted the concepts of "sense of calling", "meaning and purpose", which leads to greater levels of commitment at individual level and organizational level. Giacalone&Jurkiewicz (2003) validated the employees' sense of being with happiness and joy that could be felt at the workplace by inculcating spirituality. Different scholars have highlighted various dimensions of spirituality at workplace. Some of them are as follows: Jurkiewicz&Giacalone (2004) highlights justice, mutuality, respect and trust. Kinjerski&Skrypnek (2004) highlights meaningful work and sense of collective purpose. Khasawneh, Alrjoub&Zawahreh (2010) highlights the role of family and community. Liu & Robertson (2011) highlights the role of spirituality in developing the interconnection among human beings and nature aligned with all living creatures.

Organizational Commitment

Employee commitment remains one of the challenge aspects for organizational researchers in the domain of organizational behaviour (Cooper &Viswesvaran, 2005). Organizational commitment is a central prognosticator of employee's attitude towards organization and indicates about turnover intentions, withdrawal tendencies and organizational citizenship behaviour (Mathieu &Zajac, 1990). Organizational commitment divided in to three distinctive scales i.e. affective, normative and continuance (Meyer & Allen, 1993).

Organizational Citizenship Behaviour (OCB)

Hierarchical structures and individualized jobs have provided a scope for initiative and cooperation in individual context (Ilgen&Pulakos, 1999). This sort of development induced OCB towards higher growth and alignment regarding the social systems (Organ, 1997). It has derived attention of scholars and practitioners (Motowidlo&Schmit, 1999). For the last couple of decades' management scholars widely investigated on the basic factors which affect OCB along with its antecedents and consequences by adopting theoretical as well as empirical methodologies. According to Podsakoff et.al. (2014), there are more than 2100 papers published on OCB. In the context of OCB, most of the OB scholars have studied on the performance on various contexts (Borman, White & Dorsey, 1995).

Workplace Spirituality and Organizational Citizenship Behaviour

The researchers in Workplace Spirituality (WPS) have acknowledged OCB can be a possible outcome of practicing spirituality at workplace. Organizational citizenship behaviour is defined by extra-role behaviour displayed by an

individual which is beyond the basic job responsibilities. Movassagh&Oreizi, (2014) validated a substantial relationship between WPS and OCB. They have also identified significant relationship which is established partially between workplace spirituality and OCB. Nur& Organ (2006) displayed a relationship between the companies who practice virtues and OCB. Based on the above studies, the author(s) propose

H1: Workplace Spirituality (WPS) is positively related with Organizational Citizenship Behaviour (OCB).

Workplace Spirituality and Organizational Commitment

Milliman, Czaplewski& Ferguson (2003) has identified and validated through their research that workplace spirituality dimensions can be aligned with commitment which relationship is much more significant. Nur& Organ (2006) substantiated the role of commitment by highlighting the effect of virtuous firms who have practiced religious beliefs. Crawford, Hubbard, Lonis-Shumate & O'Neill (2008) had done a comparative analysis of levels of commitment in a hotel management group. Their study revealed that employees who practice workplace spirituality, has higher levels of commitment and those who don't practice workplace spirituality, has marginal level of commitment. Positive strong correlation of workplace spirituality has been explored and validated with normative and affective commitment by several studies in different contexts (Ahiauzu&Asawo 2009, 2010; Ahiauzu&Asawo 2012). Another study in the same context found positive relation between WPS and OC (Nwibere&Emecheta, 2012). Rego& Pina e Cunha (2008) found positive correlation between five dimensions of WPS with affective and normative forms of OC. Furtherance to the above studies, the author(s) propose

H2: Workplace Spirituality (WPS) is positively related with Organizational Commitment (OC).

Organizational Commitment and Organizational Citizenship Behaviour

There is a significant relationship between OC and turnover intentions (Randall,Fedor&Longenecker, 1990), with substance to these supplementary relationships has been explored between organizational commitment and extra-role behaviours (Meyer &Herscovitch, 2001) along with OCB (Organ & Ryan, 1995). Levels of commitment may differ from individuals to individuals but increased commitment leads to reduced withdrawal cognitions at the organization and individual level that results in various outcomes for employees' discretionary extra-role behaviour i.e. OCB (Gautam van Dick &Wanger, 2001).

In continuance with the above studies, the author(s) propose

H3: Organizational Commitment (OC) is positively related with Organizational Citizenship Behaviour (OCB).

From the above literature review this conceptual model is emerged. Figure .1

METHODS

An internet based survey has been designed to collect data from employees working in different private manufacturing and service based industries operating in India. The survey has been shared via LinkedIn, a professional networking platform, to the concerned employees. The survey has been shared to 850 employees and out of which 310 employees provided their responses (110 males and 200 females). All the respondents are graduates having work experience of more than five years and serving in different managerial positions.

Measures

Workplace Spirituality

This survey adapted Ashmos and Duchon's (2000) instrument for measuring workplace spirituality. There are three dimensions i.e. inner life, meaningful work and sense of community spreading over 21 items in the instrument. Out

of the 21 items, inner life has5 items (e.g. "I feel hopeful about life"), meaningful work has 7 items (e.g. "I experience joy in my work"), and sense of community has 9 items (e.g. "I feel part of a community in my immediate workplace-Department/Unit"). α=0.91 for the overall workplace spirituality construct. Participants responded to all workplace spirituality items in a 7-point scale. The 7-point scale ranged from 1= *strongly disagree to* 7=*strongly agree.*

Organizational Commitment

This survey has adapted Cook and Wall's (1980) instrument for measuring organizational commitment. It has a total 9 items (e.g. "I am quite proud to be able to tell people who it is I work for"). α=0.87 for the overall organizational commitment construct. Participants responded to all psychological empowerment items in a 7-point scale. The 7-point scale ranged from 1= strongly disagree to 7=strongly agree.

Organizational Citizenship Behaviour

This survey has adapted Spector and Fox (2010) OCB-C instrument for measuring organizational citizenship behaviour. It has got total 10 items (e.g. "Took time to advise, coach or mentor a co-worker"). α=0.81 for the OCB construct. Participants responded to all OCB items in a 5-point scale. The 5-point scale ranged from 1= Never to 5=Every day.

Control Variables

The demographic factors i.e. age, gender and experience, are controlled. Age and experience are measured in years, whereas gender is measured as a dichotomous variable and coded as 1 for male and 2 for female.

RESULTS

Measurement Model

Partial Least Squares (PLS) structural equation modelling (SEM) is used to analyse the data. PLS-SEM has derived significant attention of scholars in social science disciplines including organizational management (Sosik et al., 2009) and human resource management (Ringle et al.,2018). PLS SEM generates factor loadings for each scale item which can be used to assess the measurement model. Indicator loadings, Convergent Validity (AVE) and Composite Reliability (CR) are provided in Table I. Each construct should have an AVE greater than 0.5 (Chin, 1998). All the coefficients are showing homogeneity with scales (Thompson, 1997) and showing good convergent validity. Table-1

Table 2. explains the Heterotrait-Monotrait (HTMT) ratio as proposed by (Henseler*et al.*,2015) to check the discriminant validity. HTMT can be examined by looking at the upper bound of the 95 percent confidence interval of HTMT is lower than 0.90 or 0.85 (Hair *et al.*,2019). In this case, the CI low (2.5%) and CI up (97.5%) are mentioned in the columns. Since all HTMT are significantly different from 1, discriminant validity is said to be established between these reflective constructs. Table 2.

Structural Model

Table 3. explains the collinearity statistic (VIF) of each predictor in the structural model. It should be higher than 0.20 and should be lower than 5 (Hair *et al.*,2016, p.208). The author(s) haven't found any collinearity issues with the structural model, as all the values are above the threshold levels. Table 3.

Table 4. explains the path coefficients of the structural model that helps to evaluate the model (Hair *et al.*,2011). For reflective measurement models, outer loadings should be taken in to consideration (Hair *et al.*,2016, p.84). As we can observe from the table, all the outer loadings are significant at P<0.05. The 3 hypotheses proposed by the researchers are accepted. Table 4.

Table 5. explains the R^2 value that represents the amount of explained variance of the endogenous constructs in the structural model. R^2 values of 0.25, 0.50 and 0.75 for target constructs are considered as weak, medium and substantial (Hair *et al.*,2016, p.222). The researcher(s) have found the target endogenous constructs' predictive accuracy is substantial. Table 5.

For mediation the author(s) have used The Preacher and Hayes (2008) procedure. Initially the significance of the direct effect has been checked by using bootstrapping, excluding the mediator OC (Organizational Commitment) and then included the mediator OC (Organizational Commitment) along with the path coefficients. In Table-VI has explained the process of mediation along with the values. We have found that there is substantial relationship of workplace spirituality with organizational citizenship behaviour, thus H1 is accepted. We have also found that there is substantial indirect effect of workplace spirituality on organizational commitment and the effect of organizational commitment on organizational citizenship behaviour, thus both H2 and H3 are accepted. Organizational commitment fully mediates between workplace spirituality and organizational citizenship behaviour in this study. Table 6. For testing Goodness of Fit Model (GoF), the author(s) have used the Standardized Root Mean Squared Residual (SRMR) for approximate fit criterion (Hu &Bentler, 1999). If the SRMR value is zero, it is perfect fit, if the value is 0.08 or lower, it is acceptable and value more than 0.08 depicts absence of fit (Henseler et al. 2014). In Table-VII, the author(s) have mentioned the SRMR. The author(s) have found that the model has acceptable fit. Table 7.

Practical Implications

Workplace spirituality has been empirically recognized in several organizations and outreached globally. There is a positive effect of WPS on job fulfilment, ethical behaviour, productivity, increased self-esteem, work-life balance, reduced counterproductive behaviour, subjective well-being, meaning, psychological-wellness etc. which has been validated by empirical studies. Managers can follow the best practices that is established and validated by organizational researchers to improve the innate abilities of the individual employee at workplace or workplace as a whole.

DISCUSSION

Various disciplines identified spirituality as one of the important construct to be researched upon. By operationalizing workplace spirituality through two of its major constructs, organizational commitment and organizational citizenship behaviour, much knowledge can be gained in understanding and articulating spirituality at workplace. Conceptualizing a model and deriving hypotheses on the role of OC and OCB appears to be stronger than empirical evidence at this stage. It can be strengthened by further empirical investigation. The proposed hypotheses between WPS, OC and OCB are the significant opportunities that can be explored in the domain of workplace spirituality. The existence of such relationships should now be examined through empirical researches to confirm or refute the hypotheses appealed/ applied between WPS, OC and OCB. It will be also a significant contribution if further studies can provide insight on causality in such relationships.

CONCLUSION

Spirituality is a highly multifaceted and multidimensional concept. The dimensional aspects of spirituality can be conceptualized and operationalized by the organizational researchers. The scholars of workplace spirituality usually concentrate on further development and extension of the construct (Altman, 2013). There are lot of opportunities to be explored in researching workplace spirituality. Various mediators and moderators have been introduced to establish the connections, to explore relations and to envisage the impact on organizations.

DISCLAIMER

Preliminary version of the manuscript has been presented by the Corresponding Author at 1st PAN IIT International Management Conference, Roorkee, INDIA on November 2018 and highly appreciated by the distinguished panel.

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Table 1. Coefficients for the measurement model

Construct	No. of items	Cronbach's α	Variable	Factor Loadings	Composite Reliability (CR)	AVE
Workplace Spirituality	21	0.91	WPS1(IL) WPS2 (IL) WPS3 (IL) WPS4(IL) WPS5 (IL) WPS6(MW) WPS7(MW) WPS8(MW) WPS9(MW) WPS10(MW) WPS11(MW) WPS12(MW) WPS13(SOC) WPS14(SOC) WPS15(SOC) WPS16(SOC) WPS17(SOC) WPS18(SOC) WPS19(SOC) WPS20(SOC) WPS21(SOC)	0.89 0.89 0.98 0.77 0.90 0.89 0.83 0.89 0.91 0.92 0.89 0.84 0.86 0.87 0.89 0.84 0.86 0.87 0.89 0.89 0.90	0.89	0.83
Organizational Commitment	9	0.87	OC1 OC2 OC3 OC4 OC5 OC6 OC7 OC8 OC9	0.89 0.84 0.86 0.87 0.89 0.90 0.89 0.83 0.89	0.78	0.97

Organizational Citizenship Behaviour	10	0.81	OCB1 OCB 2 OCB 3 OCB 4 OCB 5 OCB 6 OCB 7 OCB 8 OCB 9 OCB10	0.77 0.76 0.70 0.71 0.76 0.73 0.76 0.84 0.71 0.80	0.76	0.97

Note: IL: Inner Life, MW: Meaningful Work, SoC: Sense of Community (Dimensions of Workplace Spirituality).

Table 2. Heterotrait-Monotrait Ratio (HTMT)

	Original Sample (O)	Sample Mean (M)	Bias	2.5%	97.5%
WPS→OCB	0.793	0.807	0.014	0.748	0.815
WPS→OC	0.790	0.799	0.009	0.740	0.819
OC→OCB	0.340	0.315	0.025	0.231	0.470

Note: WPS: Workplace Spirituality, OC: Organizational Commitment, OCB: Organizational Citizenship Behaviour. Confidence Interval: lower: 2.5% and upper: 97.5%

Table 3. Collinearity Assessment

	Inner VIF Values
WPS→OCB	2.660
WPS→OC	1.000
OC→OCB	2.660

Note: WPS: Workplace Spirituality, OC: Organizational Commitment, OCB: Organizational Citizenship Behaviour

Table 4. Path Coefficients of the Structural Model

Path	Loadings	Std. Dev	T Statistic	P Values
WPS→OCB	0.553	0.066	7.998	0.000
WPS→OC	0.799	0.019	41.227	0.000
OC→OCB	0.316	0.068	4.989	0.000

Note: WPS: Workplace Spirituality, OC: Organizational Commitment, OCB: Organizational Citizenship Behaviour

Table 5. Co-efficients of determination (R^2)

	R-Square	R-Square Adjusted
OC	0.778	0.777
OCB	0.820	0.819

Note: OC: Organizational Commitment, OCB: Organizational Citizenship Behaviour

Table 6. Mediation Analysis

Hypothesis	Procedure	Path	Path Coefficient	Indirect Effect	Std. Dev	Total Effect	VAF	T Statistic	P Values
Accepted	Step-1: Direct Effect (without mediator)	WPS→OCB	0.553	n/a				7.998	0.000
Accepted	Step-2: Indirect Effect (with mediator)	WPS→OCB	0.535	n/a		0.554	0.089	4.989	0.000
		WPS→OC	0.799	0.049	0.024				
		OC→OCB	0.316						

Note: P<0.05. WPS: Workplace Spirituality, OC: Organizational Commitment, OCB: Organizational Citizenship Behaviour

Table 7. Model Fit

	Saturated Model	Estimated Model
SRMR	0.085	0.086

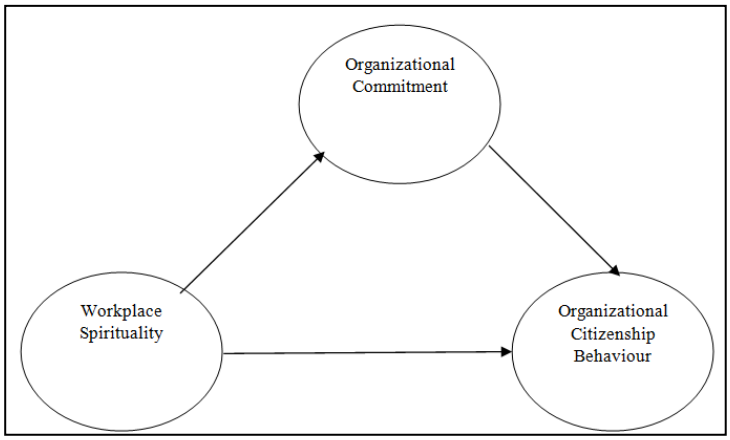

Figure - 1. Conceptual Model

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Effect of Habitat on Haematological Parameters of Two Teleosts *Mugil cephalus* and *Parastromateus niger*

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Received: 21 Feb 2020 Revised:23 Mar 2020 Accepted: 25 Apr 2020

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ABSTRACT

Aquaculture is known as the largest sector for production of foods. It is playing an important role in providing nutrition throughout the year. Blood parameters are very much sensitive to any changes in environmental factors that made easier to analyses. Haematological parameters are the well-known catalogues which have been chosen as the most important guide to identifying the health status of living organism. Blood parameters are very much sensitive to any changes in environmental factors. The main objective of this research work to provide an standard data of haematological parameters for these two teleost species on different habitats. These two species namely *Parastromateus niger* (Bloch, 1795) and *Mugil cephalus* (Linnaeus, 1758) were taken for the experimental analysis. Haematological parameters were analysed by standard protocol. Packed cell volume, total red blood cell and white blood cell count have shown their significant statistically at $P<0.001$.The value of haemoglobin and mean corpuscular haemoglobin concetration value show statistically non-significant at $P>0.05$. These assessments of haematological parameters will show the influence of habitat on physiological status of a species.

Key words: Haematology, habitat, *Mugil cephalus, Parastromateus niger*

INTRODUCTION

Fishes have high nutritional value and are the rich source of protein that provides a range of health benefits. There are approximately 54,771 species are recognized living vertebrates and fish constitute slightly more than one-half of these. (Nelson 2006). Haematological parameters are considered to determine the status of a fish or fish population (Gabriel et al. 2004).These haematological parameters of fishes mainly depend upon the variation in sex, nutrition and environmental condition (Hrubec et al. 2001; Fazio et al. 2016). These variations are highly sensitive to environmental

conditions like oxygen content, pH and salinity of water as well as the ecological factors (Parrino et al., 2018). Fish haematology helps us to find out the relationship with phylogeny,habitat and adaptation to specific environment (Wilhem et al. 1992).

Mugil cephalus (Linnaeus, 1758) are Grey mullets belongs to the family Mugilidae have a worldwide distribution. These species are generally inhabitating in tropical estuaries. Though they are spawning in sea, but can live in wide range of salinities like from marine to estuaries (Cappello et al. 2016). Mugil cephalus is important for fishing as it is widely consume throughout the world. (Whitfield et al. 2012). *Parastromateus niger* comess under Class-Actinopterygii, order-perciformes and family Carangidae is commonly known as black pomfret. The *Parastromateus nigar* is a species of carangid fish available of the Indian ocean and the western pacific ocean. The fish population gradually decreases due to the increasing demand for this species, over exploitation and lack of proper management of fishing. Base lines of haematological indices provide an important detector to know the physiological changes in the fishes. These parameters provide the information about the health status and toxicological symptoms of an organism .It also shows adaptation capacityof that species to that specific environment (Piccione et al,.2010).

The relationship between the haematological parameters and different habitat are already studied in in mullet *Mugil cephalus* Linnaeus,1758 (Fazio et al 2012). The haematological parameter are comparatively studied in different marine fishes. The result represents the haematological red blood cell (RBC), white blood cell(WBC) , mean corpuscular volume(MCV), mean concentration (MCHC) are significantly correlated (Satheeshkumar et.al.,2010). Morphometry can be used to quantify a trait of evolutionary significance and by detecting change in shape, assume something of their ontogeny , function or evolutionary relationship (Langer, 2013).

MATERIALS AND METHOD

Study area

The experimental study was undertaken from the month of November 2019 to February,2020. *Mugil cephalus,and Parastromateus nigar* were selected for this experimental work. The experimental work was done in the laboratory of Zoology Department ,Centurion University of Technology and Management, Bhubaneswar campus, Odisha.

Collection of speciemen

These fish species were collected from local fishing sites of Puri and Chilika. These species were identified and then weights were measured. Collection of blood samples from the species were done by standard protocol. Then two ml of blood samples were taken into the anti-coagulant Ethylene Diamine Tetra Acetic Acid (EDTA) vial for further experiments.

Analysis of haematological parameters

The haematological analysis has done for getting knowledge about different parameters related to total number of RBC and WBC are done by using Neubauer chamber (Marienfeld, Germany). Haemoglobin is a reasonable index of red cell population which was estimated by Sahli's method by using sahli's haemometer. PCV,MCV, MCH andMCHC are examined.

RESULT AND DISCUSSION

Estimation of WBC

The total WBC count was varies from 12640±344.222 $\times 10^3/mm^3$ to 9580±470.4135 $\times 10^3/mm^3$in *Mugil cephalus and P niger* respectively. The total WBC count was high in *M cephalus* than *P niger* which is statistically significant at P<0.001.The WBC value was higher in case of *M cephalus* which is either due to their feeding habitat of the species or environment since increased in salinity (Maisano M et al,. 2016).

Estimation of RBC

A significant difference was found from the comparison between the total RBC counting at P<0.01. The TRBC value was ranges from 3.151±0.451532$\times 10^6/mm^3$ to 1.749±0.220552 $\times 10^6/mm^3$ in *Mugil cephalus* and *P.nigar* respectively. The total RBC count was high in *M cephalus*where as a lower value was found *P. nigar* which is due to requirement of oxygen for their higher metabolic rates (Engel and Davis, 1964).

Estimation of Haemoglobin

Haemoglobin percentage was higher in case of*Mugil cephalus* was high and low in *P.nigar*. The mean±SD of haemoglobin is 13.19±0.420965 and 12.67±0.509259 g/dl.The Hb value is statistically non-significant at P<0.01. The higher value of haemoglobin in case of *M.cephalus* indicates that this fish is more active than the *P. niger*. To remains more active a fish needed more oxygen which increases the Hb concentration (Engel and Davis, 1964 ; Rambhaskar and Srinivasa Rao, 1986).

Estimation of PCV

The PCV value ranges from 41.3±2.773085 and 32.9±1.882374% which is statistically significant at P<0.01. A high PCV value was found in case of *Mugil cephalus* and *P.nigar*having a lower PCV value. The increasing or decreasing values of PCV may be due to blood plasma content in different fish species. The fishes undergoing stress condition may also leads to difference in PCV values of these two different fishes (John, 2007).

Estimation of MCV

The Mean Corpuscular Volume of both the fishes shows statistically significant value at P<0.05. The values vary from 148.384±14.56912 to 216.2333±28.5336 fl. for *Mugil cephalus* and *P.nigar* respectively. **Estimation of MCH and MCHC**
The mean Corpuscular haemoglobin ranges from 51.028±8.848 to 80.11223±7.442489 pg.in *Mugil cephalus* and *P.nigar* respectively. The MCH values is statistically significant at P<0.01.The Mean Corpuscular Haemoglobin Concentration value was statistically non-significant at P>0.05. The MCHC value ranges from 33.635±3.058 to 39.859±3.120 respectively. This is due to the consumption of oxygen and swimming activity in normal condition (Stillwell and Benfey, 1995) [12]. MCHC in all the three commercially important freshwater fishes ranged between 30 % to 66% and this was similar to Peruzzi et al., (2005) .

CONCLUSION

This preliminary study of two species provides basic knowledge of haematological indices of *M. cephalus* and *P.niger* . In conclusion, this study has established influence of environment and habitat on haematological parameters of two different fishes in different habitat. These results of haematological indices are considered as important tools for determining the health status of a fish by changing in adaptive physiological changes at a particular habitat. These investigation on fish haematology will provide a better contribution towards the pisciculture and fish management. Such haematological findings from this study may represent the effective diagnostic tool for early understanding and the variability blood cells in different fish species.

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Table 1 Comparative values of haematological indices of *Mugil cephalus* and *Parastromateus niger*

SL NO	Haematological parameters (Units)	*Mugil cephalus* (X±SE) (n=10)	*Parastromateus niger* (X±SE) (n=10)	P Value
1	White blood cell ($\times10^3/mm^3$)	12640±344.222	9580±470.41	2.71E-05
2	Red blood cell ($\times10^6/mm^3$)	3.151±0.451	1.749±0.220	0.006047
3	Haemoglobin (g/dl)	13.19±0.420	12.67±0.509	0.220756
4	Pack cell volume (%)	41.3±2.773	32.9±1.882	0.01101
5	Mean corpuscular volume(fl)	148.384±14.569	216.2333±28.533	0.024186
6	MCH (pg)	51.02865±8.848	80.11223±7.442	0.010803
7	MCHC (%)	33.635±3.058	39.859±3.120	0.085712

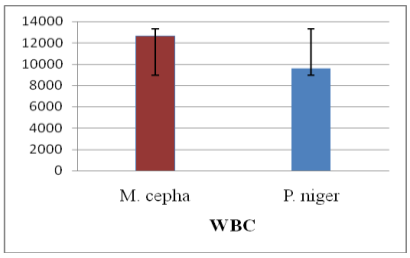

Fig. 1 Comparison between the TWBC of *Mugil cephalus* and *Parastromateus niger*.

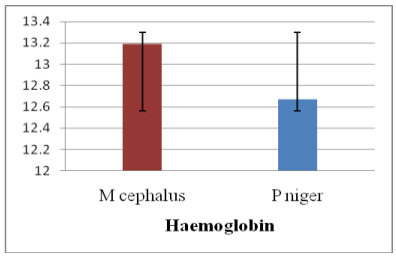

Fig. 2. Comparison between the haemohlobin conc. of *Mugil cephalus* and *Parastromateus niger*

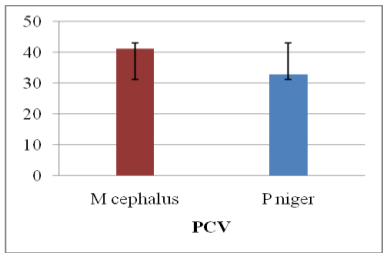

Fig. 3. Comparison between the PCV of *Mugil cephalus* and *Parastromateus.*

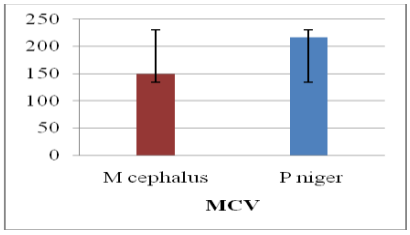

Fig. 4. Comparison between the MCV of *Mugil cephalus* and *Parastromateus niger*

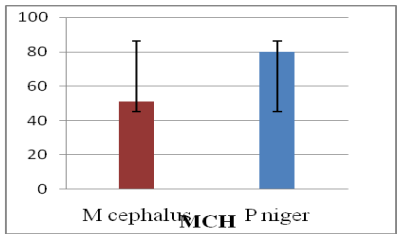

Fig. 5. Comparison between the MCH of *Mugil cephalus* and *Parastromateus niger*

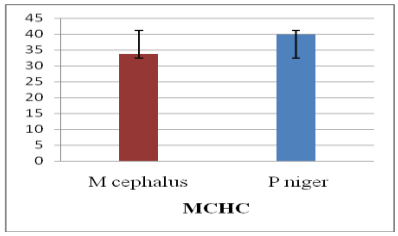

Fig. 6.Comparison between the MCHC of *Mugil cephalus* and *Parastromateus niger.*

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

REVIEW ARTICLE

A Systematic Review on Job Stress, Burnout and Coping Strategies in Police Constables

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Received: 22 Feb 2020 Revised:25 Mar 2020 Accepted: 27 Apr 2020

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ABSTRACT

There is always a huge area of extensive research going on an international level in the area of job stress of police persons and its impact on individual and professional life of police personnel. The Job stress of police personnel has activist impact on the organizational performance and that is most dangerous for police organization. The objective of this study was to review the literature on police constables stress levels with emphasis on manifestations as well as the symptoms of strain that facilitate recognition of problem, identification and delineation of the stressors experienced by law enforcement agents and coping behaviour among law enforcers. It has been observed that Job stress has leads to the increasing of negative output for the individual employee and the self-organization. Decreasing levels of general health and well-being as well as levels of satisfaction based performance and commitment to the organization has each been identified as a result of the employee experiencing occupational stress. The results of stress are harmful to people, society and organizations. High levels of stress will cause negative effect on employees physical and mental wellbeing ultimately shows effect on performance.

Keywords: Occupational Stress, Burnout, law, job demands, coping strategies.

INTRODUCTION

Police services have always been one of the most challenging and stressful services in India and with changing times it is becoming even more so. The major brunt of this job is borne by constables as they are the foot-soldiers of police in India. They have to deal with angry mobs, counter-insurgency operations, traffic control, VIP security, political rallies, religious festival crowd control, and various other law and order duties without losing their composure and sensitivity. They have to face potentially hazardous situations that can result in physical or mental trauma or even

death in the line of duty. Their work stress can be further aggravated because of their personality traits or wrong coping methods. A majority of Indian and international studies have found high stress levels in police, which is disturbing as psychiatric morbidity in police can have many direct and indirect negative consequences for society. Therefore, apart from physical fitness, they have to be mentally fit to do full justice to their duties.

Job or Work levels of police personnel are highly stressful as they always have to face challenges to their life by taking risk in their daily work. A study by Johnson et al. (2005) found that police was one of the six professions where the high stress led to maximum impact in terms of poor health and low job satisfaction. Traumatic stress is well known stress cause by physical hazards and is common in police. This kind of stress arises due to Police organizational structures and hierarchies tend to be rigidly stratified and unresponsive to individual needs. The incidence of suicide and fratricide has been rising over the years due to the physical and psychological problems faced by the police force. The high rate of suicides is just due to higher stress levels in Indian police, which is also a matter of serious concern. Stress is a complex phenomenon with multiple variables. The role played by psychologists and government in relation to coping mechanisms at every stage of service for police in India in comparison with other countries like USA, Australia etc. is negligible in spite of the fact that there is a need to alleviate stress in India has been recognized . The present study aims to fulfil these gaps.

Objective

The first objective of the study is systematically review the current evidence on job stress, burnout, and coping for the effectiveness of study on occupational stress of police constables. The second objective of this study was to review the literature on police stress with emphasis on manifestations as well as the symptoms of strain that facilitate recognition of problem, identification and delineation of the stressors experienced by law enforcement agents and coping behaviour among law enforcers

METHODOLOGY

The systematic review was completed March 2020.The study was based on the University of Pune guidelines for conducting systematic literature reviews. This review was conducted in two parts. The first part was focused on identifying sources of job stress and notice symptoms of sever job stress in police by reviewing research papers on stress, mental health and job performance. The second part of the review retrieved papers on job stress, burnout, coping measures that evaluated signs of stress at work, on performance and high risk associated with job stress. Studies included were research articles dating from 1972 to 2020 undertaken in different journals, conferences at national and international level by psychiatrists, psychologist, researchers and social science professors

Observation

Stress is an essential part of everybody's life. All Stress is not always inevitable, but some time it is good. For example, the physical exercise improves cardiovascular system, and feeling pressure of exam causes to study harder for results with high score. However Police stress refers to the negative pressures related to their work. Police officers are one of the common men. In many research study researcher exposed that police are affected by their everyday exposure to human offensiveness and pain; and that when the shift changes, the long periods of boredom, and the continuous danger that are part of police work do cause serious job stress. Dr. Hans Selye's in his book "The Stress of Life" described the effect of long-term "stressors." Dr. Selye maintains that the unrelieved effort to cope with stressors can lead to heart disease, high blood pressure, ulcers, digestive disorders, and headaches. Stressors in police work fall into four categories

- Stresses inherent in police work.
- Stresses arising internally from police department practices and policies.
- External stresses stemming from the criminal justice system and the society at large.
- Internal stresses confronting individual officers

Dr. Martin published the first study of police officer stress in 1972 in the American Journal of Psychoanalysis based on Selye's work, describing psychological effects of stress in police officers proposed by Dr. Martin (1972), which divides the sources of police stress into two broad categories: (1) the nature of police work; and (2) the nature of police organizations. In the first category of stressors, Symonds includes constant exposure to danger, facing the unknown, confronting hostility, and making judgments in rapidly changing, unpredictab1e situations. Inhissecondcategory,Dr. Martinincludesthequasi-military structure of police organizations, competition for promotional opportunities, disagreeable job assignments, and varying tours of duty. The utility of S Dr. Martins' model as a framework for understanding police stress has been demonstrated in the work of William H. Kroes and his associates.

However, the first empirical study of police officer stress was conducted by Dr. William Kroes in 1974 and his study is the foundation for the formation of modern police stress awareness. Dr. Kroes Interviewed 100 Cincinnati police officers using an obtrusive semi-structured interview technique, categorizing primary Job stressors into equipment, courts, administration, and community relations areas. His research's result clearly indicates organizational stressors, identified in the administration category, were the main sources of line officers' concern. Dr. Terry Elsenberg followed Kroes in 1975 with exploratory research based on his experiences as a psychologist and police officer, placing 33 implied sources of stress into six categories: intra-organizational practices and characteristics; Inter-organizational practices and characteristics; criminal Justice system practices and characteristics; public practices and characteristics; police work itself; and the police officer. The intra - organizational practices and characteristics category contains features within an organization which may provoke or encourage stress development of stress such as poor supervision, absence or lack of career development opportunities, inadequate reward system, offensive policies, excessive paperwork, and poor equipment.

In a study sponsored by National Institute of Mental Health, Bethesda conducted by Beehr, Terry A. and team on "Occupational Stress: Coping of Police and Their Spouses" (1991) suggest that coping activities of one might affect the strains of other. The activities in which the spouse is engaging will cause employee's own coping attempt less effective (or more). A study through questionnaires including large city police department in the Eastern US and a suburban country department in the same state who provided the voluntary participants. A unique subset of the married officers and their spouses were indulged in this study. The questionnaires were anonymous in order to assure security of the officer's identities and there were no direct way of matching each officer's questionnaire with her or his spouse's questionnaire. Its appeared to be five coping activities in which the police and their spouses engage when they experience stress: problem-focused coping, rugged individualism, avoidance, religion and self-blame. By studying the stress among the officer and their spouses, it enlightens the divorce potential as it is strongly correlated by the officer and his/her spouse. No coping strategy had an apparent effect on the divorce among the officer except for self-blame and its effect was deleterious. In the marriage life of officer and spouse blaming is a major factor cause trouble. Due to close bond between religion and marriage in our culture religion is also somewhat an affecting factor.

According to Mathur P study on "Stress in police personnel: A preliminary survey, NPA magazines, 1993:45, he found that there are few job related factors among Indian police personnel those are acting as specific stressors, for example inadequate equipment, fear of severe injury, working conditions, anti-terrorist operations, lack of recognition, being killed on duty, work overload shooting someone in the line of duty, tackle with the public, lack of job satisfaction and police hierarchy. A study by Storch and Panzarella on "Police Stress: State-Trait Anxiety in

Relation to Occupational Stress and Personal Stressors" (1996) finds that organizational factors and relationships with outsiders are major negative stressors, rather than potential violence or exposure to human misery. They find that the amount of stress or anxiety experienced by police officers is similar to other profession. The Police officers who enjoy job excitement, offense skirmishing experienced more stress than the Police officers who just focused on job compensation. Police officers who were facing changes at their work front or at family front are with more stress. The most objectionable feature in job of police officer is work schedule.

In another study according to Carson and Kuipers (1998), he divided the process of stress into three levels. The first level which contains stressors coming from external sources, e.g. high job demands, a lack of resources and lack of support from supervisors and colleagues etc., the second level suggest coping strategies those are acting as buffer against negative impact of stressors on individuals. The third level consists of the stress impact on individual which can be positive or negative. According to Schaufeli and Enzmann (1998) study organizational stressors are divided into two groups: job demands and a lack of resources, where job demands tip to the required constant physical or mental exertion characteristic of the job and can consequently be associated with certain physiological and psychological costs, like excessive paper work, shift work, working over time, meeting deadlines and handling crisis situations. And job resources are part of the job that may be efficient in achieving work goals, reducing job demands and the probable physiological and psychological costs, and motivating personal development, e.g. sufficient equipment, excellent management, an ample salary, appreciation and adequate human resources.

Anshel (2000) highlighted three underlying postulates in stress research with police officers. Firstly, excessive or strange external stimuli that are professed as threatening will be traumatic and cause major changes in psychological, physiological and behavioral responses. The second stress postulate is that the failure to cope successfully with temporary unexpected stress which leads to long-standing, chronic stress, which might in turn restrain the body's resistant system, And then it leads to an array of medical illnesses and diseases. And lastly, sources of police stress that are ongoing and long-term will result in burnout, reduced motivation, poor performance, and eventual dropout from the police profession. A National Institute of Justice (NIJ) report published in 2000 summarized the causes and effects of job-related stress on law enforcement officers and their families. The exposure to violence, suffering, and death are the source of the stress among police officers. Due to rotating shifts of work of police officers unable to spend enough time with their families annoying these stressors. Report also highlighted few more stress causing incidence like high levels of violent offense, greater public scrutiny, unfavorable publicity, and changes in law enforcement such as the advent of community policing.

One more study conducted by Sergeant Corey Haines, Madison Heights Police Department in 2003 on "Police Stress and The Effect on The Family". The objective of this research Paper was to identify the effects of stress on the Police Officer as it relate to his professional and family life and to identify how the department can assist to the officer in stress management . Another objective is to identify stressors and find the correct ways to handle the situation of stress before they become uncontrollable and cause negative impact on the officer and his/her family. Developing a counseling training program to the officers which will result in increased efficiency of the organizations has been proved by the research. The counseling process will add stress if it is conducted publically so it should be confidential. The study was concluded with the fact that there should be counseling programs for officer to balance their personal life and professional life, so that the divorce cases would reduce at an extent. The officers should be counseled time to time so that they relieve their stress and maintain a healthy relationship with not only organization but also with the family.

Pienaar and Rothmann conducted a study on South African Police Service in 2006. They found that 2145 police officers had a noteworthy impact on the occurrence of occupational stress in the SAPS. All the different groups experienced higher levels of stress due to lack of support, salary, promotion and recognition as compared to other occupational stressors. While considering rank in police department it was reported to have also impacted significantly on the experience of occupational stress in the police. Constables experienced stress less frequently

because of job demands, crime-related stressors and lack of support in comparison to other ranked police officers. A survey research conducted by Buker and Wiecko (2007) on civilian officers, police officers, and mid-level supervisors around 811 respondents working for the Turkish National Police Organization in which they founded the organizational factors are the most stressful as compared to other stressors. A study carried out by Gul (2008) examined the stressors in policing and law enforcement officers' depression on their profession. He also found that officers on duty of violent arrests feel more negative and depressed about their work. In addition to that officers who attended a police funeral were more likely to feel negative and depressed about their profession.

A study conducted by Martin Gachter and his team in 2009 on "Gender Variation of Physiological and Psychological Stress among Police Officers" with main objective is to analyse the effect of gender on reported and perceived level of stress through examination of both the physiological and physiological indicators. Data were taken for analysis from the study "SHIELD"(Study to Help, Identify, Evaluate and Limit department Stress) conducted by Gerrshon (1999) in Baltimore, Maryland. Several indices were constructed to measure different aspects and outcomes of stress for the purpose of study. Initially, t-test was ran to control whether the mean level of perceived stress levels differ significantly between males and females. After then regression was ran to explore the partial effects rather than just the raw effects. A large set of explanatory variables were taken. There was no significant difference were found between males and females concerning physiological stress but the observation shows that female officers have higher level of physical stress (covering also somatization and overall health). Furthermore, stress mitigation factors overall like social capital, and perceptions of fairness (Individual) are affecting the male officers but not helps in reducing physical stress among female officers. For both gender groups, only work-life balance and home stability show the tendency to be statistically significant. The requirement of implication of important policy for stress-reducing programs among female police officers with the aim of reducing gender gaps leads to the conclusion of research. For the police officers to find a sane difference between their tasks, both at home and the job such program should focus on overcoming stereotype about job profiles and on allowing a reasonable work-life balance. And this also leads to an environment in which female officers work have significantly hindered their stress coping abilities.

The study conducted with The Campbell Collaboration by George T. Patterson and team on "The Effects of Stress Management intervention among Police Officers and Recruits"(2012) with the objective to identify, recover, assess and produce the available facts about effects of stress management involvement offered to veteran police officers and recruits. The research arrives with conclusion that stress management interventions had no significant effect on psychological, behavioral or physiological outcomes. The 12 primary studies examined psychological stress outcomes with stress can be contribute to negative psychological and physiological outcomes. To support the efficacy of stress management interventions for police officers or recruits, the result does not provide evidence.

Among the many stressful experiences police officers are exposed to in their line of work, exposure to traumatic events (e.g. violence, seeing dead bodies, abused children, etc.) may produce some of the highest stress levels (Korre et al., 2014). One can add to this array of occupational exposures the recent negative public image that police face, resulting in public loss of confidence in police integrity (President's Task Force on 21st Century Policing, 2015). Repeated exposure to traumatic events was shown to affect performance among police officers, depending on the type of incident (Levy-Gigi et al., 2016). Results showed that trauma-unexposed civilians performed better in low (relative to high) aversive conditions. When the authors compared performance of officers who had repeated traumatic exposure to that of unexposed civilians in conditions of low intensity, they found poorer performance among the trauma-exposed officers. Other than work sited in this review, few studies have been identified that investigated associations between exposure to traumatic events at work and chronic diseases (e.g. cancer, cardiovascular disease (CVD), mortality, etc.) in police officers. Results of studies utilizing non-law enforcement populations have shown that exposure to traumatic stress is associated with higher prevalence of CVD and eyesight degeneration (Gallo et al., 2014; Karatzias et al., 2015; Walczewska et al., 2011). Research on effects of traumatic

events on chronic health conditions in police officers is warranted. Organizational stressors which include the organizational setting or design (e.g. management-autonomy, flexibility, participation in decision making, etc.) may be a greater source of stress for police officers as they represent daily routines. However, they are less studied compared to operational stressors which dominate the literature. A recent study of police officers showed that two specific organizational stressors "fellow officers not doing their job" and having "inadequate or poor quality equipment" were among the top five of 60 most frequently occurring stressors (Violanti et al., 2016). Organizational stressors could lead to negative physiological and psychological responses in officers including CVD (Goh et al., 2015; Kivimäki and Kawachi, 2015). A meta-analysis of workplace stressors and health outcomes showed that organizational stressors, such as work-family conflict, job insecurity, high job demand, low job control, and lack of social support, were associated with poor physical health, poor mental health, and physician-diagnosed morbidity (Goh et al., 2015). The study also showed that high job demands raised the odds of having a physician-diagnosed illness by 35 percent.

FINDINGS AND CONCLUSION

The occupational stress has leads to the development of negative outcomes for the individual employee and the employing organization. Degradation of general well-being as well as levels of satisfaction and commitment to the organization has each been identified as a result of the employee experiencing occupational stress. The results of stress are harmful to people, society and organizations. High levels of stress will cause negative effect on employees physical and mental well being ultimately shows effect on performance. Many studies shows that organizational factors are more responsible for stress than to physical hazards on the job. To take corrective measure police administration must take efforts to within organization and also by improving training programs, counseling session for police officers and family, good compensation and rewards policy and transparency at work place. Observations from intensive literature review are as follows

Sources of Job Stress	Symptoms of Sever Stress in Police	Signs of Stress in the Work Place	High Risk in police due to stress
Work Overload Staff Shortages Insufficient Resources Lack of Consultation Boring Administration Financial Crisis Organizational Structures Organizational Climate Non-Grant of Leaves Job/Task Conflicts Long Hours Political Pressure Neglected Family Life Handling Communal Riots Violent arrest Police Funeral etc.	Poor job performance Suicidal thoughts or plans Crying Depression Irritability Short temper Excessive indigestion or heartburn Substance abuse or increased drinking Increased use of sick time Marital problems Sleeping too much or too little Loss of sexual drive Nightmares Isolation loss of interest in social activities Startling easily Changes in weight or appetite etc.	Increasing lateness Going home early Working excessive hours Absenteeism Withdrawal from social contacts Frequent mistakes Forgetting appointments or deadlines Long lunch breaks Increased smoking or drinking Inability to manage time Frequent accidents Conflict with colleagues etc.	High blood pressure Heart problems Insomnia Suicide Post-traumatic stress disorder Depression Anxiety disorders Infection caused by immune dysfunction Panic attacks etc.

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Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

The Impact of Workplace Spirituality on Emotional Labour: Job Satisfaction as a Mediator

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Received: 19 Feb 2020 Revised: 21 Mar 2020 Accepted: 24 Apr 2020

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ABSTRACT

The purpose of the paper is to understand the impact of workplace spirituality on emotional labour among young millennials and also to check the mediating role of job satisfaction on that relationship. A survey-based research has been designed to collect data from 200 front line hotel employees who are waiters and waitresses. The relationships are tested by using Partial Least Squares Structural Equation Modelling (PLS-SEM) and for mediation Preacher and Hayes (2008) procedure has been used. The PLS-SEM and Preacher and Hayes (2008) mediation results have revealed that workplace spirituality buffers the effect of emotional labour. There is also a substantial effect of workplace spirituality on job satisfaction and job satisfaction on emotional labour. Job satisfaction is found to be mediating fully between workplace spirituality and emotional labour. To the best of the authors' knowledge, this study will be one of the firstof its type to study the impact of workplace spirituality as an antecedent on emotional labour.

Keywords: Workplace Spirituality, Job Satisfaction, Emotional Labour, Front Line Hotel Employees, PLS-SEM

INTRODUCTION

"Emotions are psychological, behavioural and physiological, behavioural and psychological episodes experienced towards an object, person or event that create a state of readiness" (McShane and Glinow, 2007). Emotional labour is

defined as "the regulation of both feelings and expressions to support the display rules promoted by an organization and its goals" (Zhan et al., 2016; Grandey, 2000). Emotional labour has gained great acclaim among researchers and literature (Ashforth and Humphrey, 1993; Morris and Feldman, 1996). Workplace Spirituality is defined as "The recognition that employees have an inner life that nourishes and is nourished by meaningful work that takes place in the context of community" (Ashmos and Duchon, 2000). spirituality is summum-bonum in enriching the work standards and thinking standards of the population (Dhiman and Marques, 2011; Gupta et al., 2014, Vasconcelos, 2013). Alignment of spirituality with organizations has resulted in reaping various benefits, which is validated by most of the organizational researchers through their empirical investigations (Vasconcelos, 2015).

Research Gap

Applying spirituality at workplace requires rigor and scientific methods. As discussed above, we have stout research evidence demonstrating that workplace spirituality brings in positive personal and organizational outcomes. Nevertheless, research is scant with respect to its influence in mitigating the effects of emotional labour and that too in Indian context, it is rare (Houghton et al.,2016).

Theoretical Underpinning and Model Development

Affective Event Theory (AET) (Weiss and Cropanzano, 1996) argues that discrete events at work and discrete occasions at work cause full of feeling responses (the two temperaments and feelings), which accordingly influence work mentalities and practices. The full of feeling responses to work occasions incorporate a double level examination process. The first evaluation surveys how important an occasion is to a person. The subsequent examination is full of feeling response, prompting discrete emotional responses (e.g. Anger or joy). AET likewise proposes the workplace has a direct influence on full of feeling responses. For instance, work occasions (for example difficult client cooperation) influence full of feeling states (for example dread, outrage, and scorn), which influence administration worker work practices (for example passionate work technique and administration quality) and work mentalities (for example work fulfilment and turnover aims). In the same line, one of the philosophical underpinning of workplace spirituality is "Intrinsic-origin view" (Krishnakumar and Neck, 2002) which focuses on inner realms of an individual which is integrated with spirituality. We propose that practicing workplace spirituality will lead to job satisfaction that can buffer the effect of emotional labour and workplace spirituality also will have direct effect on emotional labour. From the above theoretical underpinning, this conceptual model is emerged. Figure-I

Literature Review and Hypothesis Development

Workplace Spirituality and Emotional Labour

Workplace spirituality along with religion are able to buffer the drastic effects of emotional labour faced by individual employees at various workplaces (Byrne*et al.,* 2011). In the service sector context, the dimensions of workplace spirituality are able to positively influence positive outcomes at individual level and workplace level through buffering the effect of emotional labour (Lee*et al.,* 2013). From the conservation of resources theoretical framework, workplace spirituality able to buffer the effects of emotional labour through increasing employee's subjective well-being (Zou and Dahling, 2017).

Based on this, the author(s) propose

H1: There is a negative relationship between workplace spirituality and emotional labour

Workplace Spirituality and Job Satisfaction

Workplace spirituality is able to reduce job overload and increase job satisfaction (Altaf and Awan, 2011). In the context of medical practitioners i.e. doctors it is also validated that workplace spirituality is able to enhance levels of job satisfaction (Noor and Arif, 2011). Researchers also used spiritual leadership along with workplace spirituality to

predict job satisfaction where workplace spirituality is able to increase job satisfaction (Supriyanto and Soetijipto, 2016). In the context of white-collar workers, workplace spirituality is able to increase the level of job satisfaction (Walt and Klerk, 2014). All the dimensions of workplace spirituality that examined empirically with job satisfaction levels proved that workplace spirituality is able to increase the levels of job satisfaction.
Based on this, the author(s) propose

H2: There is a positive relationship between workplace spirituality and job satisfaction.

Job Satisfaction and Emotional Labour

Emotional labour has various negative effects. Job satisfaction has a negative effect on emotional labour (Pugliesi, 1999). In the context of school teachers, it is also validated that there is a negative relationship between job satisfaction and emotional labour (Cheung*et al.*,2011). An empirical investigation among nurses also evidenced in validating the negative relationship between job satisfaction and emotional labour (Joung and Kim, 2006).
Hence the author(s) propose

H3: There is a negative relationship between job satisfaction and emotional labour.

METHOD

A survey-based research has been designed to collect data from 200 front line hotel employees who are waiters and waitress. According to emotional labour literature emotional labour has been faced largely by hotel staff (waiter/waitress), airhostesses, bill collectors etc. Thus, the selection of the sample is appropriate to the context. The author(s) directly contacted the 30 hotel managers based at various regions of India through fixing appointment over phone and visited the hotel and explained the study purpose and implications to the managers. The author(s) could able to receive consent only from 15 hotel managers. After that the self-administered questionnaire has been given to a total of 300 waiters and waitress of the concerned hotels, from that 250 questionnaire are received, out of that 50 questions are partially filled, thus finally 200 complete questionnaires are retained and used for analysis.

Measures

Workplace Spirituality

This survey adapted Ashmos and Duchon's (2000) instrument for measuring workplace spirituality. There are three dimensions i.e. inner life, meaningful work and sense of community spreading over 21 items in the instrument. Out of the 21 items, inner life has5 items (e.g. "I feel hopeful about life"), meaningful work has 7 items (e.g. "I experience joy in my work"), and sense of community has 9 items (e.g. "I feel part of a community in my immediate Workplace-Department/Unit"). α=0.91 for the overall workplace spirituality construct. Participants responded to all workplace spirituality items in a 7-point scale. The 7-point scale ranged from 1= strongly disagree to 7=strongly agree.

Job Satisfaction

This survey adapted Minnesota Satisfaction Questionnaire-Weiss *et al.*,1977-short form instrument for measuring job satisfaction. It has got total 20 items (e.g. "I feel about being able to keep busy all the time", "I feel about the chance to work alone on the job"). α=0.87 for the overall job satisfaction construct. Participants responded to all job-satisfaction items in a 7-point Likert scale. The 5-point scale ranged from 1= very dissatisfied to 5=very satisfied.

Emotional Labour

This survey adapted Castro *et al.,* 2006's instrument for measuring emotional labour. It has got total 9 items. 5 items for surface acting (e.g. "I act like nothing bothers me, even when a client makes me mad or upset"). 4 items for deep acting (e.g. "To give advice, I have to make sure I say it in nice way). α=0.81 for the overall emotional labour

construct. Participants responded to all emotional labour items in a 5-point scale. The 5-point scale ranged from 1= Never to 5=most of the time.

Control Variables

The demographic factors i.e. age, gender and experience, are controlled. Age and experience are measured in years, whereas gender is measured as a dichotomous variable and coded as 1 for male and 2 for female.

RESULTS

Measurement Model

Partial Least Squares (PLS) structural equation modelling (SEM) is used to analyse the data. PLS-SEM has derived significant attention of scholars in social science disciplines including organizational management (Sosik et al.,2009) and human resource management (Ringle et al.,2018). PLS SEM generates factor loadings for each scale item which can be used to assess the measurement model. Indicator loadings, Convergent Validity (AVE) and Composite Reliability (CR) are provided in Table I. Each construct should have an AVE greater than 0.5 (Chin, 1998). All the coefficients are showing homogeneity with scales (Thompson, 1997) and showing good convergent validity. Table-I

Table II explains the Heterotrait-Monotrait (HTMT) ratio as proposed by (Henseler*et al.*,2015) to check the discriminant validity. HTMT can be examined by looking at the upper bound of the 95 percent confidence interval of HTMT is lower than 0.90 or 0.85 (Hair *et al.*,2019). In this case, the CI low (2.5%) and CI up (97.5%) are mentioned in the columns. Since all HTMT are significantly different from 1, discriminant validity is said to be established between these reflective constructs. Table II

Structural Model

Table III explains the collinearity statistic (VIF) of each predictor in the structural model. It should be higher than 0.20 and should be lower than 5 (Hair *et al.*,2016, p.208). The author(s) haven't found any collinearity issues with the structural model, as all the values are above the threshold levels. Table III

Table IV explains the path coefficients of the structural model that helps to evaluate the model (Hair *et al.*,2011). For reflective measurement models, outer loadings should be taken in to consideration (Hair *et al.*,2016, p.84). As we can observe from the table, all the outer loadings are significant at $P<0.05$. The 3 hypotheses proposed by the researchers are accepted. Table IV.

Table V explains the R^2 value that represents the amount of explained variance of the endogenous constructs in the structural model. R^2 values of 0.25, 0.50 and 0.75 for target constructs are considered as weak, medium and substantial (Hair *et al.*,2016, p.222). The researcher(s) have found the target endogenous constructs' predictive accuracy is substantial. Table V.

For mediation the author(s) have used The Preacher and Hayes (2008) procedure. Initially the significance of the direct effect has been checked by using bootstrapping, excluding the mediator JS (Job Satisfaction) and then included the mediator JS (Job Satisfaction) along with the path coefficients. In Table-VI, we have explained the process of mediation along with the values. We have found that when there is increase of workplace spirituality, there is a decrease of emotional labour, thus H1 is accepted. We have also found that there is substantial indirect effect of workplace spirituality on job satisfaction and the effect of job satisfaction on emotional labour is negative thus both H2 and H3 are accepted. Job satisfaction fully mediates between workplace spirituality and emotional labour. Table-VI. For testing Goodness of Fit Model (GoF), the author(s) have used the Standardized Root Mean Squared Residual (SRMR) for approximate fit criterion (Hu &Bentler, 1999). If the SRMR value is zero, it is perfect fit, if the value is 0.08

or lower, it is acceptable and value more than 0.08 depicts absence of fit (Henseler et al. 2014). In Table-VII, the author(s) have mentioned the SRMR. The author(s) have found that the model has acceptable fit. Table-VII.

DISCUSSION

In this study, first we have examined the correlation of workplace spirituality with emotional labour. To buffer the effects of emotional labour, there is a need to incorporate spirituality at workplaces. Further to understand and develop our understanding, we incorporated job satisfaction as a mediator between workplace spirituality and emotional labour as per our theoretical framework i.e. Affective Event Theory (AET). When spirituality at workplaces has been promoted, there is a high chance emotional labour can be buffered, that can lead to well-being of employees. It is worth noting that this is the first empirical study which humbly tried to link workplace spirituality and emotional labour and job satisfaction within AET framework. It is also very interesting to check the direct, indirect and the total effect of the independent variable on the dependent variable (with the presence of mediator and without the presence of mediator), which provided us additional insights to understand the theoretical model and its linkages to various human resource implications.

The limitation of this study sheds light on future research avenues. This study has only focused on front-level employees with reference to hotel industry. Future researchers can take different type of industries. Future research can also do comparative analysis of emotional labour faced by different set of industries and level of employees. blue-collar and white collar worker's creativity with relation to workplace spirituality. Different other theoretical frameworks can also be used to understand the relationship between workplace spirituality and emotional labour. One of the theoretical framework is Social Exchange Theory (Blau, 1964).

Practical Implications

In the context of organizations, it will be really beneficial to reduce emotional labour through inculcating workplace spirituality. Expressing genuine emotions is a prerequisite for well-being of employees. Promoting workplace spirituality at organizations are the prerequisite in organizations in this competitive business environment, which can create a sustainable and empowered workforce.

CONCLUSION

Emotional labour has the disastrous effect on the well-being of employees of service industry. Our research reveals new insight that employees and organizations can opt for buffering the effect of emotional labour. Our findings likewise to open new avenues for research in integrating spirituality and emotional management.

DISCLAIMER

Preliminary version of the manuscript has been presented by the Corresponding Author at 4th International Conference on Management, Sciences, Engineering and Applications (ICMSEA) on December 2019 organized by Centurion University of Technology and Management (CUTM), Paralakhemundi, Odisha State (INDIA) and highly appreciated by the distinguished panel.

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Table I. Coefficients for the measurement model

Construct	No. of items	Cronbach's α	Variable	Factor Loadings	Composite Reliability (CR)	AVE
Workplace Spirituality	**21**	**0.91**	WPS1(IL)	0.89	0.89	**0.83**
			WPS2 (IL)	0.89		
			WPS3 (IL)	0.98		
			WPS4(IL)	0.77		
			WPS5 (IL)	0.90		
			WPS6(MW)	0.89		
			WPS7(MW)	0.83		
			WPS8(MW)	0.89		
			WPS9(MW)	0.91		
			WPS10(MW)	0.92		
			WPS11(MW)	0.89		
			WPS12(MW)	0.84		
			WPS13(SOC)	0.86		
			WPS14(SOC)	0.87		
			WPS15(SOC)	0.89		
			WPS16(SOC)	0.84		
			WPS17(SOC)	0.86		

			WPS18(SOC) WPS19(SOC) WPS20(SOC) WPS21(SOC)	0.87 0.89 0.89 0.90		
Job Satisfaction	12	0.87	JS1 JS2 JS3 JS4 JS5 JS6 JS7 JS8 JS9 JS 10 JS 11 JS 12	0.89 0.84 0.86 0.87 0.89 0.90 0.89 0.83 0.89 0.91 0.92 0.88	0.78	0.97
Emotional Labour	9	0.81	EL1 EL 2 EL 3 EL 4 EL 5 EL 6 EL 7 EL 8 EL 9	0.77 0.76 0.70 0.71 0.76 0.73 0.76 0.84 0.71	0.76	0.97

Note: IL: Inner Life, MW: Meaningful Work, SoC: Sense of Community (Dimensions of Workplace Spirituality).

Table II. Heterotrait-Monotrait Ratio (HTMT)

	Original Sample (O)	**Sample Mean (M)**	**Bias**	**2.5%**	**97.5%**
WPS→EL	0.793	0.807	0.014	0.748	0.815
WPS→JS	0.790	0.799	0.009	0.740	0.819
JS→EL	0.340	0.315	0.025	0.231	0.470

Note: WPS: Workplace Spirituality, JS: Job Satisfaction, EL: Emotional Labour. Confidence Interval: lower: 2.5% and upper: 97.5%

Table III. Collinearity Assessment

	Inner VIF Values
WPS→EL	2.660
WPS→JS	1.000
JS→EL	2.660

Note: WPS: Workplace Spirituality, JS: Job Satisfaction, EL: Emotional Labour.

Table IV. Path Coefficients of the Structural Model

Path	Loadings	Std. Dev	T Statistic	P Values
WPS→EL	0.553	0.066	7.998	0.000
WPS→JS	0.799	0.019	41.227	0.000
JS→EL	0.316	0.068	4.989	0.000

Note: WPS: Workplace Spirituality, JS: Job Satisfaction, EL: Emotional Labour.

Table V.Co-Efficients of Determination (R^2)

	R-Square	R-Square Adjusted
JS	0.778	0.777
EL	0.820	0.819

Note: JS: Job Satisfaction, EL: Emotional Labour.

Table VI. Mediation Analysis

Hypothesis	Procedure	Path	Path Coefficient	Indirect Effect	Std. Dev	Total Effect	VAF	T Statistic	P Values
Accepted	Step-1: Direct Effect (without mediator)	WPS→EL	-0.363	n/a				7.998	0.000
Accepted	Step-2: Indirect Effect (with mediator)	WPS→EL	-0.281	n/a		0.554	0.089	4.989	0.000
		WPS→JS	0.799	0.049	0.024				
		JS→EL	-0.223						

Note: P<0.05. WPS: Workplace Spirituality, JS: Job Satisfaction, EL: Emotional Labour.

Table VII. Model Fit

	Saturated Model	Estimated Model
SRMR	0.075	0.061

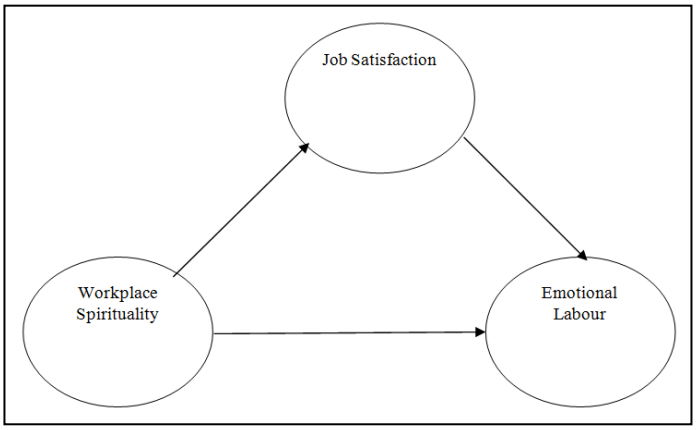

Figure - I. Conceptual Model

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

REVIEW ARTICLE

Medicinal Plants used as Diuretic by the Traditional Healers in the Ganjam District of Odisha: A Review

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Received: 23 Feb 2020 Revised: 25 Mar 2020 Accepted: 27 Apr 2020

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ABSTRACT

Diuretics are the type of antihypertensive drugs that are lesser toxic than the other types of anti hypertensive drugs. Diuretics which are obtained from plants are more safer and lesser toxic than the chemically prepared diuretics. Synthetic drugs which have been used for this are of so many side effects and adverse effects. Some specific organic and inorganic constituents are responsible for the increase or decrease of diuretic potency. There are so many plant species of various genera includes asparagus, piper, aristolochia, taraxacum, psoralea, anthocleista, rumex, clematis, eugenia and boerhaavia are mostly having diuretic constituents in their plant species. The article emphasis on the phytopharmacological investigation beneficial for emerging hypotensive agents.

Keywords: Medicinal plants; kidney; diuresis; oedema.

INTRODUCTION

Diuretics are the substance which increases rate of urine formation and its excretion from the body by inhibiting tubular reabsorption. This can be called as urine volume enhancer (UVE). UVE is a class of antihypertensive drugs used in the treatment of oedema, hypocalcaemia, diabetes insipidus, acute mountain sickness, primary hyperaldosteronism and glaucoma. [1]. According to systolic hypertension in the elderly program (SHEP) [2] and treatment of mild hypertension study (TOMHS), [3] diuretics is the first line therapy of hypertension and this was also supported by the Vth and VIth report of Joint National committee (JNC) on hypertension. The clinical trial study from the year 1967-1990, it reveals that diuretics are the effective agents for hypertension treatment and this can be used as single drug of choice which was recommended by JNC.[4] As per WHO/ISH guideline, 1999 most of antihypertensive drugs can be used for the treatment of hypertension. [5]

According to the European Society of Cardiology/European society of Hypertension a class of thiazide diuretics can be fit instead of other antihypertensive drugs like β-blockers, calcium channel blockers, ACE inhibitors and angiotensin receptor blockers [6] which was also mentioned in the VIIth report of JNC in 2003. [7]. According to WHO, 45% of deaths occur in case of hypertension and 51% of deaths occur in case of stroke throughout the world (WHO, 2013). It was estimated that approximately 40% of world population affected with hypertension and the number of hypertensive patients' increases from 0.6crore in the year 1980 to 1billion in the year 2008 and this shows a measure health problem in the world (WHO, 2011). In the year 2015, it was reached to 1.13 billion with the percentage of prevalence 24 and 20 in men and women globally [8]. The rate of prevalence is more when people crossed 60 years of age irrespective of their income status. [9] It is assessed that the prevalence of hypertension worldwide will cross 1.5 billion in the year 2025 by increasing at a rate of 15-20% per year. [10].

According to antihypertensive and lipid lowering treatment to prevent heart attack trial study it was confirmed that diuretics are less toxic than other classes of antihypertensive drugs due to less incidence of coronary vascularisation, heart failure and myocardial infarction. Synthetic diuretics on prolonged use increase the risk of renal failure, renal carcinoma and cholesterol as well as triglyceride level. [11-13]. Several natural diuretics are been used as antihypertensive agents in South America due to their potential diuretic activities. [15] Crude drugs can be used for a long time [16] and effective in health care system. [17] Natural diuretics may have also anti-inflammatory activity.

MATERIALS AND METHODS

This review was done through an online survey of the ethno pharmacological references, activities and uses of different species of plants. The data are collected from online articles, manuscripts, books, M.Sc. and Ph.D. dissertations available on websites such as Google, Google scholar, Science Direct, Elsevier.

RESULT AND DISCUSSION

From above review we have found that synthetic diuretics are toxic in nature and on prolong use harmful for the body in table-I. It shows that mostly polar extracts like alcoholic, hydro-alcoholic and aqueous extracts are showing diuretic activity (Table-II). From the table III, It shows that mainly decoction and infusion herbal preparations are active diuretic nature. Due to presence of some bioactive organic constituents in plant posses diuretic activity like 2,3-dihydroxy 1,4-dioxane, [35] delphinidin-3-sambubioside, [50] asparagine which is a strong diuretic source of folic acid and selenium ;[48] xanthenes (caffeine, theophylline & theobromine); glycoside (robinin); glucoside (coccinin and it's aglucone part coccinitin which is a dimethyl allyl ester of caffeic acid); flavonoid (isoquercitrin and equicertin); flavones glycoside (wikstroemin); saponin (equisitonin, dimethyl sulphone, thiaminase and aconitic acid); dipeptide (pyroglutamyl glutamine); coumarin derivatives (fraxin, fraxetin & fraxinol); allantoin, sterol, rhabdiol; organic acids (glyceric acid & aspartic acid); oil (terpinen-4-ol); withanolides (withaferin A & witharistatin). [48;51]

Inorganic constituents like salts of potassium e.g. potassium nitrate, potassium malate etc. are having diuretic potency. [48] High sodium content decreases the diuretic potency. Some plants have shown contrasting activity like *Bergenia ligulata* (acetone extract) and Alismatis rhizome (ethanol extract) [52] at low dose shows diuretic and at high dose antidiuretic effect. Many plant species of some specific genus are mostly having diuretic potency like asparagus (Asparagaceae) due presence of asparagine, piper (Piperaceae), aristolochia (Aristolochiaceae) due to presence of aristolochic acid, taraxacum (Asteraceae), psoralea (Fabaceae), anthocleista (Gentianaceae), rumex (Polygonaceae), clematis (Ranunculaceae), eugenia (Myrtaceae), boerhaavia (Nyctaginaceae). [48]

CONCLUSION

Diuretics are one of the best choices for the treatment of hypertension but this should not be recommended for the elderly hypertensive patients. The synthetic diuretic drugs are very potent but these are highly toxic in compare to the natural drugs prepared from the herbal extracts. Natural products which are extracted from the diuretic plants are generally soluble in polar solvents like alcohol mostly ethanol or water or mixture of these two. The diuretic activity of these natural products may be strengthen by incorporating potassium ion into it. From this study, It is observed that out of 17020 genera like asparagus, piper, aristolochia, taraxacum, psoralea, anthocleista, rumex, clematis, eugenia, boerhaavia are having potent diuretic activity as well as antihypertensive activity but till now most of plant species belongs to these are used traditionally by the practitioners as remedy for the treatment of their patients. So further scientific study is necessary for the emerge of many safe and effective diuretic and antihypertensive drugs for the benefit of society.

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Table 1: Side effects of synthetic diuretics [11, 14]

Class of diuretics	Examples	Side effects
Osmotic	Mannitol, sorbitol, glycerol, isosorbide	Hyponatremia
Carbonic anhydrase inhibitors	Acetazolamide, methazolamide, ethoxzolamide, dichlorphenamide	Hyperchloremic, metabolic acidosis, wasting of potassium
Thiazides	Dihydrochlorothiazide, chlorthalidone, metolazone, quinethazone	Hyponatremia, hypokalemia
Loop/high ceiling	Furosemide, torsemide, bumetanide	Hyponatremia, Hyperuricemia Abnormalities of fluid & electrolyte balance
Potassium sparing	Spironolactone, aldactone, canrenone, amiloride, triamterene	Hyponatremia, metabolic hyperkalemic acidosis, chronic renal failure
Mercurials	Meralluride, mercaptomerin sodium, mersalyl	Mercurialism, hypersensitivity, electrolyte depletion, vascular complications

Table II: Herbal diuretics with herbal extract

Plant	Family	Parts used	Extract showing Diuretic activity	Activities other than diuretic
Senna septemtrionalis	Fabaceae	aerial parts	ethanol extract	Anxiolytics, anticonvulsant [18]
Lagopsis supina	Lamiaceae	whole plant	ethanol extract	Anti-inflammatory, Antioxidant [19]
Talinum paniculatum	Portulacaceae	leaves	ethanol extract	Hypotensive [20]
Celosia argentea	Amaranthaceae	leaves	ethanol extract	Hypotensive [21]
Fabiana imbricate	Solanaceae	aerial parts	aqueous ethanol extract	Gastro protective, Anthelmintic [22]
Urtica dioica	Urticaceae	leaves & roots	aqueous extract	Blood purifier, emmenagogue, antifungal, antieczematic [23]
Petroselinum crispum	Apiaceae	seed	aqueous extract	Hypotensive, Antioxidant, anti-inflammatory [24]
Gomphrena celosiades	Amaranthaceae	whole plant	ethanol extract	Anti-hypertensive, antilithiatic [25]
Luehea divaricate	Malvaceae	whole plant	ethanol extract	Hypotensive [26]
Physalis alkekengi L.	Solanaceae	calyxes and fruits	ethanol extract	Expectorants, antieczematic, antidiabetes, antiasthmatic [27]
Piper amalgo	Piperaceae	whole plant	ethanol extract	Antipyretic, Antiinflammatory, Analgesic, Anti-hypertensive [28]
Eugenia brasiliensis L.	Myrtaceae	bark & leaves	hydro-alcoholic extract	Antirheumatic, anti-inflammatory, antinociceptive, astringent [29]
Bidens odorata C	Astreraceae	whole plant	aqueous extract	Antidiabetic, anti-inflammatory, antipyretic, antitussive [30]
Dioscorea septemloba	Diocoreaceae	rhizome	ethanol extract	Anti rheumatic [31]
Eysenhardtia polystachya	Fabaceae	bark	Aqueous extract	Anticancer, analgesic, anti-inflammatory, antidiarrhoeal [32]
Achillea millefolium	Asteraceae	whole plant	aqueous & hydro ethanolic extract	Anti-inflammatory, antidiabetic, antispasmodic [33]
Asphodelus tenuifolius	Asphodelaceae	whole plant	aqueous ethanolic extract	hypotensive, antioxidant, antibacterial [34]
Capsicum annum L.	Solanaceae	fruits	Aqueous extract	diaphoretic, hepatoprotective, antiasthmatic, appetizer [35]
Atractylodes macrocephala K.	Asteraceae	rhizomes	Aqueous extract	Anticancer, neuroprotective, anti-obesity, antiaging [36]
Aloysia Citrodora P.	Verbenaceae	leaves	Aqueous extract	Antidiarrhoeal, antiflatulence, antirheumatic [37]
Alibertia edulis	Rubiaceae	whole plant	aqueous extract	Anti-hypertensive, Antioxidant [38]
Euclea divinorum	Ebenaceae	roots	aqueous extract	Anti-hypertensive, Antioxidant [39]
Rudgea viburnoides	Rubiaceae	leaves	Ethanol extract	Antirheumatic [40]
Terminalia arjuna r.	Combretaceae	bark	hydro alcoholic extract	Cardioprotective [41]
Helichrysum stoechas	Asteraceae	flowers	Aqueous extract	Antiurolithiatic [42]
Hibiscus sabdariffa	Malvaceae	whole plant	Aqueous extract	Anti-inflammatory [43]

Neolamarckia cadamba R.	Rubiaceae	whole plant	Aqueous extract	Antihepatotoxic, antimalarial, analgesic, anti-inflammatory, antipyretic [44, 45]
Scutia buxifolia	Rhamnaceae	bark	hydro ethanolic extract	Cardiotonic [46]
Lippia nodiflora	Verbenaceae	whole plant	Methanol extract	Antirheumatic, antipyretic, analgesic, antimicrobial [47]
Ajuga bracteosa W.	Lamiaceae	leaves	Aqueous extract	Astringent, Febrifuge, Stimulant, Antirheumatic [48]
Andrographis echioides N.	Acanthaceae	whole plant	50% ethanol extract	Febrifuge [48]
Asteracantha longifolia N.	Acanthaceae	whole plant	Aqueous extract of herb ash	Spasmolytics, Hypotensive, Antibacterial, antihepatotoxic [48]
Celosia argentea L.	Amaranthaceae	seeds	alcoholic extract	Antidiarrhoeal, Antibacterial, antiscorbutic [48]
Ceropegia bulbosa R.	Asclepiadaceae	tuber	Aqueous extract	Analgesic [48]
Chukrassia tabularis A.	Meliaceae	bark	50% ethanol extract	Spasmolytic, hypotensive [48]
Cyperus rotundus L.	Cyperaceae	tuber	aqueous alcoholic extract	Hypotensive, Diuretic, Antipyretic, analgesic [48]
Datisca cannabina L.	Datiscaceae	seeds & flowers	50% ethanol extract	Sedative, Anti-inflammatory Analgesic, antipyretic [48]
Dendrophthoe falcate L.	Loranthaceae	whole plant	Alcoholic extract	Antilithiatic [48]
Dysoxylum binectariferum H.	Meliaceae	fruit	50% ethanol extract	CNS depressant, Antiinflammatory [48]
Eruca sativa M.	Brassicaceae	leaf & seed	Ethanol extract	Stimulant, Stomachic, Antiscorbutic, antibacterial [48]
Erycibe paniculata R.	Convolvulaceae	aerial parts	50% ethanol extract	Hypotensive [48]
Eryngium foetidum L.	Apiaceae	aerial parts	50% ethanol extract	Antistrychnine, anti-inflammatory [48]
Jatropha curcas L.	Euphorbiaceae	aerial parts	50% ethanol extract	CNS depressant [48]
Mesua ferrea L.	Clusiaceae	whole plant	Ethanol extract	Hypotensive, astringent, Haemostatic [48]
Sombucus ebulus L.	Caprifoliaceae	whole plant	Aqueous extract	Expectorant [48]

Table III: Herbal diuretics with herbal preparation

Plant	Family	Part used	Herbal formulation showing diuretic action	Activities found other than diuretic
Abelmoschus esculentus L.	Malvaceae	Immature pods	decoction	Emollient, Demulcent [48]
Acanthus ilicifolius L.	Acanthaceae	whole plant	decoction	Antacid [48]
Acanthus mollis	Acanthaceae	Leaves & root	infusion	Analgesic, Anti-inflammatory, Antioxidant [48]

Benincasa hispida	Cucurbitaceae	fruit	decoction	Laxative [48]
Blumea eriantha DC.	Asteraceae	whole plant	cold infusion	Hypotensive [48]
Chenopodium ambrosioides L.	Chenopodiaceae	whole plant	infusion	Sudorific [48]
Clitoria ternatea L.	Fabaceae	root	infusion	Hypotensive [48]
Cucurbita maxima	Cucurbitaceae	seeds	infusion	Antihypertropic of prostate [48]
Cucurbita pepo	Cucurbitaceae	seeds	infusion	Antihypertropic of prostate [48]
Cupressus sempervirens L.	Cupressaceae	whole plant	tincture	Vasoconstrictor, Antiseptic, Sedative, Antispasmodic [48]
Cyperus esculentus L.	Cyperaceae	whole plant	tonic	Indigestion, Antiflatulent Antidiarrhoeal [48]
Dolichos biflorus L.	Fabaceae	seeds	decoction	antilithiatic[48]
Elephantopus scaber L.	Asteraceae	Whole plant	infusion	Antipyretic [48]
Eleusine indica G.	Poaceae	Whole plant	decoction	Stomachic, Febrifuge [48]
Ervataemia coronaria	Apocynaceae	leaves	decoction	Antihypertensive [48]
Helianthus annuus L.	Asteraceae	seeds	decoction	Anticold, Expectrant, Febrifuge [48]
Indigofera enneaphylla L.	Fabaceae	Whole plant	juice	Antiscorbutic [48]
Ipomoea sepiaria	Convolvulaceae	Whole plant	juice	Deobstruent, Hypotensive, Antidote to arsenic poisoning [48]
Morus nigra L.	Moraceae	leaves	infusion	Antidiabetes, Hypotensive [48]
Mussaenda frondosa L.	Rubiaceae	leaves	infusion	Expectrant, Antiasthmatic [48]
Opuntia dillenii H.	Cactaceae	flowers	decoction	Astringent, Haemostatic [48]
Ruellia tuberosa L.	Acanthaceae	Whole plant	decoction	Antibronchitis [48]
Sauropus androgynus M.	Euphorbiaceae	Whole plant	decoction	Antipyretic [48]
Terminalia argentea	Combretaceae	bark	decoction	Antibronchitis, Antiulcer [48]

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Investigation of Mechanical Properties of Concrete with Partial Replacement of Coarse Aggregates by Ferrochrome Slag Aggregates

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ABSTRACT

The lack of lime content encourages the problem of manufacturing of cement in near future. To avoid the consequences many methods and strategies are adopted by researchers to replace cement by different industrial wastes. Similarly, the availability of natural aggregates in earth is also required to be conserved. Hence ferrochrome slag aggregate is used in this project to replace the natural coarse aggregates in different percentages (0%, 15%, 30%, 45%, 60% and 75%) in OPC concrete mixture of M30 grade of concrete. The highest compressive strength achieved from the ferrochrome slag concrete is found out to be 43.95 Mpa with 40% replacement of natural aggregates by ferrochrome slag.

Keywords: ferrochrome slag aggregates, compressive strength, flexural strength, split tensile strength.

INTRODUCTION

It has been noticed that the utilization of waste materials and industrial by-products in place of cement in construction industry is highly appreciated in today's world. Another serious reason behind cement replacement is to reduce carbon dioxide emissions to the atmosphere to dissuade the global warming problem worldwide. [1] These materials can be used in the building construction, bridges, pavements, foundation works, etc. The extensive use of industrial wastes not only reduces the landfill blocks but also helpful in saving the natural raw materials for environmental health. Ferrochrome slag is a waste by-product generated from the chrome manufacturing industry by calcination process at 1700 °C at certain environmental conditions [5,2]. Ferrochrome slag is a high carbon content retainer hence the disposal of this in open environment can be prove to be hazardous to living beings and their surroundings.[4] Around one million tonnes of ferrochrome slag is produced globally in a year and the increasing rate is supposed to be 3-5%. In India, the current production capacity of FeCr is 3.36 million tonnes per annum from 118 plants operating more than 229 furnaces. The replacement of natural aggregates with ferrochrome slag saves the

materials and time consumption in construction by 30-35%.[6] Reddy (2015) stated that the properties of the ferrochrome slag are similar to that of natural aggregates provided for construction purpose.[9]
Prusty et al ., (2017) investigated the structural behaviour of RC beams by using ferrochrome slag as an alternate to conventional aggregates and compare it with ordinary RC beam and concluded that the ferrochrome slag RC beams are possessing high load carrying capacity.[10] The use of ferrochrome slag improves the thermal resistivity and climatic wear and tear.[12]

MATERIALS AND METHODS

Cement

The cement used in the concrete was Portland slag cement with trade name 'ULTRA TECH cement. Physical properties of the cement have been obtained from the tests carried out in the structure laboratory of B.I.T Sindri as per specification laid down in IS: 455-1989. The chemical properties of cement have been obtained from IS: 455:1989. The composition of cement used in the project is provided below in table 1.

Aggregates

Aggregate properties greatly influence the behaviour of concrete, since they occupy about 80% of the total volume of concrete. In this study it was used the sand of zone -II, known from the sieve analysis using different sheet size(10 mm, 4.75 mm, 2.36 mm, 1.18 mm, 600 Micron, 300 Micron, 150 Micron) adopting IS 383:1963. Crushed Stone maximum size of 20 mm and down has been used as coarse aggregate. The properties of the coarse aggregates have been obtained from the tests carried out in the structure laboratory of B.I.T Sindri as per specification laid down in IS: 383-1970. The Indian Standard Recommendation of IS 383: 1970 has been used to find out the properties of coarse and fine aggregate. The proportion in which the coarse aggregate of <20mm size and <10mm size has been used is 60% and 40% respectively.

Ferrochrome slag

The properties of Ferrochrome Slag aggregate used in the present investigation are procured from Ninita Enterprises, Bhubaneswar (INDIA). Physical property of ferrochrome slag aggregate has been obtained from the tests carried out in the structure laboratory of B.I.T Sindri as per specification laid down in IS: 383:1970. The chemical composition data of ferrochrome slag aggregate have been collected from Mugmasteel plant. The chemical properties of ferrochrome slag used in the project are provided below in table 2.

Mix design and curing

Mix design method is preferred to manufacture ferrochrome slag concrete here. IS 456 : 2000 is used as a reference to design the concrete mix. The water-cement ratio is considered from IS 10260 : 209. The mix design is done for M30 grade of concrete.Ferrochrome slag aggregate (FSA) is mixed with the concrete mixture by replacing the coarse aggregate material in 0%, 15%, 30%, 45%, 60% and 75% respectively with adopting the W/C ratio as 0.44. The mix proportion data is detailed below in table 3 and 4. The dry ingredients are all mixed first and then water is added to it slowly to make a homogenous mixture and then casted in cubes of 150 mmX150 mmX150 mm and cylinders of 150 mm diameters. 36 cubes and 36 cylinders were casted in total for the hardened state tests of ferrochrome slag concrete samples in 7 and 28 days.

RESULTS AND DISCUSSIONS

Workability

Workability of concrete defines the ease of handling the manufactured concrete. Here in this project slump test is performed to identify the workability of concrete. The ferrochrome slag concrete achieves a slump value of 67 mm-74 mm for all the three mixes. The acceptable slump is obtained from 0.45 W/C ratio. The slump values obtained from replacing ferrochrome slag by coarse aggregate with 0.44 W/C ratio are given below in table 5.

Compressive Strength

The compressive strength of the ferrochrome slag concrete is examined in CTM machine. The compressive strength of concrete with partial replacement of natural coarse aggregate @ 0%, 15%, 30%, 45%, 60% and 75% by ferrochrome slag aggregate increases up to 40% and beyond 75% it decreases. Up to 75% replacement of coarse aggregate by ferrochrome slag aggregate dust gives good result in strength than normal concrete for M-30 grade. The compressive strengths of ferrochrome slag concrete are given below in table 6.

Split Tensile Strength

The split tensile strength of concrete is investigated with addition of ferrochrome slag aggregate. It has seen that the addition of ferrochrome aggregate increases split tensile strength up to 60%. The split tensile strengths are provided below in table 7.

Flexural Strength

The flexural strength of concrete is investigated with addition of ferrochrome slag aggregate. It has seen that the addition of ferrochrome slag aggregate increases strength up to 60% and beyond 75% it decreases. The flexural strength values are given below in table 8.

CONCLUSIONS

Slump value of concrete with partial replacement of coarse aggregate by ferrochrome slag aggregate decreases as increases the percentage of ferrochrome slag aggregate from 0% to 50%. Compressive strength of concrete with partial replacement of coarse aggregate by ferrochrome slag aggregate increases up to 40% at both 7 days and 28days and beyond 40% starts decreases. The compressive strength of concrete attained maximum at 40% ferrochrome slag aggregate. The increased in the compressive strength is 5.85% and 9.62% at 7 days and 28 days respectively. The slump value of concrete decreases with addition of coarse aggregate by ferrochrome slag aggregate. The compressive and split tensile strength of concrete with replacement of coarse aggregate by ferrochrome slag aggregate increases up to 60% and beyond 75% it decreases. The compressive strength of concrete with constant replacement coarse aggregate by ferrochrome slag aggregate increases the compressive strength by 15.44% and 25.96% at 7 days and 28 days respectively with respect to normal concrete.

The split tensile strength of concrete with constant replacement of coarse aggregate by ferrochrome slag aggregate increases in the split tensile strength by 31.27% and 34.36% at 7days and 28 days respectively in comparison to normal concrete. A huge quantity of ferrochrome waste can be managed in preparation of good quality and sustainable green concrete that has ecological benefits.The basic properties of ferrochrome mixture like relative density, Bulk density and impact worth area unit higher than standard coarse mixture that indicates that the standard of fabric is nice and therefore the concrete made victimization the ferrochrome mix can have high Density.

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Table 1: composition of cement

Components	SiO2	$Al2O_3$	$Fe2O_3$	CaO	MgO	SO_3	Na_2O_3
%	19.71	5.20	3.73	62.91	2.54	2.72	0.25

Table 2: chemical composition of ferrochrome slag

Oxide composition	SiO_2	Fe_2O_3	Al_2O_3	Coo	MgO	SO_3	K_2O	Na_2O	Cl	Loss in ignition
%	59.7	1.4	7.9	23.2	5.7	0.3	0.12	0.02	0.05	0.60

Table 3: mix proportions for trial mixes

Mix	M1	M2	M3
W/C ratio	0.45	0.44	0.43
Cement (Kg/m³)	425.73	643.00	1123.99
Fine aggregates (Kg/m³)	643.00	636.52	627.74
Coarse aggregates (Kg/m³)	1123.99	1122.69	1120.30

Table 4: mix proportions of ferrochrome slag concrete in kg/m³

Mix	% FSA	Cement	Fine aggregates	Coarse aggregates	FSA
30	0	435.41	636.52	1122.69	-
	15	435.41	636.52	954.28	168.40
	30	435.41	636.52	785.88	336.80
	45	435.41	636.52	617.47	505.21
	60	435.41	636.52	449.07	673.61
	75	435.41	636.52	298.67	824.01

Table 5: slump value of ferrochrome slag concrete in mm

% FSA	0	15	30	45	60	75
Slump in mm	25	27	29	33	35	37

Table 6: compressive strength in 7 and 28 days in Mpa

% of FSA	CS in 7 days	CS in 28 days
0	27.85	39.40
15	28.74	40.74
30	29.03	41.33
45	29.18	42.52
60	29.48	43.19
75	29.33	42.67

Table 7: Split tensile strength in 7 and 28 days in Mpa

% of FSA	STS in 7 days	STS in 28 days
0	2.59	3.58
15	2.83	3.82
30	3.20	4.25
45	3.35	4.53
60	3.40	4.81
75	3.20	4.34

Table 8: Flexural strength in 7 and 28 days in Mpa

% of FSA	FS in 7 days	FS in 28 days
0	6.0	7.6
15	6.4	8.0
30	7.6	9.6
45	8.4	10.4
60	9.6	11.2
75	8.4	10

Fig. 1: Ferrochrome Slag Aggregate

Fig. 2: Slump Test

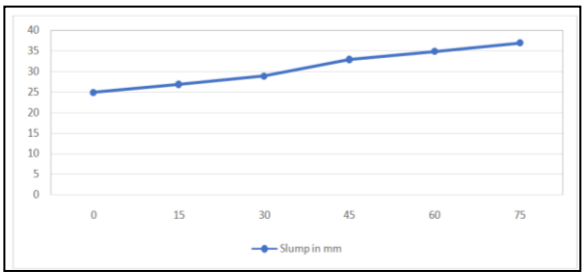

Fig. 3: Slump in mm

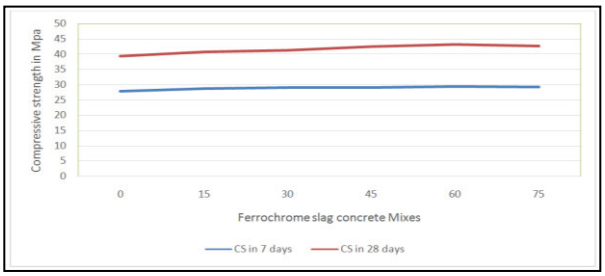

Fig. 4: Compressive strength of ferrochrome slag concrete in 7 and 28 days

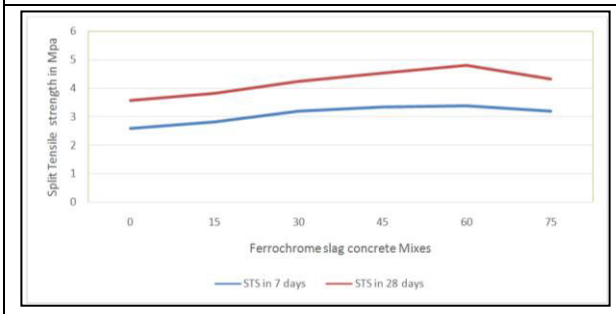

Fig. 5: Split tensile strength in Mpa

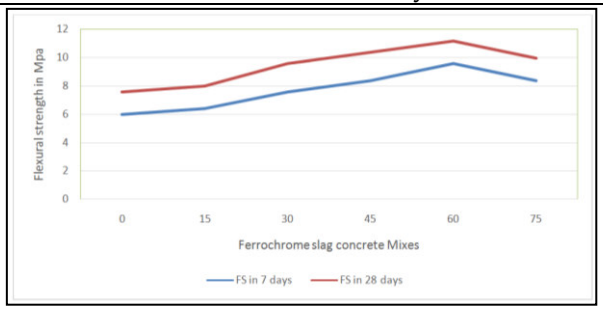

Fig. 6: Flexural strength in 7 and 28 days in Mpa

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Performance Testing of Hybrid Type Solar Dryer by using Multiple Regression Technique

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Received: 17 Feb 2020 Revised: 20 Mar 2020 Accepted: 24 Apr 2020

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ABSTRACT

The present paper represents the experimental investigation on performance testing of Hybrid type of solar dryer for drying food products in a hygienic way. The food products such as ginger, potato, tomato, bread were taken for drying and it was used during day as well as night purpose. The main components were solar flat plate collector (1m^2), drying chamber. The four numbers of DC blowers were used such as at inlet, outlet of collector and also at exhaust of drying chamber. The blowers were operated by solar panel, battery, and charge controller with a proper size. The main objective of the research to have a multi regression analysis in order to get R^2, adjusted R^2 and estimated standard error. Further by using baffle in between the glass plate and absorbing plate, the convective heat losses was reduced as well as turbulence of the heated air inside the collector was increased which was main objective of this research. The Instantaneous efficiency of solar collector and overall loss coefficient were calculated as 48.73% and 2.11 W/$m^2$0c by taking Bhubaneswar Latitude as (20.2961^{o}N, 85.8245^{o}E) .The maximum coefficient of performance was calculated as 0.789 by taking maximum outlet collector temperature and maximum drying chamber temperature.

Keywords: Hybrid dryer, flat plate collector, transparent plate, multiple regression

INTRODUCTION

Traditionally people dry the food products, vegetables and sea foods in an open and unhygienic condition which results poor prices realizations in the market. It also requires more times for drying .Also diesel or electricity operated dryer is mostly cost effective and not environmental friendly. So solar dryer is an alternative and effective method of drying food products in a hygienic way and quick process of drying.

Regression: A way of predicting the value of one variable from another.

- It is a hypothetical model of the relationship between two variables.
- The model used is a linear one with a single input variable
- Therefore, we describe the relationship using the equation of a straight line.
- Test model with R-Square (Coefficient of Determination). R-squared gives you the percentage variation in y explained by x-variables.
- Use the predicted R-square to determine how well the model predicts new observations. Probability of future events occurrence.
- R^2: Coefficient of determination: The proportion of variance accounted for by the regression model.
- ANOVA (Analysis of variance): If the model results in better prediction than using the mean, then we expect SS_M to be much greater than SS_R.
- Multiple Regression is a natural extension of this model:
- We use it to predict values of an outcome from several predictors.
- It is a hypothetical model of the relationship between several variables.
- R: The correlation between the observed values of the outcome, and the values predicted by the model.
- R^2: the proportion of variance accounted for by the model.
- Adj. R^2: An estimate of R2 in the population (shrinkage). It takes into account the number of variables in a data set.

To design a hybrid type solar dryer previous mathematical modeling and simulation have been reported. A.A.EI.Sebaii et al. [1] had done an experimental investigation of an indirect –mode forced convection solar dryer for drying thymus and mint. A blower was provided to circulate heated air inside the drying chamber. A mathematical regression analysis (chi square x^2) was used to calculate the moisture ratio. L.Cano et al [2] had done a mathematical model was done for evaluation of the temperature and specific humidity in the drying chamber. A.A.EI.Sebaii et al. [3] had done experimental work on Indirect solar dryer for drying Thymus cut leaves without using phase change material and also using phase change material (paraffin wax). A new mathematical model (four parameter logistic model) was used to calculate the moisture ratio of Thymus leaves. M.S. Manjunath et al. [4] found that due to the presence of Pin fin, the fluid turbulence increases and also heat transfer rate increased up to 53.8%.F. Tedesco et al. [5] had done an analysis on Passive indirect solar dryer which is integrated with chimney.C. Pardhi et al. [6] had done an performance testing of mixed mode type solar dryer with forced convection. From the experiment he found the absorber plate temperature up to 69.2^0C under no load condition. O.Prakash et al. [7] had done laboratory test of greenhouse dryer under two conditions (firstly covered with concrete floor and secondly when floor was not covered).S. Sunthikun et al. [8] found that the moisture content of rubber sheet reduced from 34.6% to 0.34%.A.Elkhadraoui et al. [9] had done experimental investigation on solar mixed mode greenhouse dryer with forced convection for drying red pepper and grape and found that the drying time for red pepper and grape reduced by 7 and 17 hours. D.Jain et al. [10] had done performance test on natural convective solar crop dryer by using phase change material.

Nomenclature

1. m_w = Mass of water to be removed from fruits
2. m_i = Weight of fruits in kg

3. M_i= Initial moisture content in fruits
4. M_f = Final moisture content in fruits
5. $\dot{m}_a$ = Mass flow rate of dry air in kg/sec
6. C_p = Specific heat in kJ/kg-K
7. T_1 = Inlet temperature of collector
8. T_2 = Outlet temperature of collector
9. L = Latent heat of vaporization in kJ/kg-K
10. ρ_a = Density of air in kg/m^3
11. A_c = Collector area in m^2
12. H_o = Monthly average daily global radiation on horizontal surface kJ/m^2-day
13. H_d = Daily diffuse radiation on a horizontal surface in kJ/m^2-day
14. H_g =Daily global radiation on a horizontal surface in kJ/m^2-day
15. n = Number of days of the year
16. δ = declination angle in degree
17. φ = Latitude angle of Bhubaneswar.
18. w_s= Sunrise hour angle
19. S_{max} = Day length
20. E_L = Elevation from sea level
21. a_1 and b_1 = Constants
22. I_o = Monthly average of the hourly extra-terrestrial radiation on a horizontal surface (kJ/m^2-h)I_g = Monthly average of the hourly global radiation on a horizontal surface (kJ/m^2-h)
23. w= Hour angle
24. LAT = Local apparent time
25. I_d = Hourly diffuse radiation on a horizontal surface in kJ/m^2-h
26. θ = Zenith angle
27. r_b = Tilt factor for instantaneous/hourly beam radiation
28. r_d = Tilt factor for instantaneous/hourly diffuse radiation
29. I_t = Instantaneous/hourly flux incident of tilted surface.
30. Q_U= Rate of useful heat gain in Watt
31. η_i= Instantaneous efficiency of solar collector
32. $(\tau\alpha)_e$= effective transmittance absorptance product
33. τ= Transmittance of glass plate
34. α= Absorptance of the absorbing plate
35. U_L= Overall loss coefficient in
36. T_p= Absorbing plate temperature in ^{0}C.
37. T_{sc}= maximum Solar collector temperature in ^{0}C
38. T_{rm} = maximum drying chamber temperature in ^{0}C
39. T_{am}= Atmospheric temperature in ^{0}C
40. F_R= Heat removal factor
41. d= diameter of DC blower at outlet of collector
42. Mathematical Modelling

m_i = 20 kg
M_i= 71%
M_f = 20%

$$m_w = \frac{m_i (M_i - M_f)}{(1 - M_f)} = \frac{20(0.71 - 0.2)}{1 - 0.2}$$ =12.75 kg of water to be removed from 20 kg of wet fruits D.D Behera et.al [14]

$$\dot{m}_a C_p (T_2 - T_1) = m_w L$$

$$\dot{m}_a = \frac{m_w L}{C_p (T_2 - T_1)} = \frac{12.75 \times 2260}{1.005(72-45)} = 1061.912 \text{ kg of dry air}$$

Assuming 24 hours to be dried

$$\frac{1061.912}{24 \times 3600} = 0.01229 \text{ Kg/sec of dry air}$$

Volume flow rate = $\frac{\dot{m}_a}{\rho_a} = \frac{0.01229}{1.225} = 0.01003 \text{ m}^3/\text{sec}$

Bhubaneswar latitude = 20.2961°N, 85.8245°E on 1st May

n = 31+28+31+30+31 = 151

$$\delta = \text{Declination} = 23.45 \sin\left[\frac{360}{365}(284+151)\right] = 21.8984^o \quad [14]$$

Ws = $\cos^{-1}(-\tan\phi \tan\delta) = \cos^{-1}[-\tan(20.2961)\tan(21.8984)] = 98.5458$ = sunshine

Hour angle = 1.719 radian

Day length $S_{max} = \frac{2}{15}(98.5458) = 13.139$ hours

H_o = Monthly average daily global radiation

$$= \frac{24}{\pi} \times 1.367 \times 3600 \times \left(1 + 0.033 \cos\left(\frac{360}{365}\right) \times 151\right) \{1.42 \times \sin 20.2961 \times \sin 21.8984 + \cos 20.2961 \times \cos 21.8984 \times \sin 98.5458\}$$

= 234694.72 kJ/m²-day

By taking E_L = Elevation from sea level = 45 m = 0.045 km

Average sunshine hours per day => $\overline{S}$ = 11 hours

$a_1 = -0.309 + 0.539\cos\varphi - 0.0693 \times E_L + 0.29\left(\frac{\overline{S}}{S_{max}}\right)$

= 0.436

$b_1 = 1.527 - 1.027\cos\varphi + 0.0926 E_L - 0.359\left(\frac{\overline{S}}{S_{max}}\right)$ = 0.267

$$\frac{\overline{H}_g}{H_o} = a_1 + b_1\left(\frac{\overline{S}}{S_{max}}\right) = 0.436 + 0.267\left(\frac{11}{13.139}\right)$$

= 0.659

$\overline{H}_g$ = 154862.63 kJ/m²-day

$$\frac{\overline{H}_d}{H_g} = 1.411 - 1.696\left(\frac{\overline{H}_g}{H_o}\right) = 1.411 - 1.696\left(\frac{154862.63}{234694.72}\right)$$

= 0.292

$\overline{H}_d$ = 0.292 × 154862.63 = 45219.88 kJ/m²-day

$$w = 11.49 \times \frac{15}{60} 2.8725^o$$

a = 0.409 + 0.5016 sin (w_s - 60)

= 0.721

b = 0.6609 - 0.4767 sin (w_s - 60)

= 0.3639

$$F_c = a + 0.5b\left[\frac{\frac{\pi w_s}{180} - \sin(w_s)\cos(w_s)}{\sin(w_s) - \frac{\pi w_s}{180}\cos(w_s)}\right]$$

= 0.895

$$I_o = 1.367\left[1 + 0.33 \times \cos\left(\frac{360}{365}\right) \times n\right] \times \left[\sin\phi\sin\delta + \cos\phi\cos\delta\cos w\right] = 532.765 \text{ kJ/m}^2\text{-h}$$

$$\frac{I_g}{H_g} = \frac{I_o}{H_o}(a + b\cos w)/F_c$$ = 425.59 kJ/m²-h

$$\frac{I_d}{H_d} = \frac{I_o}{H_o}$$

I_d = 102.65 kJ/m²-h

Zenith angle = cos θ = $\sin\phi\sin\gamma + \cos\phi\cos\gamma\cos w$ θ = 20.495°

$$r_b = \frac{0.936}{\sin\phi\sin\delta + \cos\phi\cos\delta\cos w}$$

= 0.9378

$$r_d = \frac{1 + \cos\phi}{2} = \frac{1 + \cos(20.296)}{2} = 0.9689$$

$$r_r = \frac{0.2(1-\cos\phi)}{2} = \frac{0.2(1-\cos(20.296))}{2}$$

$= 6.20 \times 10^{-3}$

$$\frac{I_T}{I_g} = \left(1-\frac{I_d}{I_g}\right) r_b + \frac{I_d}{I_g} r_d + r_r = \left(1-\frac{102.65}{425.59}\right) 0.9378 + \frac{102.65}{425.59} 0.9689 + 6.20\times10^{-3}$$

$I_T = 404.94$ W/m^2

Assuming 50% instantaneous efficiency

$$\eta = \frac{\dot{m}_a C_p (T_2 - T_1)}{A_c \times I_T}$$

$$0.5 = \frac{0.01229 \times 1005(72-45)}{A_c \times 404.94}$$

$$A_c = 1.647\,m^2$$

$$A_c = L \times B = 1.28 \times 1.28$$

EXPERIMENTAL SET UP

The figure 1 shows the experimental set up of Hybrid type of solar dryer. The sizing of the collector and drying chamber were carried out by considering various parameters such as atmospheric temperature, relative humidity and the amount of food products to be dried. The collector was tilted as per the latitude of Bhubaneswar location (20.2961^{o}N, 85.8245^{o}E).So the Collector was tilted with 20^0 and facing due south so that more amount of sun radiation incident on the collector. There was proper circulation of heated air inside the drying chamber due to the presence of two numbers of dc blowers at outlet of collector. The test was conducted in the month of February by taking food products such as Potato, tomato, ginger, bread. The two numbers of glass plates were provided to enhance the transmittance power as well to reduce the losses due to re radiation. The baffle was provided to reduce the convective heat losses as well as to increase the turbulence effect of heated air inside the collector. The dryer was used during the night purpose by using heated coil which was run by inverter and battery.

RESULT ANALYSIS AND DISCUSSION

To do the performance testing of Hybrid type of Solar Dryer, the dryer was taken in an open shadow free location where the maximum sun radiation was incident on the surface. It was facing due south and was tilted according to the latitude of that location. The various parameters like temperature, Absolute humidity, wind velocity, velocity of blowers at various point of dryer such as at inlet of the collector, on glass plate outlet of the collector, in the drying chamber and at outlet of drying chamber at a regular interval of time. It was tested throughout the day in a sunny day to dry the ginger, bread, potato, tomato. The Infrared Thermometer, hygrometer, Anemometer, weight measuring machine were taken to measure the temperature, Absolute humidity, atmospheric wind velocity, velocity of blowers and percentage of weight reduction. The experiment started first day to measure various parameter like relative humidity, temperature, wind velocity and diameter of ginger. The relative humidity, temperature and wind velocity obtained from the experiment are shown in table below

Initial weight of ginger= 233 gm and Final weight of ginger= 55gm
Determination of Moisture content on weight basis

$$=\frac{initial\ weight-final\ weight}{initial\ weight}\times 100=\frac{233-55}{233}\times 100=76.39\text{gm/m}^3$$

Time required for drying potato=5 hours

Rate of reduction of ginger $=\frac{initial\ weight-final\ weight}{time\ taken}=\frac{233-55}{5}=35.6\%$

The experiment started 3rd day to measure various parameters like relative humidity, temperature, wind velocity and diameter of potato. The relative humidity, temperature and wind velocity obtained from the experiment are shown in table below.
Determination of Moisture content on weight =76.83 gm/m³
Rate of weight reduction of potato or drying rate = 46.1%

The experiment started 4th day to measure various parameter like relative humidity, temperature, wind velocity and diameter of tomato. The relative humidity, temperature and wind velocity obtained from the experiment are shown in table below
Determination of Moisture content on weight basis = 92.77gm/m³ = 95.8%
This experiment has been conducted to measure temperature at night. The experiment started to measure temperature of bread. Parameter temperature is observed hour to hour interval.

Performance analysis of Hybrid solar Dryer

3.1.1 Calculation of Instantaneous Efficiency= $\eta=\frac{\dot{m}_a C_p(T_2-T_1)}{A_c\times I_T}$

$\dot{m}_a = \rho_a\times A\times V_{air}=\rho_a\times\pi/4\times d^2\times V_{air}$

η= Instantaneous efficiency of solar collector=48.73%
Q_u=Useful heat gained=$\dot{m}_a\times C_p\times (T_2-T_1)$ =197.356 W
$Q_u = A_c [I_T(\tau\alpha)_e - U_L(T_P-Ta)]$ [12]
=0.811
197.356=1[404.94×0.811- U_L (95-33)
U_L=2.11W/m²⁰C
$Q_u/A_c=F_R[I_T(\tau\alpha)_e-U_L(T_2-T1)]$ = 0.8
Heat utilization factor= $[T_{sc}-T_{rm}/T_{sc}-T_{am}]$ = 0.22
Coefficient of performance (COP) = $[T_{rm}-T_{am}/T_{sc}-T_{am}]$ =64-33/72-33=0.7948

Table 1.Regression Statistics (Output Model summary

Multiple R	0.58962853	ANOVA				
R Square	0.347661803		*df*	*SS*	*MS*	*F*
Adjusted R Square	0.021492705	Regression	2	7.375397	3.687698	1.065894363
Standard Error	1.860032855	Residual	4	13.83889	3.459722	
Observations	7	Total	6	21.21429		

	Coefficients	***Standard Error***	***t Stat***	***P-value***	***Lower 95%***	***Upper 95%***
Intercept	-10.30555556	32.19902	-0.32006	0.764945	-99.7044	79.09326738
Time(AM/PM)	-1.733333333	8.621752	-0.20104	0.850475	-25.6712	22.2044871
Atmospheric Humidity(Gm/m3)	1.511111111	1.037477	1.456525	0.218967	-1.36939	4.391608379

Observation	Predicted Drying Chamber Humidity (Gm/m3)	Residuals
1	35.81666667	-1.316666667
2	36.5	-1
3	37.18333333	2.316666667
4	37.86666667	0.133333333
5	34.77222222	1.727777778
6	37.72222222	-0.222222222
7	36.13888889	-1.638888889

Table 2. Regression Statistics(Output Model summary)

Multiple R	0.975934715	ANOVA				
R Square	0.952448567		*df*	*SS*	*MS*	*F*
Adjusted R Square	0.857345701	Regression	4	325.4653	81.36632044	10.01493024
Standard Error	2.850351201	Residual	2	16.249	8.124501972	
Observations	7	Total	6	341.7143		

	Coefficients	Standard Error	t Stat	P-value
Intercept	-18.77571127	40.69749941	-0.46135	0.6759419
Time(AM/PM)	-56.1114265	26.69200005	-2.10218	0.1263027
Atmospheric Temperature in 0c	0.596161463	1.440664675	0.41381	0.7068176
Inlet collector Temperature in 0c	2.331556538	0.860883188	2.70833	0.0732678

RESIDUAL OUTPUT

Observation	Predicted Outlet collector Temperature in 0c	Residuals
1	55.59845534	-1.598455344
2	59.11591524	1.884084758
3	64.96493168	2.035068321
4	70.7608588	1.239141197
5	61.42821308	-5.428213083
6	53.83096244	-0.83096244
7	50.30066341	2.69933659

Table 3. Regression Statistics (Output Model summary)

Multiple R	0.99634952	ANOVA					
R Square	0.99271236		*df*	*SS*	*MS*	*F*	*Significance F*
Adjusted R Square	0.98542471	Regression	3	14.86286	4.954287014	136.2186	0.001053847
Standard Error	0.19070954	Residual	3	0.10911	0.036370129		
Observations	7	Total	6	14.97197			

Intercept	1.01637426	0.69660855	1.459	0.2407	-1.20055	3.23329
Time	21.7673808	1.37946808	15.78	0.0006	17.3773	26.1575
Atmospheric air velocity in meter/sec	0.72092211	0.20280681	3.5547	0.038	0.0755	1.36634
Inlet collector air velocity in meter/sec	-3.3424808	0.25019801	-13.36	0.0009	-4.13872	-2.5462

Observation	Predicted Outlet collector air velocity in meter/sec	Residuals
1	4.48909753	-0.13909753
2	4.57028204	0.20971796
3	5.22427621	-0.01427621
4	7.85622873	-0.05622873
5	8.4800793	0.0899207
6	6.57805502	-0.16805502
7	6.73198118	0.07801882

Table 4. Regression Statistics (Output Model summary)

Multiple R	0.981416696	ANOVA					
R Square	0.963178732		*df*	*SS*	*MS*	*F*	*Significance F*
Adjusted R Square	0.779072389	Regression	5	244.3722	48.87444	5.231643	0.319796
Standard Error	3.056481935	Residual	1	9.342082	9.342082		
Observations	7	Total	6	253.7143			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	-9.550742067	67.26755677	-0.142	0.9102	-864.26609	845.1646
Time(AM/PM)	-3.914898687	30.22891993	-0.1295	0.918	-388.009744	380.1799
Atmospheric Temperature in 0c	0.085026845	2.121416287	0.04008	0.9745	-26.8701228	27.04018
Inlet collector Temperature in 0c	0.360740429	0.270814558	1.33206	0.41	-3.08028479	3.801766
Outlet collector Temperature in 0c	0.816786063	0.208330659	3.92062	0.159	-1.83030595	3.463878

Observation	Predicted Drying Chamber Temperature in 0c	Residuals
1	49.91185297	0.088147028
2	63.81442731	0.185572691
3	55.82370594	0.176294057
4	52.67943286	-1.67943286
5	46.35304236	1.646957636
6	33.41753856	-0.41753856

DISCUSSION

The figure 1 shows the drying of ginger for making ginger powder in a sunny day (on 18th February, 2019). It was taken 6 hours for drying the ginger. The initial and final weight of ginger was measured to calculate the percentage of weight reduction. The table 1 and 2 represents the model summary output of multiple regression analysis and R^2, Adjusted R Square and standard error. The predicted value of drying chamber humidity, outlet collector temperature, drying chamber temperature were calculated by taking independent variable such as time, inlet collector temperature and atmospheric temperature.

CONCLUSION

The maximum temperature reached with 72^0C at outlet of solar collector where as 64^0C inside the drying chamber. So there were some heat losses from the collector to drying chamber and over all heat loss was calculated as 2.11W/m2^0C.Further it was observed that at exhaust of drying chamber the temperature lost by 55^0C for which it was again recirculated inside the collector by connecting a pipe from exhaust of drying chamber to the inlet of collector. The maximum absorbing plate was found as 95^0C. R^2, Adjusted R Square and standard error were found as 0.98899634, 0.9449817 and 2.404285618. The coefficient of performance was calculated 0.7948 where as Percentage of heat with respect to ambient air temperature was calculated as 0.55.

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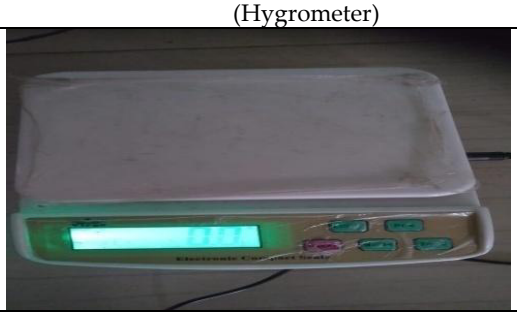
Figure 1. Experimental set up of front side of Hybrid type of Solar dryer.
1. DC blower at inlet of collector, 2. Absorbing plate, 3. Baffle 4. Drying Chamber, 5. Solar Panel

(Anemometer)

(Hygrometer)

(Infrared Thermometer**)**



(Weight Machine)

Figures 2 showing various types of measuring instruments during testing and analysis

Figure 3.showing drying of ginger in 1st day of observation

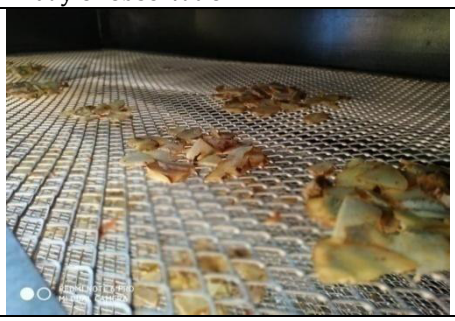


Figure 4.showing drying of potato in 2nd day of observation

Figure 5.showing drying of tomato in 3rd day of observation

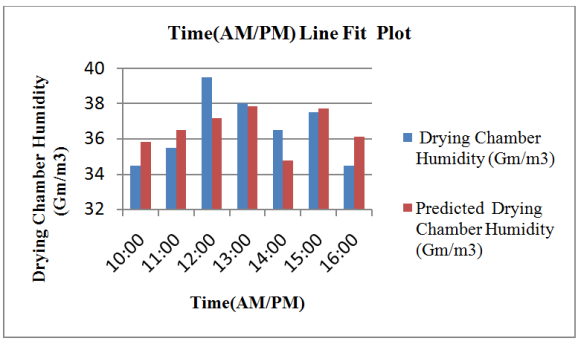


Figure-6 shows the predicted drying chamber humidity and time

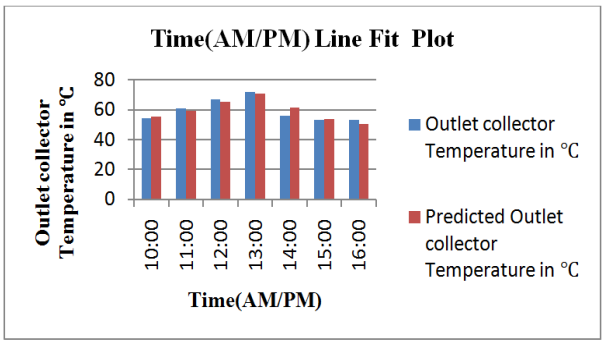


Figure 7. Shows the predicted outlet collector temperature with time

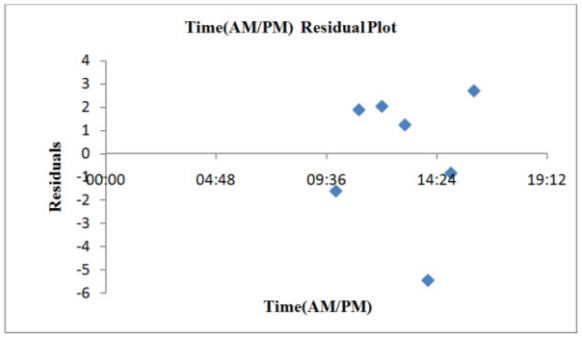


Figure 8. Shows the residual plot for predicted outlet collector temperature with time

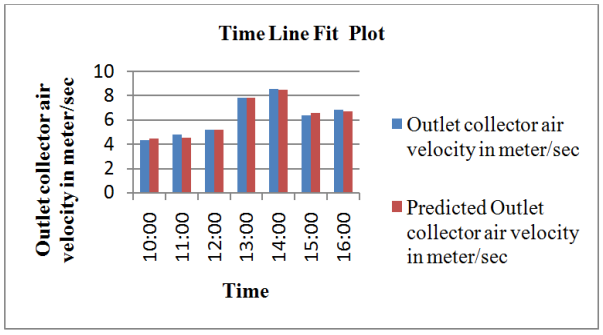


Figure 9. Shows predicted outlet collector air velocity with time

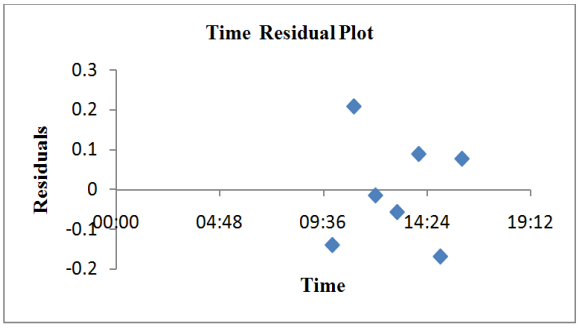


Figure 10. Shows residual plot of predicted outlet collector air velocity with time

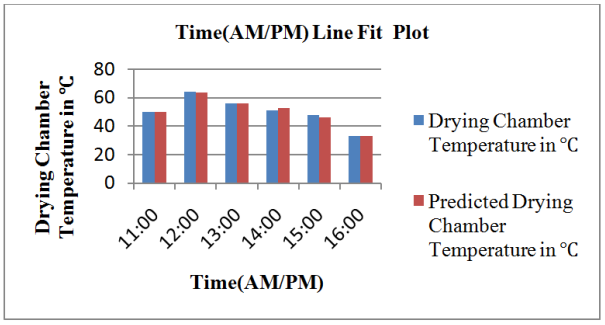


Figure 11. Shows predicted drying chamber temperature with time

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Detecting Malicious URLs Using Machine Learning Techniques

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Received: 21 Feb 2020 Revised: 24 Mar 2020 Accepted: 27 Apr 2020

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ABSTRACT

The World Wide Web supports a wide range of activities such as financial frauds, malware dissemination etc. People visit these sites unaware of the danger. These visits can be driven by email, web search results or links from web pages of social media sites. From these sites attackers or hackers can steal the important data of the user such as, Personal information, Bank Account details, Business Information etc. and can destroy anyone's Pc, mobile which leads to data loss and become a big problem. However in such cases users is required to take some actions such as clicking the URLs.In order to identify these links a full-stack website is proposed. This study prposes the detection of malicious URLs. It is a binary classification problem.It shows only two outputs either the URL is malicious or not. Several classifiers namely K-Nearest Neighbor, Support Vector Machine, Logistic Regression, Decision Tree and Random Forest have been studied, simulation has been done using Python and the results are compared. We used a Kaggle Dataset having 411275 samples. It contains URLs and Labels. Random Forest attains the highest accuracy. We compare the Accuracy Score, Precision score and Recall Score.

Key words: Malicious URLs;Classification; Random Forest; Decision Tree; Logistic Regression; SVM and KNN

INTRODUCTION

Now-a-days the human understandable URLs are used to identify billions of websites presented over the internet. Rivals or Opponents who try to get illegal access to the confidential data may use malicious URLs and present it as a legitimate URL to naive user. Such URLs that act as a path for the uninvited activities are called as malicious URLs. These malicious URLs can cause unethical activities such as theft of private and confidential data, ransom ware installation on the user devices that result in huge loss every year globally. Even security agencies are alert about the malicious URLs as they have the ability to compromise confidential and sensitive data of private organizations and government. With the improvement of social networking platforms, many allow its users to publish the illegal URLs.

Many of these URLs are related to the promotion of business and self-advertisement, but some of these unprecedented resource locators can present a vulnerable threat to the naive users. The naive users, who use the malicious URLs, are going to face serious security threats initiated by the Rivals or opponents. The detection of URLs is very essential in order to ensure that user should be prevented from visiting malicious websites. Many mechanisms have been proposed to detect the malicious URLs.

In this project, a Website is proposed to check whether a given URLs are malicious or not using Machine Learning Techniques. In this we are using a classification problem which only detects whether the urls are malicious or not. Classification Algorithmssuch as Support Vector Machine (SVM), K-Nearest Neighbor (KNN), Random forest, Decision Tree and Logistic Regression is used for the prediction of malicious URLs. In Detection of Malicious URL, the aim is to predict the given input and shows the result whether it is malicious or not. The project focuses on the use of Classifier Model based Machine learning to predict the URLs given as input. And it also compares the Accuracy score, Precision Score and Recall Score to choose the better Algorithm to detect the Malicious URLs.

RELATED WORKS

Vanhoenshoven, Frank(2016) [1] used six algorithm techniques to detect the malicious URLs and and demonstrated that Random Forest appears to be the most appropriate classification algorithm for this problem, followed by MLP. And also achieves high scores for both precision and recall. Sahoo, Doyen, Chenghao Liu, and Steven CH Hoi (2017) [2] categorized the existing contributions for malicious URL detection in literature and identified the challenges developing for malicious URL detection as a service for real world. Also highlighted some practical issues for the application domain and indicated some important open problems. Le, Hung, et al (2018) [3] proposed character CNN and Word CNN for malicious URL detection and jointly optimized the network and also proposed advanced word-embedding techniques which are used to deal with rare words. Islam, Mazharul, and Nihad Karim Chowdhury (2016) [4] used various classification algorithms and demonstrated that Random Forest Gives better accuracy than the other classifiers with accuracy rate 97.47%. Kulkarni, Arun (2019) [5] used four algorithms and found that decision tree needs to be pruned to work well with the testing dataset. The pruned decision tree provided the highest classification accuracy 90.39 percent.

PROBLEM DESCRIPTION

Now a days, attackers and hackers have an advantage to the Internet as their channel to attack the client/users device to gain information using fake URLs.In this project, we have developed a full stack website using machine learning Technique to identify the malicious URL's to avoid the attacker and hackers.Data must be taken from a valid source. Now a day data are already available preprocessed. So we directly used the kaggle data then tokenize it. These are URLs so got to use own functions then Vectorization is done. After Feature extraction splitting of data takes place. Here 70% of data used for training purpose and 30% of data used for testing purpose. Then various models are applied and tested by the accuracy score and which model gives best accuracy is used to perform prediction. Fig 1 shows the flow diagram.

METHODOLOGY

Classification Techniques

Classification is a common scenario in real world problems and most available models are inspired by human reasoning and behavior when facing such scenarios. This problem consists in assigning the correct category to a new pattern of the problem, given a set of alternative responses. Patterns are normally described by a set of attributes which could be numerical, categorical or both. Formally speaking, the pattern classification problem is about building a mapping $:\mathcal{U}\rightarrow\mathcal{D}$that assigns to each pattern/object $x\in\mathcal{U}$described by the attribute set $\Psi=\{\psi 1\ ...,\psi M\}$ a

decision class d from the k possible ones in $\mathcal{D} = \{D1,...,Dk\}$. Generally, the mapping is learned using a supervised approach, i.e., using an available set of historical data about the classification problem for training the model.

Decision trees

A decision tree is a tree-like graph structure, where each node represents a test on an attribute. Each branch represents the outcome of the test and the leaf nodes represent the class label obtained after all decisions made through that branch. The paths from root to leaf represent classification rules. The goal is to represent the data while minimizing the complexity of the model. Several algorithms for constructing such optimized trees have been proposed. for example, derives from the well-known divide-and-conquer technique and have been widely used in several application fields, being one of the most popular machine learning algorithms. This scheme builds decision trees from a set of training data using the concept of information entropy. At each node of the tree,chooses the attribute of the data that most effectively splits its set according to the normalized information gain (difference in entropy). It is important to note that this simple, yet efficient technique, is capable of handling missing values in the data sets and both numerical and categorical attributes.

K-nearest neighbor

The k-Nearest Neighbors algorithm (kNN) is a method used for classification and regression. For the inference process, this technique determines the k closest training examples to the testing instance. The output in a classification context is the mode of the class values of the selected neighbors. In case of regression is the mean of the values of its k nearest neighbors. Therefore kNN is a type of instance-based learning, or lazy learning, where the function is only approximated locally and all computation is deferred until classification. The use of weights for the neighbors is a common technique and can be based on the distance to the instance in question. A commonly used distance metric for continuous variables is Euclidean distance, or Hamming distance for discrete features. The neighbors are chosen from historical data, but no learning algorithm is performed. A shortcoming of the kNN algorithm is that it is sensitive to the local structure of the data.

Random Forest (RF)

It is a well-known ensemble learning method for supervised classification or regression. This machine learning technique operates by building an ensemble of random decision trees at training time and outputting the class that is the mode of the classes (classification) or means prediction (regression) of the individual trees. Therefore a RF is a classifier consisting in a collection of tree structured classifiers which uses random selection.We set the hyper parameter for test size is 0.2 and random state is 42 and then trained the model. It takes some time based upon your CPU and GPU performance. For us it took 2 minutes to complete the process. And finally we got the accuracy rate of 0.97 or 97%.

Support Vector Machine

Support Vector Machines (SVM) is another powerful supervised learning technique that can be used for classification and regression analysis. The base idea of SVM training algorithm starts from a given set of training examples (support vectors), where each one is marked for belonging to one of two categories, as a non-probabilistic binary linear classifier. Then, the SVM can be seen as a representation of the examples as points in space, with the goal of separating examples in categories divided by a clear gap. More formally, SVM tries to find a hyperplane or set of hyperplanes in a high- or infinite-dimensional space, such as a good separation is achieved by the hyperplane that has the largest distance to the nearest training-data point of any class (functional margin), minimizing the generalization error of the classifier. Unclassified instances then are mapped into the same space for classification.

Logistic Regression

Logistic regression is a classification algorithm used to assign observations to a discrete set of classes. It transforms its output using the logistic sigmoid function to return a probability value. It is a predictive analysis algorithm and based on the concept of probability.Logistic Regression uses a more complex cost function, this cost function can be defined as the '**Sigmoid function**' or also known as the 'logistic function' instead of a linear function. The hypothesis of logistic regression tends it to limit the cost function between 0 and 1.

FEATURE EXTRACTION

The data set is already preprocessed in Kaggle. To extract the features Tokenization and vectorization has to be done.Tokenization means splitting the data into small-small tokens. And Vectorization is the technique of converting the tokens into Sparse Matrix. Because these are URLs so Tokenization is done by the functions created by us. Split the URLs on the basis of Dash, Dot or slash. Then by using that tokenization,Vectorization is done. Where TF-IDF vectoriser is used. It converts the URLs into Sparse matrix.

PERFORMANCE EVALUATION, SIMULATION RESULTS AND DISCUSSION

Performance Evaluation

We tested the System by giving some inputs. In this paper, URLs of facebook.com and google.com are considered as the inputs.

Project Phases and Schedule

Phase-1: Preparing the System with Machine Learning Environment.
Phase-2: Getting the Datasets.
Phase-3: Develop code using ML code using Python.
Phase-4: Develop a website using html and css .
Phase-5: Link Python code to website using Flask
Phase-6: Testing the Project.

Simulation Results and Discussion

Given the massive of data available, 5000 rows were taken, where good and bad urls were shuffled. And 70% of data added to the training set and 30% of data is employed for testing purpose. Testing set contains all URLs that have not been used for training. The experimental results have been achieved by calculating the prediction accuracy.In order to gauge the models, we use a metric. Table 1 shows the simulation results obtained from different models used in this paper

Accuracy can be seen as the overall success rate of the method in terms of predictions.

$$accuracy = TP + TN / TP + TN + FP + FN \quad (1)$$

Precision is the ratio of positive predictions that are correctly classified.

$$precision = TP / TP + FP \quad (2)$$

Recall is a measure of how many truly relevant results are returned.

$$recall = TP / TP + FN \quad (3)$$

The formula's used in Eq. 1, 2, and 3 uses the following symbols:
TP: number of true positives, malicious URLs that are classified as such ·
TN: number of true negatives, benign URLs that are classified as such ·
FP: number of false positives, benign URLs that are classified as malicious ·
FN: number of false negatives, malicious URLs that are classified as benign.

CONCLUSION

In this work, we implemented five classifiers using Machine Learning, which are the decision tree, Random Forest, Support Vector Machine (SVM), K-Nearest Neighbor and logistic Regression. The classifiers were used to detect Malicious URLs. In detecting Malicious URLs, there are two steps. The first step is to extract features from the URLs, and the second step is to classify URLs using the model that has been developed with the help of the training set data. In this work, we used the data set that is already preprocessed. The Classifiers classifies the training set data very well but yields average results with a testing dataset. But among these five classifiers Random forest gives better accuracy and also can better recognize the malicious and non-malicious URLs. It provided the highest classification accuracy 97.8 percent. We have shown that it is possible to construct a Random forest to classify malicious URLs.

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Table 1.Results from SVM, Random Forest, Logistic Regression, KNN and Decision Tree models

Algorithms	Accuracy Score	Recall Score	Precision Score	Average Precision-Recall Score
SVM	0.972	0.960	0.9704	0.97
Random Forest	0.973	0.962	0.9796	0.95
Logistic Regression	0.962	0.9	0.9354	0.93
KNN	0.972	0.94	0.9408	0.91
Decision Tree	0.964	0.95	0.9757	0.95

The performance of all the Classification Algorithms are shows in Figure2. The results for *k*NN are obtained with k =5. In SVM, LinearSVC is used.It is observed that Random forest Gives better Accuracy than other Algorithms. So Random Forest is used to predict the URLs.

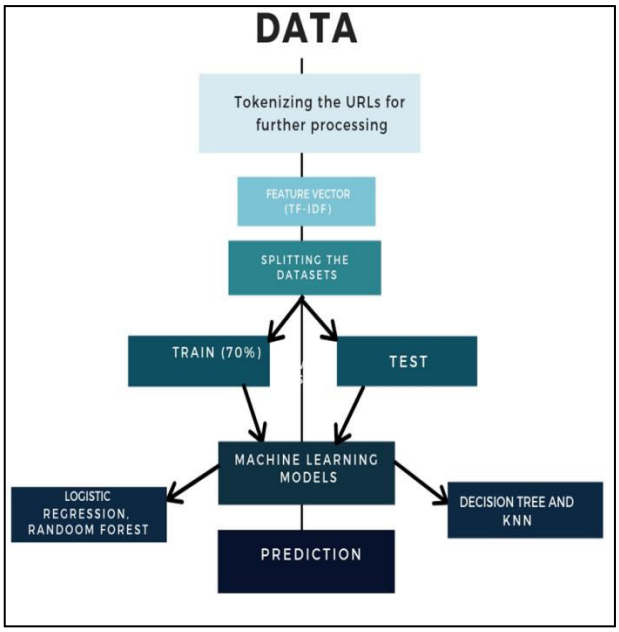

Fig. 1 Flow Diagram

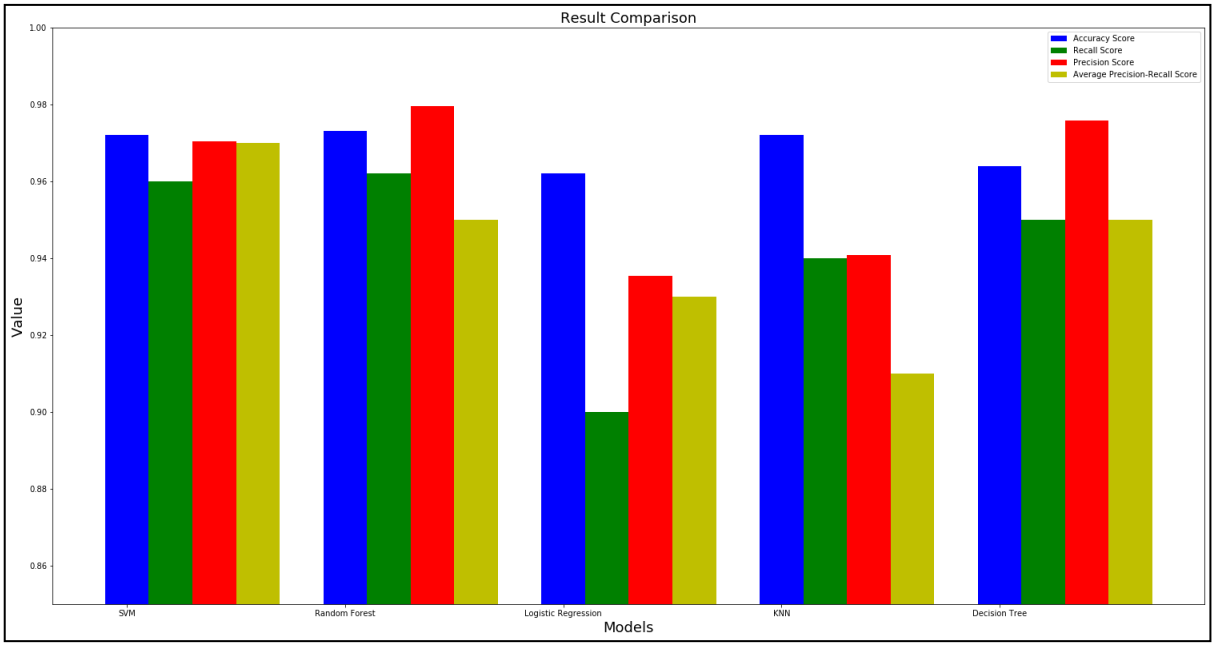

Fig.2.Comparison of Results of SVM, Random Forest, Logistic Regression, KNN and Decision Tree

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Workplace Spirituality: A Researcher's Perspective

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Received: 21 Feb 2020 Revised: 24 Mar 2020 Accepted: 25 Apr 2020

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ABSTRACT

Spirituality provides optimal solution for every critical problem of mankind and thus transpires as a prominent area for researchers. It has considerable importance in diversified fields i.e. social sciences, psychology and other ailed fields because of its scientific approach towards most of the issues. This paper gives on overview of various qualitative studies adopted by researchers for conducting scientific inquiry on spirituality at workplace. A metaphorical description of workplace spirituality has been attempted through three prime lenses viz. – Intrinsic-origin view, Religious view and Existentialist view.

Key-words: Workplace Spirituality, Social Sciences, Intrinsic-Origin, Religious View, Existentialist View

INTRODUCTION

Workplace spirituality has become one of the emergent research domains in organizational behaviour which intrigues many organizational researchers to explore spirituality as a field of inquiry (Duchon & Plowman, 2005; Fry, 2003). According to Giacalone & Jurkiewicz (2003, p.13), "It is a framework of organizational values evidence in the culture that promotes employees' experience of transcendence through the work process, facilitating their sense of being connected to others in a ways that provides feelings of completeness and joy". Spirituality at workplace mainly focuses in "meaningful work", "transcendence" and "sense of community". Most definitions of workplace spirituality include the "notions of meaning", "purpose" and "being connected to others" (Ashmos & Duchon, 2000; Conger, 1994).

Literature Review

"Employees have an inner life that nourishes and is nourished by meaningful work that takes place in the context of community" (Ashmos & Duchon, 2000). In recent times, there is much interest paid by researchers upon workplace spirituality and its consequences on individual and organization as a whole. Now-a-days, most of the researchers use 'Workplace Spirituality' as a mediator, moderator and agent in their studies. Researchers are also developing various theoretical models by using different constructs of Workplace Spirituality.

Petchsawang and McLean (2017) had conducted a relationship based study of Workplace Spirituality, Mindful Meditation and Work engagement in Thai context, where the authors validated the role of workplace spirituality as a mediator in the relationship between meditation and work engagement. Karakas and Sarigollu (2019) had conducted an emergent analysis by developing case studies of 5 Turkish organizations. It explored different patterns of individual and organizational dimensions of spirituality with respect to positive and negative outcomes. Garg (2017) conducted a research by taking different mediators like employee commitment, employee engagement and work motivation to establish a relationship with workplace spirituality. Devendhiran and Wesley (2017) highlighted the concept of Workplace Spirituality for its underlying benefits and cope-up strategies regarding various issues in the context of employee and organization. Molloy and Foust (2016) explored Work Calling from interdisciplinary literatures and connected with Meaningful Work and Organizational Spirituality. Houghton et.al. (2016) extended the literature of workplace spirituality for the span of 14 years by covering most of the contributions in the field and they examined various dimensions of workplace spirituality. Chawla (2016) developed a conceptual model on workplace spirituality governance from an extended literature review which positively impacts customer orientation and salesperson performance. Roof (2015) studied the role of Spirituality on Employee Engagement, which was positive and explored different dimensions of engagement and spirituality. Lee et.al. (2014) developed a conceptual integrated model of workplace spirituality to address the issues of emotional labor in the service organizations. Gupta et.al. (2014) analyzed the effect of spirituality at workplace towards development of job satisfaction among private insurance companies located in Punjab. Walt and Klerk (2014) studied workplace spirituality in the dimensions of attitude, which helps to bring positive outcomes at the workplace.

Overview of methodologies for scientific inquiry in the field of Workplace Spirituality

Action Research

"Action research is an interactive inquiry process that balances problem solving actions implemented in a collaborative context with data-driven collaborative analysis or research to understand underlying causes enabling future predictions about personal and organizational change." (Reason & Hilary, 2001). Example: Benefiel, Fry & Geigle (2014) empirically investigated the role of spirituality and religion in workplace and explored various challenges and opportunities for future research and towards integrated model building.

Case Studies

"Case study should be defined as a research strategy, an empirical inquiry that investigates a phenomenon within its real-life context." (Yin, 2014). Example: Nelson et.al. (2017) studied the group of students belong to Millennial Generation (Generation Y) born in 1980s and1990s. In the study the researchers investigated the unethical behaviour patterns of the group and how different tragedies at that time shaped the life of the population.

Conversational Analysis

"It is an approach to the study of social interaction, embracing both verbal and non-verbal conduct, in situations of everyday life" (Atkinson et.al.1984).Example: Rafferty et.al. (2015) studied the group of Americans who are living with chronic illness with an objective to explore the relationship between religion, spirituality and psychological

well-being. In their study, the researchers used a mixed method viz. qualitative (conversational analysis) and quantitative (survey).

Discourse Analysis

"Discourse analysis is a combination of various approaches viz. written, verbal communication, non-verbal communication etc. for exploring a particular theme"(Strauss, 1963).Example: Karakas et.al. (2015) in their study revealed six emergent discourses around "collective spirituality and Islamic business ethics: Flying with both wings; striving to transcend egos; being devoted to each other; treating people as whole persons; upholding an ethics of compassion; and leaving a legacy for future generations." These discourses are organized around three themes of collective spirituality, respectively: "Transcendence, connectedness, and virtuousness."

Ethnography

"Ethnography consists of the observation and analysis of human groups considered as individual entities (the groups are often selected, for practical and theoretical reasons unrelated to the nature of the research involved, from those societies that differ most from our own). Ethnography thus aims at recording as accurately as possible the perspective modes of life of various groups"(Strauss, 1963).Example: Esmeralda (2015), in his study focused on Spirituality of Leadership of the Alangan Mangyans of Sablayan, Occidental Mindoro, Philippines. Ethnography is used in this research utilizing immersion, participant-observant, and in-depth interview of the key informant as well as of some elders and members of the community. The major findings of the study were Spirituality developed the level of unity and peace of the Alangan Mangyan communities

Field Research

"Field Research examines the personal meanings of individuals' experiences and actions in the context of their social and cultural environment". (Nelson et.al.2017). Example: Lucchetti et.al. (2016) had conducted an extensive field research on the attitudes of physicians from 2010 to 2012 in Brazil, India and Indonesia. The study revealed different orientations of attitudes towards spirituality, religiosity and health. It revealed that ethnicity and culture played an important role in approaching spirituality at medical profession.

Grounded Theory

"Grounded theory (GT) is a systematic methodology in the social sciences involving the construction of theory through methodic gathering and analysis of data"(Faggiolani, 2011; Strauss, 1994).Example: Oxhandler (2017) had developed *Namaste Theory* for understanding the role of mental health practitioners' religion and spirituality in clinical practice by using Glaser's (2008) formal quantitative grounded theory approach. The paper draws inferences from matter and spirit "the sacred in me recognizes the sacred in you", provided a framework to explain the emerging theme.

Hermeneutics

"Hermeneutics is the theory and methodology of interpretation, (Webster definition)especially the interpretation of biblical texts, wisdom literature and philosophical texts". Example: Merwe et al. (2015) used a hermeneutic phenomenological theoretical framework for developing a conceptual model of spirituality in music education. This article develops an awareness of spiritual experience in pedagogical contexts.

Narrative Inquiry

"Narrative inquiry uses field texts, such as stories, autobiography, journals, field notes, letters, conversations, interviews, family stories, photos (and other artifacts), and life experience, as the units of analysis to research and understand the way people create meaning in their lives as narratives" (Clandin et al. 2007)Example: Grimell (2016) used a narrative approach with a dialogical framework for deeper understanding of service member's self in transition from active service in to the civilian population.(Grimell, 2017).

Prime lenses of Workplace Spirituality

Intrinsic-Origin View

Spirituality is concerned with inner-self of an individual self (Krishnakumar & Neck, 2002). According to Guillory "Our inner consciousness and that which is spiritual comes from within-beyond our programmed beliefs and values". Connection with the self, others and with the universe is the real meaning of spirituality (Neck & Milliman, 1994; Mitroff & Denton, 1999). The way we connect our work with ourselves, others and universe is also coming under the purview of Intrinsic-origin (Ashmos & Duchon, 2000).

Religious View

This view is specific as per the particular religion (Krishnakumar & Neck, 2002). But it has received criticisms due to contradiction with religious freedom (Gunther & Diamond, 2001). In different religions, the significance of work has been highlighted. In Hinduism, as per The Bhagavad Gita, it's "Karma Yoga" (Menon, 1997), in Buddhist view, it is total enrichment of life and work (Jacobson, 1983), In Christianity, there is an emphasis on refraining from greed and immoral actions (Naylor et al. 1996), Islam emphasises on commitment towards the organization and displaying values and justice in actions (Yousef, 2000).

Existentialist View

This view focuses on the quest to find meaning in work at workplaces (Naylor et al. 1996). As per Krishnakumar & Neck, 2002, some of the existential questions are: "Why am I doing this work", "What is the meaning of the work I am doing", "Where does this lead me to"? "Is there a reason for my existence and the organization's"?

CONCLUSION

Workplace Spirituality has got a positive impact on the organized population in the context of individuals, groups and organizations. There are various segments which can be focused for the concerned implications. For example, in the field of Organizational Behaviour, there are key emergent themes like emotion, commitment and justice so on and so forth. Development of analogy on philosophy needs to be strengthened for the research population, as it helps to develop various constructs and draw variables and conclusions. It need to be studied from various contexts by applying various methodologies i.e. qualitative, quantitative and mix methods, as it broadens the scope of research and helps to generate different insights for diversified contexts. Spirituality at Workplace had also got implications for other functional domains of the organization like- Finance, Production, Operations, Information Technology and Project etc. Still there is lacuna of thought in the mind of researchers' regarding the integration of spirituality to their respective domain.

Future Research Directions

Development of various propositions on workplace spirituality for mitigation of different factors at individual and organization level can be increased. Studies on workplace spirituality in Indian context are scant and requires rigor. Various Indian Philosophical systems of research like- Yoga Systems (Karma, Bhakti, Raja, Jnana), Philosophy (Vedanta, Sankhya, Nyaya) can be integrated to study workplace spirituality and to address critical issues at workplace.

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Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Evaluation of Mechanical Properties of Fly Ash and GGBFS Based Geopolymer Concrete

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Received: 29 Feb 2020 Revised: 31 Mar 2020 Accepted: 02 May 2020

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ABSTRACT

In this paper the various strength characteristics and mechanical behaviour of the Fly ash and GGBFS based geopolymer concrete is studied. The geopolymer concrete mixes are prepare by using different percentage of GGBFS (0%, 10%, 20%, 30%, 40% and 50%) powder in simple Fly ash based geopolymer concrete mixes to study its effects. It is concluded from the above experimental work that the Strength properties increases with increase in GGBFS and the strength of ambient temperature cured geopolymer concrete is comparatively less then that of elevated temperature concrete.

Keywords: Geopolymer Concrete (GPC), Alkali Activator Solution, Compressive strength, Flexural strength, Split tensile strength.

INTRODUCTION

Concrete, from way back highly appreciated construction material have major component OPC as the chief raw material. The increasing living standards demands numerous growth in construction volume hence increasing demand for concrete.[9]This increasing demand also increases the consumption of natural aggregates more than 70% of volume which further reduces the natural mineral resources. Due to continuous urbanization methods and practices a lot of demolitions of old roads and structures take place which can be treated as waste globally [29]. The percentage of waste construction and demolition materials (C&D) is noted higher than the recycled waste materials in China in 2016 [10]. The usual CaO-Al_2O_3-SiO_2 system of manufacturing process of Portland cement requires the processing of calcium minerals involving high heat consumption and CO_2 emission. So to reduce the same industrial waste materials are used as binder in present days [11]. The industrial waste bi-products which are having pozzolanic properties can be used for construction purpose as a replacement to the cement. According to Luhar et al.

fire is a pivotal threat to the society and the future constructions are needed to be fire and elevated temperature resistantand geopolymer concrete plays an important role in this [12].

Geopolymer is a revolution in construction which is also stated as a Third Generation Cement by Asim et al. [14] with less carbon dioxide emitting which is referred as a main component of Green House Gases [21] to the environment against global warming problems. Geopolymers are the inorganic alumina silicate polymers with less energy intake making it eco-friendly [22]. These materials are not only environment friendly but also sustainable in nature. According to the studies of Luhar et al. [12] OPC cannot withstand in elevated temperatures and fire damages hence to overcome the problem GPC is introduced which retains its physical and mechanical properties even after exposed to elevated temperatures. It is previously stated that water release while the reaction takes place provides generous workability to the concrete mixture during its manufacturing process [20]. Fly ash (FA) is an industrial waste generated by the combustion of a large volume of coal fuel in thermal power plants and got its name due to its lighter fine particles that literally have ability to fly with direction of wind. Generally two types of fly ash are used for the manufacturing of concrete which are Class C and Class F. Class C FA is classified as the high calcium fly ash with carbon content less than 2%. Whereas Class F fly ash are low calcium fly ash having carbon content between 5 to 10%. According to Luhar et al. [21] the global production of fly ash as a waste material is around 390 million of tonnes from which only 15% of the total waste is utilized and other are altering the environment immensely.

Ground Granulated Blast Furnace Slag (GGBFS) is also a bi-product waste material extracted from iron and steel manufacturing industry. Due to its fine particles it poses some pozzolanic properties and acts as a replacement to the ordinary cement in construction industry. In this paper Geopolymer Concrete (GPC) is prepared by complete replacement of cement with FA and GGBFS in different ratios as binder and to activate the binding properties of the mixture an Alkali Activator (AA) is used which is prepared by diluted Sodium Hydroxide/NH (Caustic soda) solution along with Sodium Silicate solution/NS solution (also known as 'Liquid Glass') in 14 M molarity. This GPC is investigated under different strength parameters like compressive strength, flexural strength and splitting tensile strength for both ambient and elevated temperature conditions.

MATERIALS AND METHOD

Materials

FA used in this paper are collected from Jindal, Odisha and GGBFS is transported from MESCO, Odisha. As per IS : 3812 -1970 the specific gravity of FA is found to be 2.42. The chemical composition of FA and GGBFS used in the study are derived by XRF test and mentioned below in table 1 and the properties of fine and coarse aggregates are mentioned in table 2. The fine aggregates of zone II and coarse aggregates of 20 mm passed and 10 mm retained samples are utilized in it confirming to IS standards. The tests for fine aggregates are carried out according to IS 2386 (part III) – 1963 [1]. The size of fine aggregates is considered not more than 2 mm. AA solution prepared with NH crystals diluted in water with pH value not less than 6.5 and not excess than 7.5. An exothermic reaction takes place when the NH crystals are mixed with water so it is very necessary to use a thermal resistive vessel for the preparation of solution. After mixing the crystals for 2-3 minutes or until complete liquefy NS is added to the reactive solution in a ratio of 2.5 which provides the binding properties to the concrete mix.

Conplast SP430 is an admixture used which is chloride free and based on selected sulphurated naphthalene polymers. It highly improves the strength of concrete at early stage without adding extra water to it. It also appreciate the workability factor of concrete.

Mixing and Curing

Design mix procedure is considered for the GPC manufacturing where the AA solution is prepare and used only after 24 hours of mixing for best results. The solution is taken in molarity. For example if 1M is considered, we know the molecular weight of NH is 40 gm. So for 1M the amount of NH is 40x1 = 40 gm. Similarly for 14 M solution the weight of NH required is 40 x 14 = 560 grams. And as the ratio of NH to NS is considered as 2.5, the amount of NS is 560 x 2.5 = 1400 ml. First of all the dry ingredients; FA, GGBFS, fine aggregates and coarse aggregates are mixed well similarly as in case of ordinary cement concrete. Then majority of extra water calculated is added to it and mixed. After the mixture gets moist the required AA solution is added with half of the remaining water and mixed thoroughly following which superplasticizer along with the remaining part of extra water is added to it forming a homogenous workable GPC mixture ready to cast. 150 mm x 150 mm x 150 mm moulds were taken for the casting of the GPC. The mould are filled with GPC in three layers with 25 blows of tamping rod in each layer. The top surface of the cube is levelled well by using the remaining GPC mortar and left to set well. Similar procedures are carried out for 160 x 300 mm cylinders and 100 mm x 100 mm x 500 mm prisms.

Once the GPC cube sets it is demoulded and half of the samples were kept in oven for elevated temperature curing and another half of samples were kept in room temperature for ambient curing of the GPC samples. After curing the cubes are examined for compressive strength (CS), the prisms are examined for flexural strength (FS) and the cylinders are tested for splitting tensile strength (STS) respectively. For each test result the mean of three samples are assumed. The ratio of AA to the binder is taken as 0.25 and the extra water is considered as the 0.2 of total binder. The percentage of superplasticizer is considered as 5% of the total AA solution added to the GPC mix. Design mix proportions of different ratios of binders are provided below in table 3.

RESULTS AND DISCUSSIONS

Workability

The workability test of the fresh geopolymer concrete sample is done by slump test method. As the geopolymer concrete mix contains alkaline solution of NaOH and Na_2SiO_3 which makes the mix sticky and hence extra amount of water is need to be added to make it workable. In this test the measurements of flow dimensions in both X and Y directions and the time of the flow reaching 500 mm diameter is considered. The maximum workability of the fly ash and GGBFS based geopolymer concrete mix is observed to be between 122 mm – 152 mm.

Compressive strength

The compressive strength of the samples of geopolymer concrete with different ratios of fly ash and GGBFS is found out in UTM machine after 7 and 28 days of ambient temperature curing respectively. The compressive strength values of the samples are given in table 4 below. It is observed that the maximum CS of the geopolymer concrete cube samples is achieved from the sample with the ratio of Fly ash to GGBFS is 50 : 50 i.e., 41.6 Mpa in 7 days and 48.8 Mpa in 28 days. The geopolymer concrete sample with 100% Fly ash and no GGBFS gains the least CS value. Hence it is considered that the addition of GGBFS helps to increase the CS of the geopolymer concrete.

Split tensile strength

The splitting tensile strength (STS) of the geopolymer concrete samples are tested inUTM machineat 28 days of ambient temperature curing. The split strength details are provided below in table 5.

Flexural Strength

The flexural strength or three point load test is carried out by UTM machine for all the different ratios of geopolymer concrete mixes and it is observed that the values varies from 5.52-5.91. The figure below provides the graphical variation of the flexural strengths.

CONCLUSIONS

This paper appraised various experimental study on Fly ash and GGBFS based geopolymer concrete. In this study it is concluded that the geopolymer mix without using GGBFS delivers less strength as compared to the geopolymer concrete mixes with GGBFS in different ratios. It is also observed that the increase in GGBFS ratio increases the strength and additional water is required for its workability. The change in behaviour of GPC is observed by change in ratio of alkaline solution to binder. The solution is preferred to be used in warm state instead of room temperature as due to sodium silicate solution the Alkaline Activator Solution becomes hard and unmanageable.

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Table 1: Chemical composition of Fly ash and GGBFS obtained from XRF test

Compound	Composition (%)	
	Fly ash	GGBFS
Al_2O_3	28.809	18.928
SiO_2	59.483	29.580
P_2O_5	0.955	-
Cl	0.118	0.141
K_2O	1.782	0.913
CaO	1.661	45.714
TiO_2	2.048	1.366
V_2O_5	429.6	92.1
Cr_2O_3	259.0	29.9
MnO	409.5	0.281
Fe_2O_3	4.780	0.974
SO_3	-	1.716

Table 2 : Physical properties of aggregates

Properties	Fineness Modulus	Specific Gravity	Water Absorption	Zone
Fine Aggregate	0.1	2.62	2.6%	II
Coarse Aggregate	-	2.615	3.9%	-

Table 3 : Mix proportions of FA-GGBFS GPC

GPC mix	FA (kg/m³)	GGBFS (kg/m³)	Fine aggregates (kg/m³)	Coarse aggregates (kg/m³)	AA (kg/m³)	NH (kg/m³)	NS (kg/m³)	M	Curing method	Additional water (kg/m³)	SP in Kg/ m³
G1	414	-	555	1293	138	39	99	14M	Ambient	82.8	5.52
G2	372.6	41.4	555	1293	138	39	99	14M	Ambient	82.8	5.52
G3	331.2	82.8	555	1293	138	39	99	14M	Ambient	82.8	5.52
G4	289.8	124.2	555	1293	138	39	99	14M	Ambient	82.8	5.52
G5	248.4	165.6	555	1293	138	39	99	14M	Ambient	82.8	5.52
G6	207	207	555	1293	138	39	99	14M	Ambient	82.8	5.52

Table 4 : compressive strength of geopolymer concrete samples in Mpa.

GPC Mix	*7 days CS*	*28 days CS*
G1	22.8	32.6
G2	24.4	33.2
G3	26.7	38.5
G4	32.5	42.3
G5	38.7	45.7
G6	41.6	48.8

Table 5 : Split tensile strength of geopolymer concrete samples

GPC Mixes	*28 days STS (Mpa)*
G1	2.8
G2	2.94
G3	3.2
G4	3.34
G5	3.42
G6	3.6

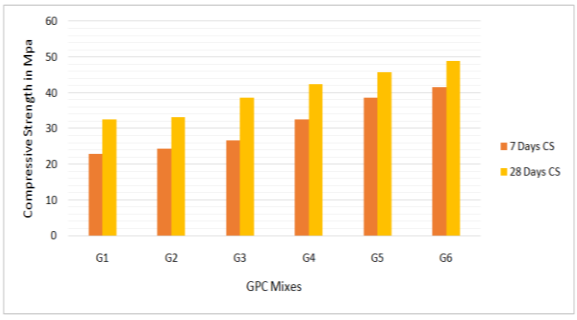

Fig. 1: Compressive strength of GPC in 7 and 28 days

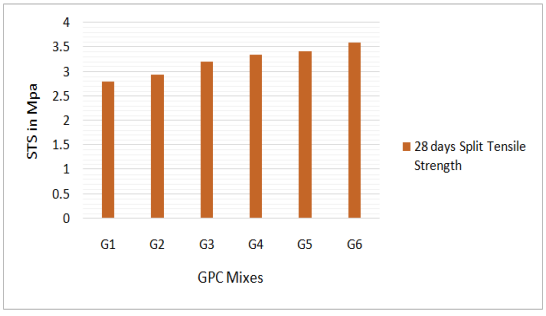

Fig. 2: Split tensile strength of GPC in 28 days

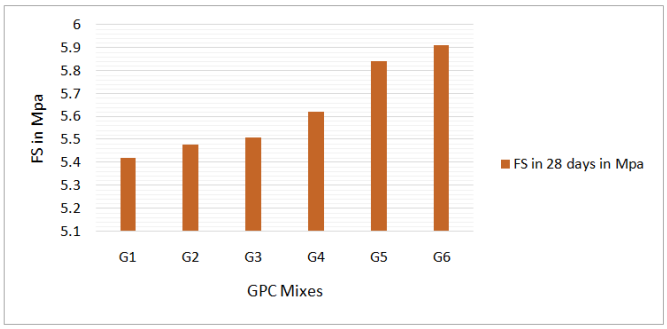

Fig. 3: Flexural strength of GPC in 28 days

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Automated Elephant Detection System to desist Uncertain Railway Accidents by Unifying AI and IoT

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Received: 24 Feb 2020 Revised: 27 Mar 2020 Accepted: 29 Apr 2020

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ABSTRACT

Wild life is one of the wonder that exits in this world from millions of years. As many people think, the wild animals and their species exist abundantly in the Earth but that is not true. They are already at the verge of extinction and their numbers are dwindling rapidly which need significant attention. The wild life rich country like India has also its list of endangered species among which Indian Elephants are in critical crisis, be it poaching, electrocution or railway and road accidents. The tragic train accidents have been caused untimely death of more than 250 Elephants in India since 1989. So without serious attention, these gigantic creatures will eventually vanish from the Earth. By taking advantage of the recent intensive research of deep learning in the Computer Vision, we have proposed a deep learning based Elephant detection system. The purpose of proposed solution is to save Elephants from the train accidents by designing a suitable setup which can detect and track Elephants in the Elephant trespassing areas near the track by giving signals to the train driver beforehand about the presence of Elephants. The whole setup is an AI and IOT integrated approach in which the power of Artificial Intelligence will enhance the Elephant detection system with the combination of sensing technology of IOT. In our methodology, the process followed is to detect and track the Elephants by using Cameras and Sensors using deep learning based YOLO V3 object detection algorithm with the help of Raspberry pi 3 and AVR board which will accurately detect whether the animal is Elephant or not and will send signals to the Railway driver with the help of three coloured signal systems; yellow, orange and red and simultaneously will send SMS notifications to the nearest Railway Department and Driver. An additional setup is also there which will use to generate high frequency noises in order to keep all other animals including Elephant away from the Railway track.

Key words: Elephant Detection System, Internet of Things (IoT), Deep Learning, Convolution Neural Network, YOLO V3

INTRODUCTION

Tragic and unfortunate death of Elephant has been witnessed too many times in India and all over the world. In the recent 4 years almost 49 Elephants has been killed due to railway accidents only in India. We can't avoid Elephants to come near the railway tracks as it is a part of their migration. So the only possible solution is to design a safer pathway for them across the track or to implement certain technologies to get the information of their presence in advance, so that we can avoid such type of accidents beforehand. Detecting and tracking elephant system has been proposed previously using sensors and cameras with most effected image processing techniques [1, 2]. Traditional image processing techniques are needed a lot of manual works like segmentation, noise reduction, feature extraction by making the process more complicated. But Artificial Intelligence has already overcome these issues of manual processing and furnishing of images by outperforming them with advanced features. From machine learning and deep learning point of views, there are some immediate techniques to detect and track Elephants such as applying linear support vector machine (SVM) classifier or K-Nearest Neighbour (KNN) with manual object bounding on hand-crafted features or fine-tuning CNN models inheriting model weights pre-trained on a very large scale dataset such as the ImageNet [3], certain deep learning based object detection algorithms like SSD, and Region based CNN, Faster RCNN and so on. These approaches have been performed promisingly by giving some potential results.

However there are certain challenges to which still have to overcome such as the manual pre-processing of images which have to feed into the classifier and to detect and bound the identified objects. The Second obstacle is the speed and accuracy of detection which is more important in Elephant detection system in order to notify as soon as possible to the Railway department. So by avoiding all kinds of complicated manual processing, the detection system will become more feasible from the working perspective. The faster the detector, the faster the detection system will notify the presence of Elephants. In our proposed model we have taken it to another level with a promising approach that is deep learning based detection technique by installing an autonomous detection system combining sensors and cameras for a efficient object detection using the most advanced deep learning based object detection technique; YOLO V3 object detection algorithm which is totally based upon the concept of convolution neural network and much more faster as compared to other traditional techniques. Above all that Camera trap videos and sensors are more conventional due to low price, easy to use and low maintenance features with the capability of detecting wild life and environment without any disturbance [4]. To make the system wholly automated we have used Raspberry pi 3 and AVR development board which will directly send the signals from the detection system to the signal board with the 3 security layers in order to track the position of Elephants from the railway track within a range of 5 KMs by monitoring their activities. We have integrated sensors like Infrared(IR), Passive infrared(PIR) and Piezoelectric sensors for enhancement of detection capacity respectively in 3 different security levels as Internet of Things (IoT) and sensors are the most traditional sensing techniques with lesser complication and faster productivity. Similarly Traditional notification system is time consuming which generally needs certain time for the concerned personnel to get notified and take immediate action. So for overcoming the time gap we have proposed the coloured signal system of three different colours to give signals directly to the train.

The system has designed basically with two objectives (i) to detect the presence of Elephant in advance by implementing cameras and sensors and (ii) to notify the driver by triggering some signals using traditional railway signal system of yellow, orange and red signals by notifying the particular position of Elephants from the track. In addition to this, the notification system has also implemented by using GSM module to notify the nearest Railway Department and Driver about the location of Elephant.

RELATED WORKS

In this section we went through a brief description of related works performed on Elephant detection system such as different setup architectures, detection techniques using sensors and controllers, use of different Image processing and some object detection algorithms. When it comes to wild life detection and tracking, IoT system using very cost effective sensors have always been the first choice because of their reliability and simple architecture. Implementing the vibration sensor in orders to detect the Elephants and processed the camera with the triggering of vibration sensor in order to send the message or notification has already done and proposed in concerned paper [3]. The process of image processing has involved image matching process by uploading collected images using Google API service. Similar kind of work has been done by S. J. Sugumar et al. [2], a detection system to track elephant intrusion in forest border areas in which wireless cameras along with PIR sensors have been used and controlled by Atmega controller with sending signals to the local center through RF network and the whole image processing has been done by comparing the collected images with the stored database images sending SMS to forest officials. With the advancement of technology, the field of machine learning and computer vision has increased drastically. So many recent works upon detection and tracking system has witnessed the emerging machine learning and deep learning. Region based convolutional neural network approach in order to detect Elephants for the purpose of Human-Elephant conflict management has been proposed by K.M.S.Madheswaran et al. [5] has mainly focused upon the region based object detection algorithms like SSD mobilenet v2 model, SSD litemobilenet v2 model and Fast R-CNN inception models by installing raspberry pi and cameras with showing a keen comparison between various models in the context of speed and accuracy.

PAST DATA ANALYSIS

An elephant named "Bholu" is the official mascot of Indian railways. But it's quite jolting to see India is on top of the table in the globe, where the maximum number of elephants has been dying each year by train accidents. The far-flung network of Indian railways cuts across the dense forest, habitats. The railways track nearer to the elephant habitat led to numerous elephant accidents and death. According to the Ministry of Environment, Forest and Climate Change of India in the year of 2016-19 total of 60 elephants died by railway accidents India as shown in Fig. 1 [6].Four elephants were killed on the Jharsuguda (Odisha) elephant corridor railway accident on 6th April 2018. Like that in the year 2017-18 twelve child and pregnant elephants killed in various parts of the Asam just for the over speed of the train. West-Bengal Dooras train accident 2019 was the witness of another regrettable example of elephant-man conflict. While the elephant crossing the railway track the Intercity express hit the elephant and after watching the viral video, where that victim elephant was doing struggle for returning back to the jungle made us think how India's railway tracks have turned into a death trap for elephants.

MATERIALS AND METHODS

The whole system is fully autonomous andhas been divided into 2 parts. One is the detection part and another is the notification part. For detection part cameras and sensors are implemented to get the perfect images and signals to detect the Elephants using deep learning object detection techniques.For the notification part different coloured signals like yellow, orange and red are used as the indicators by giving the possible location of Elephants and GSM module for giving message notification to the railway personnel. The Buzzer system is designed to alert the nearest animals including Elephants to avoid them to come near the track. For control and computation, Raspberry pi 3 B+ has been used which itself is a mini computer in order to perform all kind of processing of deep learning algorithms and signal or data transformation from cameras and sensors to the signal board.

Hardware Architecture with Circuitry representation

For our recommended model the following hardware and software are used:

Hardware

Minicomputer Raspberry pi 3 B+ model with 1.4 GHz processor and 1 GB RAM, additional WiFi, Bluetooth and Ethernet connection feature; USB Web cam of 20 megapixel resolution with XVVGA video recording; IR (Infrared) Sensor with 3.0- 5.0 V operating voltage with detection range of 2- 30 CM (Adjustable using potentiometer); HC-SR501 PIR (Passive Infrared) Sensor with a wide range of voltage from 4 V to 12 V and covering distance of 120^{o} and 7 meters;

Piezoelectric sensor of metallic disk shape; SIM900A GSM/GPRS Module with Dual-Band 900/1800 MHz, compliant to GSM phase 2/2+ class 4 and class, control via AT commands and SIM application toolkit; Required connecting wires; Signal Tower with 3 different coloured signals Red, Yellow, and Orange; Buzzer

Software

Raspbian operating system for Raspberry pi 3 B+; BalenaEtcher to flash the OS to the SD card;Anaconda3(64-bit) distribution; Tensorflow 2.0 for building and deploying deep learningalgorithms.

The USB webcam and sensors like IR, PIR and Piezoelectric has connected to the raspberry pi 3 B+. The whole hardware setup is finely illustrated in the Fig. 2.Both IR and PIR sensors are connected to GPIO 23 and GPIO 24 respectively with individually connected to +5V and Gnd. Piezo is connected to GPIO 25 and Gndwhere as 5VR, 5VT, Gnd of SIM900A GSM module is connected to respective TXD, RXD and Gnd of pi. The different lights in the Signal towers are connected to GPIO 7, 27, 22 respectively. An additional buzzer is there in order to connect with GPIO 6 for creating sound as a prototype of real noise generating speaker.

Field Setup Architecture

The setup design is implemented in order to track the Elephants starting from a distant location to the track nearest areas by covering a range of 5 KMs in order to get the early notification of their presence by which the train driver can be forewarned and can take the necessary action. From a better safety perspective, the range between 5 KMs from the railway track is divided into 3 different security levels in order to get the Elephants' location as shown in Fig. 3.

First Security Layer

In the First security level both Cameras and IR Sensors are installed up to 5- 4 KMs range in trespassing zone where camera captures the video sequence by detecting the Elephant in the video frames using object detection algorithms and IR senses the presence of Elephant by capturing the infrared radiations [7] coming from their body in order to send it to the Raspberry pi which transmitsthem to the signal tower to trigger the**yellow**light by indicating the location of Elephant that is within 5- 4KMs. Additionally a notification isforwarded by using SIM900A GSM module with the use of AT commands to the nearest forest and railway officials by alerting them about the Elephants' presence in the accident prone areas.

Second Security Layer

The Second security level enhances the detection system by providing further information like whether the Elephants enter into the 4- 3KMs range or not which must be more nearest to track. Here Cameras are implemented along with PIR sensors which sense the presence of Elephant by sensing their warm body which generate infrared radiation. PIR sensors along with cameras are preferred because when it comes to detect or sense a living body with larger structure PIR can work efficiently to sense the presence of animal as compared to other sensors because with the small, inexpensive and low-power features it makes a good option along with the sensitivity range which is quite wide spread up to 20 feet in 110^{o} x 70^{o} direction range [8] which is a best fit for a larger animal like Elephant and can easily sense the approaching of them from a decent range. If Elephant will be detected, the pi sends the signal in order to trigger the **orange** light in signal tower indicating the presence of Elephants in the second security zone (up to 4- 3 KMs).

Third Security Layer

Third Security level has designed with the combination of both cameras and piezoelectric sensors which not only detect the Elephants from the video frames, it also senses the body vibration and pressure by installing piezoelectric sensors on the ground. Aside from camera which will detect the Elephant using deep learning algorithm, the piezoelectric sensors make use of piezoelectric effect in order to measure any kind of changes in pressure, temperature and force by converting it into electrical voltage [9] and triggering the **red** signal by notifying that Elephant is somehow much closure to railway track that is within 3Kms range, so that the driver can take required action by putting the emergency brakes in order to avoid accident. As pressure and force created by Elephant is quite larger as compared to other animals, a threshold value is set, so that the sensor won't be affected by any vibrations less than the threshold value [9].As per normal speculation, the general speed of Elephant is up to 25 KM/Hr [10], it will take almost 2.5 to 3 Minutes for them to cover the distance of 1 KM which is quite minimum time. So if the final level will be limited to small distance like within 1 KM, then it will become quite difficult for the train to stop immediately in case of any emergency. So we have designed this security zone to be installed within 3 KMs, as the train after getting the notification will get ample amount of time to be stopped.

Elephants and other animals are more sensitive to small frequency sounds which cause certain irritation in them. The buzzer in our model is used to create such sound to refrain all animals including Elephant to come near the track.

ILLUSTRATION OF OBJECT DETECTION APPROACHES

Object detection is unlike of traditional classification system in which the images only have to classify into different classes, it involves some more advanced features like precisely differentiate class in images and locating the position of object by identifying the nature of object in the images [11]. Detecting and tracking Elephants in deep forest areas is surely challengeable but instead of using some traditional image processing, we have tried for implementing some advanced techniques by comparing each and every involved techniques in order to find out the most feasible one starting from Convolutional Neural Network for Image classification to Single Shot Detector (SSD) object detection algorithm and finally the object detection technique YOLO V3 which we have used in our proposed model.

Convolution Neural Network for Image Classification

Convolutional Neural Network (CNN) is a variance of neural network which can capable of differentiating images by assigning importance to various aspects and features by considering the spatial structure of input images like width, height and depth [3]. CNN has certainly overpowered conventional image processing due to minimal

requirement of pre-processing. When we see a dog or a cat, we classify by considering certain small features like ears, paws or legs. CNN also performs in same manner by considering these low level features in images like edges and curves through a couple of convolutional layers [12]. In case of artificial neural network the input layer for larger images will make the structure of neural network complicated as each layers are connected to every next layer requiring a humongous umber of weight values. CNN outperforms this normal ANN by connecting to the small region of the preceding layers by reducing the complexity which is more feasible to deal with images [3, 12].

Dataset Preparation

For performing the CNN classification, various raw images are collected from different websites consisting of 4 different classes of Elephant, Horse, Cat and Dog. Each class contain 1438 raw images. The images are labelled and resized in order to reduce the invariance. In order to get more number of images avoiding any kind of undeviating features, data augmentation is performed by increasing the number of images of each class to 3000 which has been represented in Table 1. The quality and quantity of raw images are not worthy enough to perform CNN operations as the collected images varies greatly in colour, size, orientation, brightness and amount of possible noises. So for defying these challenges, data augmentation is used for getting some uniform images by implementing rotation of images like vertical and horizontal flip, Gaussian blur or increasing the brightness and contrast level [5]. This process will generate ample amount of images with stable unwavering features by making our dataset more consistent and uniform. Train and test images are divided into 4:1 ratio.

Field Setup Architecture

The setup design is implemented in order to track the Elephants starting from a distant location to the track nearest areas by covering a range of 5 KMs in order to get the early notification of their presence by which the train driver can be forewarned and can take the necessary action. From a better safety perspective, the range between 5 KMs from the railway track is divided into 3 different security levels in order to get the Elephants' location as shown in Fig. 3.

ConvNet operation

CNN (ConvNet) consists of two parts that are Feature extractor and Classification part. The feature extractor is responsible for extracting different features from images which are actually considered as the features for classification purpose. In every convolutional layer the filters are applied to the images and the convolved output is used as the input for the next layer [13]. CNN consists of input layer, an output layer and hidden layers between input and output. As illustrated in Fig. 4, a CNN is designed by a couple of sequential layers consisting of Convolution layers in addition with non linear activation function Rectified Linear Unit (ReLU) and layer for sub sampling of images that is by using max pooling layer with a couple of classification layers of fully-connected layers where the last one is the output layer with predictions like whether the input image is an Elephant, Horse, Cat or Dog [1]. We have fed images into the ConvNet with the size of 64 x 64 and filter or kernel of dimension 3 x 3 in order to perform convolution operation to get convolved output of the size followed by the conversion result as per the Eqs 1 shown below.

$$(\boldsymbol{N} \times \boldsymbol{N}) \times (\boldsymbol{N} \times \boldsymbol{N}) = (\boldsymbol{N} - \boldsymbol{F} + \mathbf{1}) \times (\boldsymbol{N} - \boldsymbol{F} + \mathbf{1}) \qquad (1)$$

N x N is the size of image and F x F the size of filter. By putting this, we got the convolved output of size 60 x 60 [14]. We have provided 32 convolutional layers for better feature mapping. This convolved output serves as the input to the further layers. In order to maintain the non linearity of the images during convolution operation, a non linear

activation function most likely ReLU that is rectified linear unit is used by following the mathematical concept as shown in Eqs 2,

$$F(x) = max(0, x) \quad (2)$$

ReLU rectifies the feature map by making use of above concept by removing any negative values present amongthe pixel values [15] and by passing values 0 or more by making the required input more uniform and positive which is kind of faster as compared to equivalent tanh [16]. Pooling layer after the convolution layer is also one of the major features mapping process which is responsible for reducing the structural size of convolved feature in order to reduce the dimensionality leading to a better and smooth computation. From the types of pooling, we have opted Max pooling which performs the sub sampling by selecting the maximum value from the portion of image covered by kernel which also responsible for reducing additional noise [17]. Both convolution and pooling layer are performing feature mapping by extracting the feature required for further classification.

In order to perform classification the input data from feature mapping section is converted to flatten data so that the data can be fed to the fully connected layer for the classification purpose. The fully connected layer involves forward and backward propagation in order to train and test the images from dataset. We have provided 28 dense layers with ReLU activation function and 1 unit of dense layer with sigmoid activation function. For updating of weights and biases, we have given 5 epochs as the amount of Loss in case of our model is somehow above threshold. After the completion of training the dataset, the test data is fed into the model to calculate the accuracy which is 67% in our case illustrated in Table 2.

Algorithm

1. Batch size =32, no of classes 4, number of epochs = 5
2. Dimension of input image 64 x 64
3. Loading the input images from the data set
4. Variable exploration: X=test data set (9000), Train data set (3000)
5. Creating and compiling the models
6. Training the network

The above algorithm explains the whole steps involved in the Image classification using CNN.

SSD based object Detection Technique

Single shot detector (SSD) is quite faster and efficient in accuracy if considering any other object detection algorithm because of simultaneous occurring of region proposal and region classification by taking only one single shot in order to detect multiple objects [9]. SSD is feed forward neural network approach in which pixels of the images are divided into same size bounding boxes and each bounding box contains certain score for the presence of required object classes inside the boxes [18]. As SSD is a type of convolution neural network object detection algorithm, conventional way of feature extraction will be performed in the case of SSD also by retrieving a convolved output with each features surrounding by the bounding boxes. SSD does not make use of delegated region based proposed network. It performs on concept of region proposal by performing and computing class scores and the location with the help of convolutional filters which is a very simpler method. After producing the feature maps, SSD predicts for each cell by making use of convolution filters. Each applied filters calculate the output channels and scores for each respective classes with corresponding boundary box.At first, we describe how SSD detects objects from a single layer [19]. We have used SSD version with mobilenet with an average speed of 30 ms and minimum average precision (mAP) of 21 which is somehow average in performance. For this we have installed the SSD model

ssd_mobilenet_v1_coco which has trained and tested using coco dataset consisting of 80 different image classes. We got a very remarkable output with a very high accuracy level which is kind of key feature of SSD detector which is shown in Fig. 5.

YOLO V3 Object Detection Algorithm

YOLO stands for "You Only Look Once". The name itself suggests that it only looks at the image once at training and testing time unlike of moving a sliding window through the whole image in order to get contextual information about different classes. It works on the concept of single shot detection strategy which takes images as input by performing all kind of feature extraction operation and simultaneously learns bounding boxes and calculating the probability of class labels of different objects by making it the perfect candidate for real time object detection [20].

YOLO V3 is more desirable from the perspective of functional simplicity with single network evaluation system for efficient prediction where other algorithms perform 1000s of network system in order to predict which make it extremely fast. In order to predict the bounding boxes it makes use of anchor boxes by using fully connected layers besides convolutional layers for feature mapping purposes [21]. The coordinates of bounding boxes are predicted as t_x, t_y, t_w, $t_h$by considering the offset value of the top left corner of cells as (c_x, c_y) and width and height of prior bounding boxes as p_w, p_h, the coordinates are predicted by following the below Eqs. 3, 4, 5 and 6 [22].

$$b_x = \sigma(t_x) + c_x \quad (3)$$

$$b_y = \sigma(t_y) + c_y \quad (4)$$

$$b_w = p_w e^{t}{}_w \quad (5)$$

$$b_h = p_h e^{t}{}_h \quad (6)$$

After prediction of coordinates the bounding box prior are assigned to the object on the basis of objectness score. In YOLO V3, a multi-label classifier and cross entropy is used for class prediction in order to avoid overlapping labels in multi-label classification in more complex domain of dataset [22]. Softmax is not used for classification. Here 3 different scales are used by adding several convolutional layers to the feature detector Darknet- 53 for feature mapping. YOLO V3 is much better at accuracy with advanced feature mapping techniques due to involvement of a much deeper network Darknet- 53 which performs in 53 convolutional layers giving fine tuned features with fine grained information.

So in order to visualize the overall performance, we can refer to the graphical representation of performance measures of various detection algorithm on the detection metric of 0.5 IOU proposed by Joseph Redmon, Ali Farhadi in [21] illustrated in Fig. 6.In comparison of other detection algorithm, YOLO V3 is good if consider the inference time or accuracy. Though the level of accuracy diminishes in the 0.5 and 0.95 IOU metric but still it is one of the fastest object detection algorithms performing wonderfully in coco dataset with remarkable map [22]. When it comes to real time object detection it is the fastest and more reliable working per 40 to 50 fps which basically we need in our object detection system.we have opted YOLO V3 object detection algorithm by comparing the speed, accuracy and simplicity of techniques among the above mentioned techniques. We have used the continuous video sequence collected from our installed cameras to detect the presence of Elephants in the video sequence. We have modified the configuration file in order to consider only one class Elephant by avoiding any other objects. The results are kind of promising with a greater accuracy showing incredible speed which can be visualized in the below Fig. 7. As we have used Raspberry pi the, YOLO V3; even the fastest one lags behind due to computationally low device like portable embedded systems having limited amount of memory and processer while complex deep learning algorithms like YOLO need high performance GPU devices [23]. After performing YOLOV3, neglecting the accuracy measurement, the algorithm works almost 1 frame in 3 to 4 seconds by detecting a single image within20 seconds which is extremely low as compared to the actual YOLO V3. So we have tried another approach to get maximum outcome

that YOLO V3 tiny version that is TinyYOLOv3 which much more lighter and simpler as compared original YOLO v3 [24] and also it works somehow efficiently for computational limited devices like raspberry pi with 20 to 25 FPS which is much better and trust worthy.The whole comparison can be clearly visualized in the Fig. 8 where it can be envisaged that Tiny YOLOv3 gives satisfactory outcomes with better accuracy and speed like 25 FMS with raspberry pi as compared to others.

CONCLUSION

An efficient Elephant detection system is the main purpose in order to protect and save Elephants from any kind of tragic death due to railway accidents. The key factor is the reliable detection system for which we have tried different approaches like convolution neural network by performing the image classification, using SSD object detector for real time detection and YOLO V3 with the variant Tiny YOLOv3 by getting some remarkable results. From the computation and complexity view, CNN is alittle bit time consuming and complex as itinvolves some manual works like furnishing the raw images and preparing datasets by setting weights and biases in case of back propagation with a comparatively low accuracy of 67%. SSD is no doubt one of the finest detectors with great accuracy like 90-98% and decent speed. YOLO v3 is also remarkably good at accuracy just like SSD but it is kind of more fast and simple by making it more reliable for real time detection. The variant Tiny YOLO v3 is the most suitable for small computational devices like raspberry pi and other controllers with a accuracy of 60-75% with 25 FPS by performing promisingly in our proposed real time object detection system. So we have opted Tiny YOLO v3 for our proposed system. We have tried to enhance the system by keeping an eye on the movement and migration of Elephants. From the better safety perspective, we have come up with idea of 3 security zone. Implementation of a combining architecture of cameras and sensors simply enhance the safety features in case of any malfunction. The Colored Signal Tower notification system has the advantage by bridging the time gap which sometimes affect due to late receiving of SMS by Railway officials and network issues. Driver can easily alert by seeing the signals across the railway track by getting the actual location of Elephant.

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Table 1. Image Distribution in Dataset

Classes	No of images collected from internet	No of Augmented Images
Elephant	1438	1512
Horse	1438	1512
Cat	1438	1512
Dog	1438	1512

Table 2. Loss and accuracy of each epoch

EPOCH	LOSS	ACCURACY
1/5	0.5520	0.4512
2/5	0.3641	0.5116
3/5	0.3321	0.5843
4/5	0.1833	0.6400
5/5	0.1120	0.6711

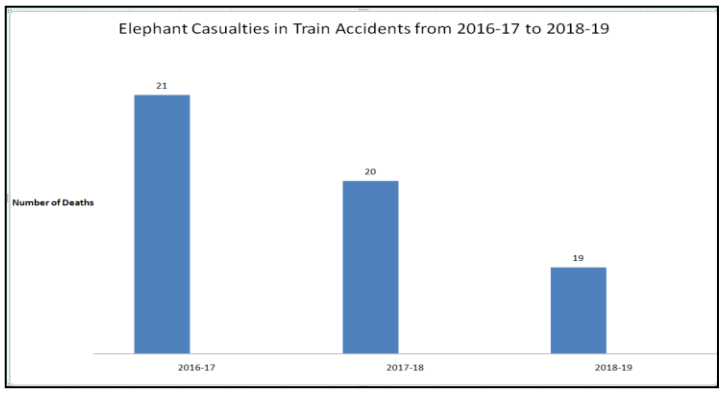

Fig 1: Death Analysis graph of Elephants died due to Railway accidents in 2016-19

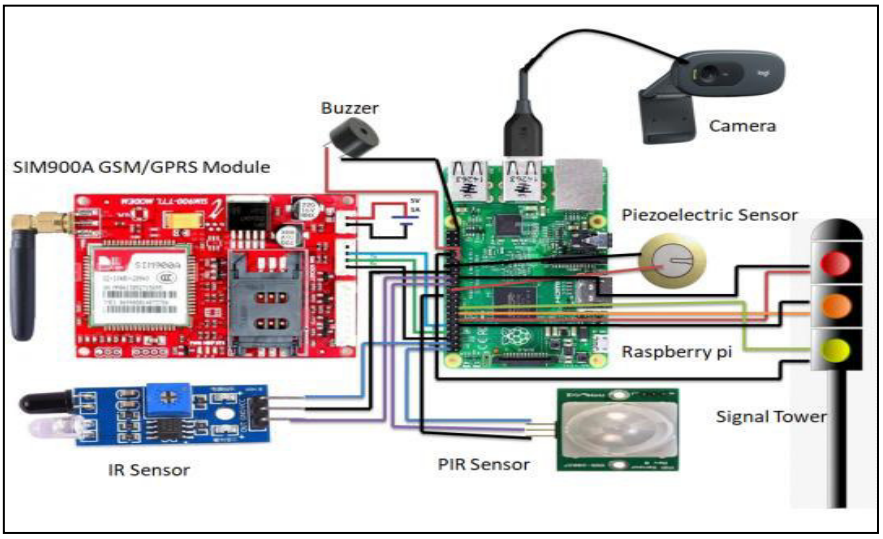

Fig. 2: Circuitry Diagram of whole Hardware Structures

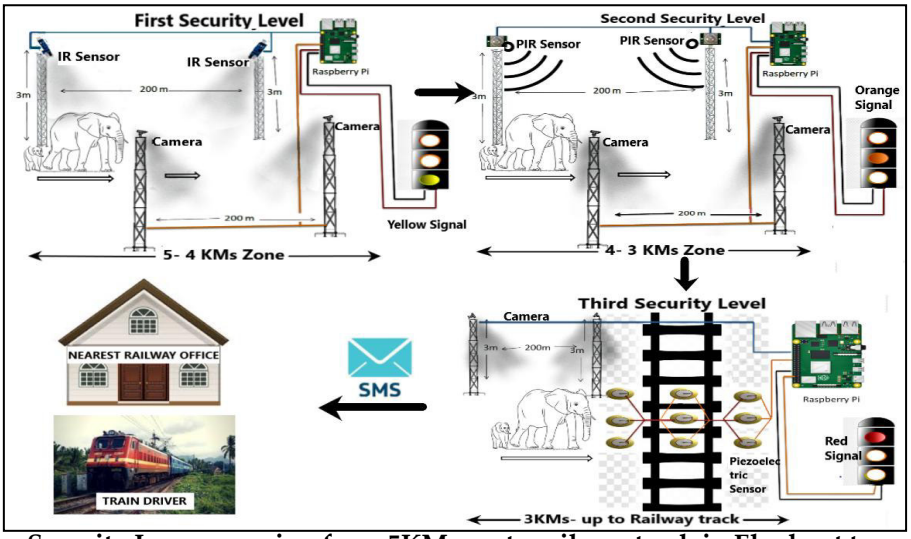

Fig. 3: Three Security Layers ranging from 5KMs up to railway track in Elephant trespassing zone

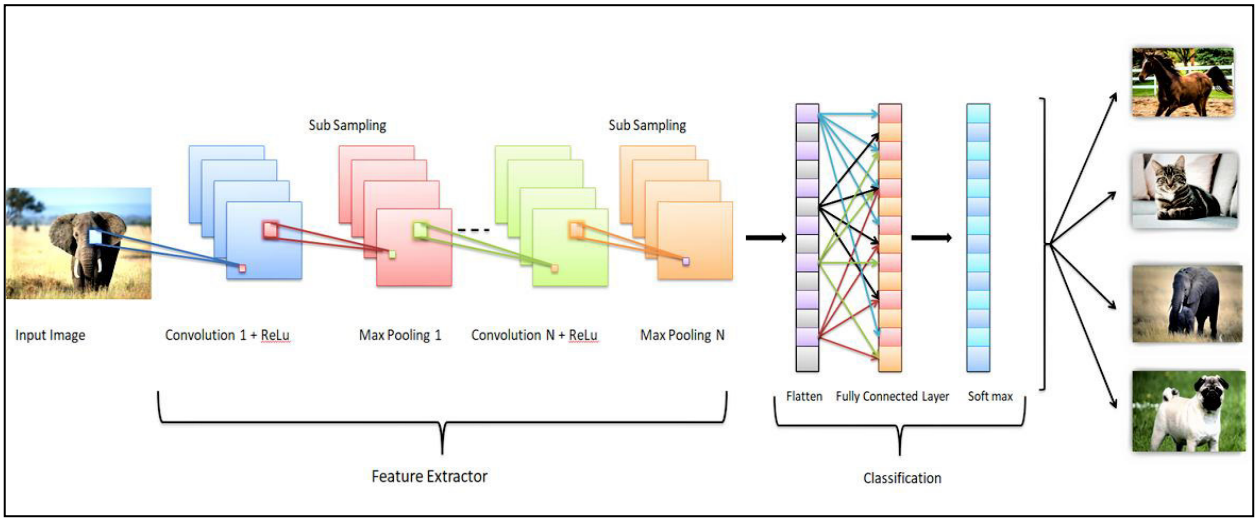

Fig. 4: Phases of Classification using Convolution Neural Network

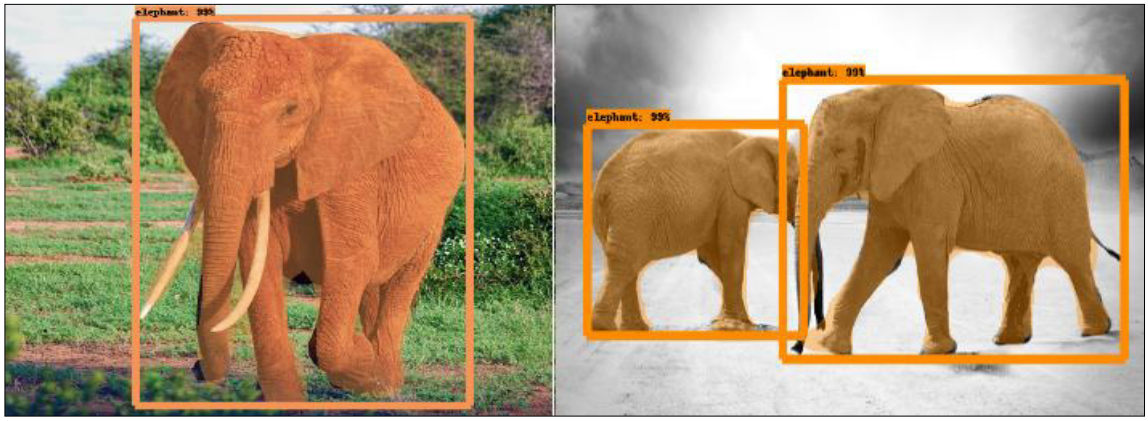

Fig. 5: SSD detected Elephant test images

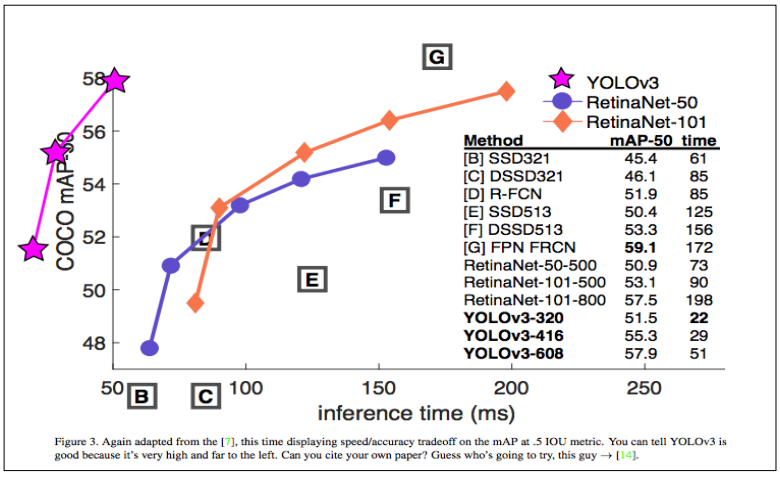

Fig. 6. We adapt this figure from the yolov2 paper [21]

Fig. 7: YOLOv3 detected Elephant images collected from test video sequence

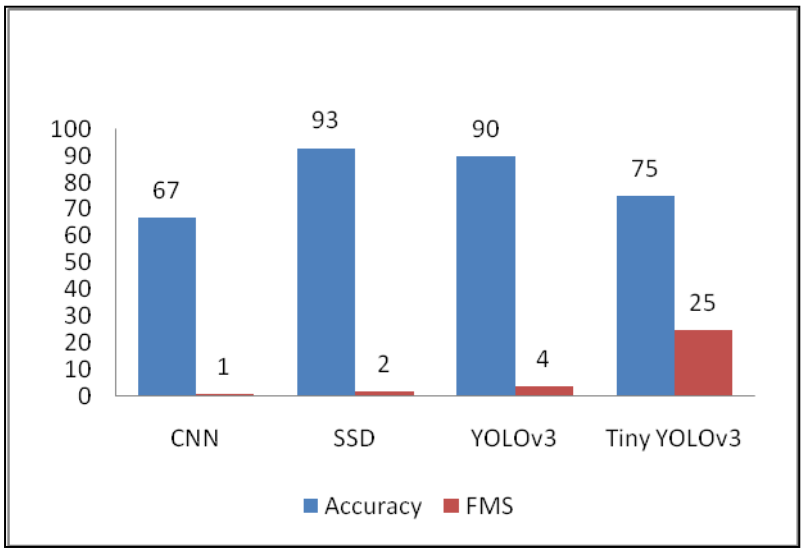

Fig.8.Comparison chart of accuracy and FMS for each object detection technique

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Comparative Study of Biochemical Parameters of Blood Serum of Male and Female Black Rock (*Gallus gallus domesticus*)

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Received: 26 Feb 2020 Revised: 28 Mar 2020 Accepted: 02 May 2020

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ABSTRACT

Black rock hen wasunique strain chicken, they are unique strain chicken. They were maintaining their essential qualities. Black rock was very popular breed of chicken. This study calculates areference intervalfor thebiochemical profile of sugar, total protein cholesterol, albumin, *s. globulin.* Blood samples were collected randomly from this male & female black rock hen, and this serum value was analysed. This value was shows significant difference betweenthe male and female hens. No significant sex based differences were observed for the other values.

Key word: Blood serum, biochemical parameter, male and female black rock hen

INTRODUCTION

Changing food habits, globalisation, industrialisation and urbanization have created a favourable atmosphere for development of poultry sector. Poultry farming is the process of raising domesticated birds, chickens, duck and gees are the example of it. Domestic birds kept by humans for their eggs, meat or feathers. Poultry science is the study of practices and principles involved. In production and marketing of poultry and its products are include breeding nutrition, management, housing, disease control and marketing for commercial benefit. Mostly chickens are farmed in great numbers. In united state, the national organisation overseeing poultry products is the food and drug Administration (FDA) and in UK, the national organisation is the department for environment, food and Rural Affairs (Anonymous, 2018).

Black rocks are one of the several breeds of chicken known as “sex links”. sex link chickens are unique because they are well known by their colours(Hanna, 2017).it commonly known that black rock are used for meat ,egg which is more helpful for human health. That original stock is said to be have come to England from the USA (Peter and

Siddons, 1950) and started raising black rocks on their farm, the Muirfield hatchery .this unique strain is improved over several decades .in 2010 ,Eddieand Calum Lovett (father and son) they cultivation of these birds. Nazifi (2011) studied about Reference values of serum biochemical parameters in adult male and female Iranian chukar partridge (*Alectoris chukar*). Khawaja (2012) studied comparative study of growth performance, egg production, egg characteristics and haemato-biochemical parameters of desi, fayoumi and Rhode Island Red chicken. Adriana and Leite (2018) studied serum biochemical profile of emus (*Dromaius novaehollandiae*) reared in captivity. The present study was an attempt to study the comparative biochemical parameters of blood serum of male and female black rock (*gallus gallus domesticus*)

MATERIALS AND METHODS

Healthy male and female rock hen were used for this for this study. animal were reared from CPDO Jaydev Vihar, Bhubaneswar. Both male and female were used to determine the serum biochemical parameters. Blood sample were collected from the brachial vein by using a sterilized disposal 25-ml syringe and needle (Beltsvile,1992) within a minutes and the sample were capture to ensure that the levels of the monitored were not affected by any stress induced by pre sampling handling (Chloupek *et.al.,* 2009).Blood sample were collected same hen after 1week. The collected blood was immediately centrifuged at 837x g at 4 C for 10 min, and plasma sample was stored at -20^{0}C in a test tube until the analysis were preformed. Blood plasma were selected for biochemical parameters the sample were analysed for total protein (Biuret method), cholesterol (Modified Abell-kendall/Levey Brodie (A-K) method), glucose, RBS, Albumin serum globulin were carried out a semiautomatic spectrophotometer and specific reagent kits(Labtest)

Data were analysis by independent t test, using SPSS/PC software for windows. Male and female were analysed separately because sex may affect the parameters studied (Rodringuez *et al.,* 2008). All values were expressed as mean ± standard error (SEM) was determined as statistically significant.

RESULTS AND DISCUSSION

In the current study, biochemical factors including sugar, protein, albumin, serum albumin and cholesterol were taken into consideration. Gender based analysis of the above mentioned parameters was done across five weeks. The sex basedserum chemistry values and the effect of sex on some blood parameters in black rock chicken are highlighted in the table- 1.

Sugar

In case of male black rock chicken, initially the blood sugar level was 110.7 mmol/l during the first week, which was slightly elevated in the second week of study and reached to a value of 116.4 mmol/l. Gradually, the sugar concentration was minimally decreased in the subsequent weeks but reached to the highest value of 134.9 mmol/l in the final week i.e., during the fifth week. Essentially, the blood sugar level ranges from 110.7 mmol/l to 134.9 mmol/l (Fig.1). Moreover the sugar concentrations in the male black rock chicken did not show any uniformity under the experimental conditions. Similarly in case of female black rock chicken during the first week of study the blood sugar concentration was 136.9mmol/l and gradually decreased in the second week and followed similar trend until the fourth week. Finally during the fifth week the sugar level again restored to a level as in case of the male i.e., 134.9 mmol/l (Fig.2). Suchy *et al.,*(2010) reported the sugar concentration male and female chukar partidage and by the sugar sexual difference were not observed in red-legged partridage (Rodriguez *et.al.,* 2004,2006)

Cholesterol

In case of male black rock chicken, initially the blood cholesterol level was 134.6 mmol/l during the first week, which was slightly elevated in the second week of study and reached to a value of 139.8 mmol/l. Gradually, the cholesterol concentration was increased in the subsequent weeks but reached to the highest value of 149.6 mmol/l in the

penultimate week i.e., during the fourth week and later decreased to 130.8 mmol/l in the final week of this study. Essentially, the blood cholesterol level ranges from 130.8 mmol/l to 149.6 mmol/l (Fig.1). Overall the cholesterol concentrations in the male black rock chicken did not show any uniformity under the experimental conditions. Likewise in case of female black rock chicken during the first week of study the blood cholesterol concentration was 142.6mmol/l and gradually decreased till the last week except in the third week. Finally during the fifth week the cholesterol level reached to 130.8mmol/l, similar to that of the male black rock chicken (Fig.2). There was no difference in serum cholesterol in birds at laying period as it reported by Bhatti *et al.,*(2002) They explained that serum cholesterol level in difference strain (Desi, Fayoumi) during hatching and prehatching laying period the cholesterol level lower than the values pheasant by Homswat *et al.,* (1999) and broilers by Meluzzi *et al.,*(1992)

Protein

In case of male black rock chicken, initially the blood protein level was 4.91 mmol/l during the first week, which was slightly decreased in the second week of study and reached to a value of 4.88 mmol/l. Gradually, the protein concentration was minimally decreased in the subsequent weeks and reached to a value of 4.79 mmol/l in the final week i.e., during the fifth week. Essentially, the blood protein level ranges from 4.79 mmol/l to 4.92 mmol/l (Fig.1). Moreover the protein concentrations in the male black rock chicken did not show any uniformity under the experimental conditions. Similarly in case of female black rock chicken during the first week of study the blood protein concentration was 4.2mmol/l and gradually decreased till the fourth week and elevated in the final week, where the protein concentration reached to 4.79mmol/l. Similarly in case of female black rock chicken during the first week of study the blood protein level was 4.2mmol/l and gradually decreased in the second week and followed similar trend until the fourth week. Finally during the fifth week the blood protein level again restored to a level as in case of the male i.e., 4.79 mmol/l (Fig.2). Ozek and Bahtiyarca (2004) studied the total protein showed no differences in sex and the total protein level results obtains from (Ozbey and Esen, 2007; Suchy *et al.,* 2010 and Rodriguez *et al.,* 2004) reported the total protein of male and female red leg paratridages.

Albumin

In case of male black rock chicken, initially the blood albumin level was 2.36 mmol/l during the first week, which was slightly elevated in the second week of study and reached to a value of 2.41 mmol/l. Gradually, the albumin concentration was minimally increased in the subsequent weeks but reached to the highest value of 3.02 mmol/l in the fourth week and again during the fifth week, a slight fall in the serum albumin concentration was observed. Essentially, the blood albumin level ranges from 2.36 mmol/l to 2.78 mmol/l (Fig.1). Moreover, the albumin concentrations in the male black rock chickendidnot show any uniformity under the experimental conditions. Similarly, in case of female black rock chicken during the first week of study the blood albumin concentration was 2.19 mmol/l and gradually decreased in the second week and followed similar trend until the fourth week. Finally during the fifth week the albumin level again restored to a level as in case of the male i.e., 2.78 mmol/l (Fig.2). Albumin will increase when protein intake exceed the amount required for growth and maintenance. In addition 50% of the calcium presents in blood is bound to albumin (Bell, 1971; Ross and Christie, 1978) found increases serum calcium to be a characteristic of young layer type birds.

Serum globulin

In case of male black rock chicken, initially the serum globulin level was 2.55 mmol/l during the first week, which was slightly decreased in the second week of study and reached to a value of 2.47 mmol/l. Gradually, the serum globulin concentration was decreased in the subsequent weeks but reached to 2.01 mmol/l in the final week i.e., during the fifth week. Essentially, the serum globulin level ranges from 1.78 mmol/l to 2.55 mmol/l (Fig.1). Moreover the serum globulin concentration in the male black rock chicken did not show any uniformity under the experimental conditions. Similarly in case of female black rock chicken during the first week of study the serum globulin concentration was 2.01mmol/l and gradually decreased until the fourth week. Finally during the fifth week the sugar level again restored to a level as in case of the male i.e., 2.01 mmol/l (Fig.2). Serum concentrations of

chukars are considerable higher than the values reported by Ozbey and Esen (2007). Seholtz *et.al.,* (2009) demonstrated higher values in Japanese quil. In some other avian species, higher values of AST values were reported in male males and females (Scholtz *et.al.,* 2009; Gylstroff and Grimm, 1998 and Sribhen *et.al.,* 2006)

CONCLUSION

The results concluded in this study that sugar level is slightly more in female while protein and serum globulin are little more in the male of black rock chicken.

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Table 1: Serum biochemistry values of sixteen-week-old male and female black rock chicken.

Parameter (unit)	Male (Mean±SE)	Female (Mean±SE)
Sugar (mmol/l)	117.36±1.018	118.94±1.558
Cholesterol (mmol/l)	139.48±0.726	139.84±0.524
Protein (mmol/l)	4.86±0.006	3.932±0.057
Albumin (mmol/l)	2.616±0.027	2.266±0.029
S Globulin (mmol/l)	2.244±0.033	1.666±0.029

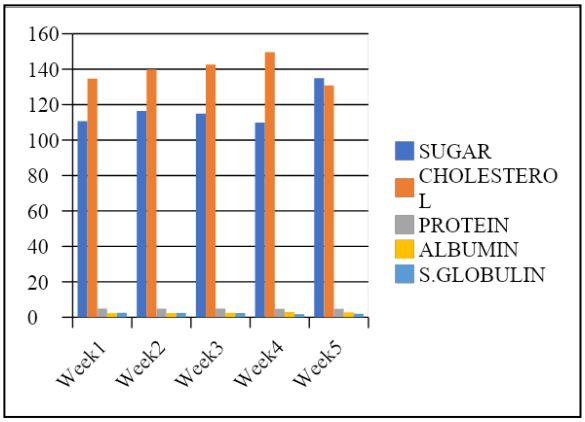

Fig.1 Serum chemistry of male black rock chicken.

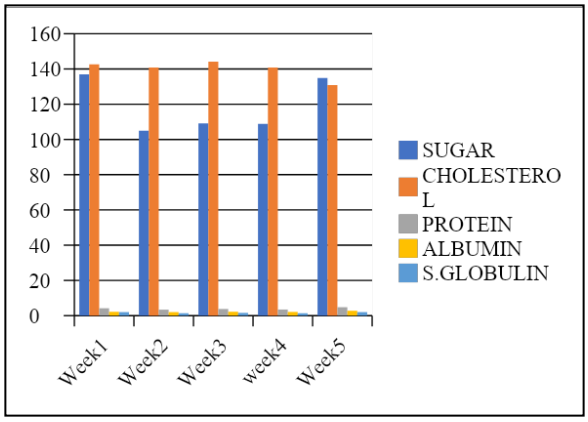

Fig.2 Serum chemistry of female black rock chicken

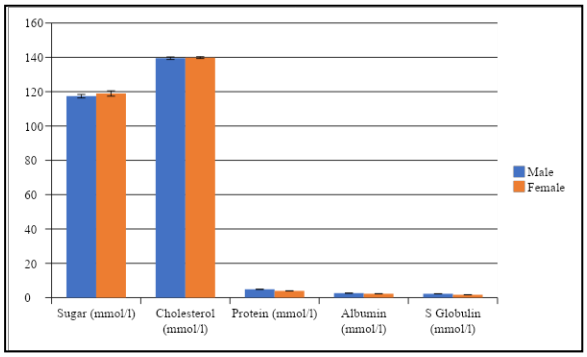

Fig.3 Serum chemistry comparison (Mean±SE) of male and female black rock chicken

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Green House Study on Growth and Productivity of Rice (*Oryza sativa* L. Var. IR64) After Combine Application of *Azotobacter vinelandii* (JQ796077) and *Azospirillum lipoferum* (JQ796078)

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Received: 29 Feb 2020 Revised: 31 Mar 2020 Accepted: 02 May 2020

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ABSTRACT

Rice (*Oryza sativa* L., Family *Poaceae*) is the staple food of 50% world, 80% Asian and 85% Indian population. Rice production highly depends upon supply of nitrogen. This investigation were to assess the growth and productivity of rice after combined application of our efficient native isolated bacteria *Azotobacter vinelandii* (JQ796077) and *Azospirillum lipoferum*(JQ796078). The growth and productivity of rice plant after the application of dual biofertilizer were studied and the phenotypic expression of rice plant was recorded. The agronomic parameters like plant height, number of tillers/plant, number of panicle/plant, number of filled grain/panicle, number of chaffy grains/panicle, straw dry weight, 100 grain weight, root length, root dry weight, leaf area and plant dry weight, endogenous nutrient contents also found to be higher as compared to control plants. These results indicated high and functional benefit of dual application of *Azotobacter vinelandii* (JQ796077) and *Azospirillum lipoferum*(JQ796078) in rice and our study provided a promising approach to improve the productivity of rice to salinity stress through overexpression.

Keywords: Nitrogen, *Azotobacter*, *Azospirillum*, Rice

INTRODUCTION

Rice is grown in almost all Indian states and West Bengal, Uttar Pradesh, Madhya Pradesh, Bihar, Odisha, Andhra Pradesh, Assam, Tamil Nadu, Punjab, Maharashtra and Karnataka are major rice growers which contribute to 92% of area and production (Seck et al. 2012). Furthermore, nitrogen most often limits rice production which requires 1 kg N/15–20 kg rice (Choudhury and Kennedy 2004). In the tropics, lowland rice yields 2–3.5 t/ha using natural N derived from biological nitrogen fixation (BNF) by free-living and plant-associated diazotrophs and from mineralization in soil N (Bashan and de-Bashan 2010). Current environmental protection requirements also make it necessary to develop ecologically clean technique of crop production that makes maximum use of natural sources of bound N. In addition, scarcity and the increasing cost of non-renewable chemical fertilizers necessitated the greater use of renewable indigenous biological N2-fixation system as source of N. Thus, biological fixation of atmospheric N, especially non-symbiotic N2-fixation in the soil, has been the subject of continuing interest in recent decades especially for low input agriculture. rice being a monocot, associative N2 fixing microbes like *Azotobacter* and *Azospirillum* would be the key components for *in situ* nitrogen fortification (Kannan and Ponmurugan, 2010). In this study we observed the phenotypic growth of rice plant after the combined application of *Azotobacter vinelandii* and *Azospirillum lipoferum*.

MATERIALS AND METHODS

Sites for pot experiments

The pot experiments were conducted in the green house of International Centre for Genetic Engineering and Biotechnology, New Delhi, India, in the year 2018-2019, to unveil the combined effect of native efficient *Azotobacter vinelandii* and *Azospirillum lipoferum* strain for formulation and production of potent indigenous biofertilizer for commercial exploitation in rice fields.

Formulation of biofertilizers

Biofertilizers were formulated aseptically using sterile (autoclaved) charcoal powder 700 g/kg, gum acacia 20 g/kg, $CaCO_3$ 100 g/kg, and liquid culture 180 g/kg (180 ml containing10^9 cfu/ml) i.e. final population 2 x10^8 cfu/g formulation (according to Bureau of Indian Standards (BIS).

Treatment of seedlings and design of pot experiments

Healthy, 21d old rice (*Oryza sativa* L. var. IR 64) seedling were dipped separately in biofertilizer suspensions (10% w/v i.e. 2 x 10^8cfu/ml) for 2 h and transplanted in different pots with three replications each viz. Control (C) without any fertilizer; Treatment 1 (T1) with *Azotobacter vinelandii.*; Treatment 2 (T2) with *Azospirillum lipoferum*; Treatment 3 (T3) with both *Azotobacter vinelandii* and *Azospirillum lipoferum.*

Growth parameters

Growth parameters like plant height (cm), tiller/hill (no), effective tiller/hill (no), panicle length (cm), leaf area (sq. cm) and panicle length (cm) were measure prior to harvest. The crop was harvested after 90d and the post harvest observations like root length (cm), root dr. wt. (g), root volume (ml), panicle weight (g), grain yield/plant (g), filled grain/panicle (no.) and 1000 grain wt. (g) were recorded.

Statistical analysis

All statistical analysis were performed using the graph and prism software. The experimental data values were mean values from three independent series, each done with three replicates, and the results presented as means ±• standard error (SE), based on three replications. The statistical significance at $P< 0.05$ has been calculated.

RESULTS

Growth observations in pot experiments

Effects of the biofertilizer formulations on the growth of the rice variety IR 64 are given in the Table 1. The treatment T1, T2 and T3 had significantly improved growth of the plants over control. T3 had relatively higher growth than all other treatments in the pot tests.

Plant height

Plant height increased progressively with time and reached maximum at harvest. The rate of increase in height was maximum between 25 and 50 DAS and thereafter plant height increased progressively with time up to harvest but a diminishing rate. Plant height was maximum in T3. At harvest there was no significant difference in plant height amongst the treatment. Highest plant height was recorded in T3 (89.65 cm).

Number of tiller/plant

Tiller number per plant increased up to 75 DAS and then height at harvest and significantly varied among the treatments observed. At the time of harvest T3 had highest number of tiller/plant i.e. 17.52 which was significantly different from rest others.

Effective tiller/plant

Number of effective tiller per plant was observed highest at harvest period. There was significant variation among the treatments observed. At the time of harvest T3 had more number of effective tiller/plant i.e. 15.21 which was significantly different from other treatments.

Panicle length

Significant variations among treatments were observed for panicle length. However maximum panicle length of 26.17 cm was observed for T3.

Root length

The data (Table 1) revealed that the root length increased up to 80 DAS. Significant differences among root lengths at different treatments was observed, the T3 showed larger roots as 25.45 cm.

Root dry weight

Root dry weight among different treatments was significantly different (Table 1). The T3 produced higher dry weight as 2.41g of root.

Root volume

A cursory look on Table1 indicated that root volume increased up to 80 DAS. There was significant difference between root volumes of different treatments at harvest. The T3 produced higher root volume i.e. 28.25 cm3 which was significantly higher than the other treatments.

Leaf area

There was significant difference in leaf area at flowering stage among the treatments. However, T3 exhibited higher leaf area (285.26 cm^2) where as other treatments were exhibited lower leaf area (Table 1).

Grain yield

Significant variation of grain yield among treatments existed (Table 1), the T3 had higher grain yield/plant i.e. 31.19 g.

Filled grain/panicle

More number of filled grains (72.13)/panicle were observed in T3. The results varied significantly among different treatments.

1000 grain weight

Significant variation between treatments for 1000 grain weight was concerned (Table 1). However higher grain weight (26.54 g) was observed for T3.

DISCUSSION

The three types of biofertilizer formulations showed differential effects on growth and yield parameters of rice (*Oryza sativa*) variety IR 64 in pot experiments in green house of ICGEB, New Delhi. The specific formulations (native) exhibited bettered growth compared to control (without application of fertilizer). The combined application of two efficient biofertilizer (*Azotobacter vinelandii* and *Azospirillum lipoferum*) showed significant effect on growth and productivity of rice in comparision to individual (*Azotobacter vinelandii*) or (*Azospirillum lipoferum*) application. Experimental evidences indicated that root length of wheat 35 days after seedling was largest when inoculated with *Azotobacter* plus *Azospirillum* but in maize root length increased only in sterilized soil when inoculated with *Azotobacter* alone and also in combination with *Azospirillum.*

Seed inoculation in combination with *Azospirillum brasilense* and *Azotobacter chroococcum* produced synergistic effect on yield of maize, sorghum and barley (Tilak and Murthy 1983). Zambre et al. (1984) stated that tiller numbers of wheat increased by the application of nitrogenous fertilizer (up to 120 kg N/ha) along with *Azotobacter chroococcum* and *Azospirillum brasilense*. Similarly, wheat seeds inoculated with *Azotobacter*, *Azospirillum* and composted refuse stimulated tiller number and plant growth. Effect is more with *Azotobacter* than *Azospirillum* (Ishac et al. 1986). Zambre et al. (1984) are of the opinion that inoculation of wheat seeds with *Azotobacter chroococcum* increased the number of effective tiller per plant. Similarly they also stated that *Azospirillum brasilense* when inoculated with wheat seeds produced more number of effective tillers per plant. Wani et al. (1988) found that continued inoculation of *Azotobacter* and *Azospirillum* in pearl millet plants for 2 or 3 years increased plant biomass yield. Dewan and SubbaRao (1979) are of the opinion that root biomass of rice seedling increased due to inoculation with *Azospirillum brasilense* and *Azotobacter chroococcum* alone or in combination.

The increase in biomass of root was better in unsterilized soil than in sterilized soil with or without inorganic N applied as urea. Grain yields of rice and wheat were increased by inoculation with *Azotobacter* and *Azospirillum* along with the application of up to 120 kg N/ha N fertilizer. *Azospirillum* gave better yields than *Azotobacter* (Zambre et al. 1984; Wani et al. 1988; Gopalswamy et al. 1989). Increased grain yields of >10% (up to 33%) over the un-inoculated control were observed in pearl millet and maize plants when inoculation with *Azotobacter* and *Azospirillum* was carried out (Wani et al. 1988; Pandey et al. 1998). Application of *Azotobacter* and *Azospirillum* to wheat crop gave the grain yield of 3.05–3.85 and 3.16–4.04 t/ha respectively over the yield of 2.90 to 3.22 t/ha without N. Application of 40 kg N plus *Azotobacter* was reported to be the most efficient fertilizer for wheat (Zambre et al. 1984). Experimental evidence indicated that combined inoculation of *Azotobacter* and *Azospirillum* produced higher grain yield of sorghum (3.32 t/ha) than inoculation with *Azotobacter* (2.53 t/ha) or *Azospirillum* alone (2.97 t/ha) or from control (2.27 t/ha). Experimental results revealed that when maize cv. Vijay. Balasubramanian and Veerabadran (1997) were of the opinion that combined application of *Azospirillum* along with 50% N as inorganic fertilizer and 25% N in the form of prickly *Sesbania* significantly increased the straw yield (588.99 t/ha) of rice over other treatments. Experimental results revealed that rice and wheat seeds inoculated with *Azotobacter* and *Azospirillum* along with increased rates of N fertilizer produced higher straw yield. *Azospirillum* gave better result than *Azotobacter* (Wani et al. 1988). Experimental evidences indicated that continued seed inoculation for 2 or 3 years with *Azotobacter* and *Azospirillum* to wheat, maize and pearl millet plants increased N uptake by the plants (Wani et al. 1988).

The finding presented here concluded that the combination of native *Azotobacter* and *Azospirillum* spp. is more effective than single application. This dual combination of fertilizer possess enhanced growth and production of rice (*Oryza sativa* var. IR 64) in different soils, especially in lateritic soils, without the recommended dose of nitrogen fertilizer.

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Table 1: Growth (Plant height, root length, root dry weight, leaf area) of rice plants at different treatments (T1, T2, T3) and control (C) after application of native biofertilizer

Attributes	C (Control, without any fertilizer)	T1 (*Azotobacter vinellandii*)	T2 (*Azospirillum lipoferum*)	T3 (*A. vinellandii*+ *A. lipoferum*)
Plant height (cm)	75±3.2^a	79±3.1^a	81±3.1^b	89.65±3.0^c
Number of tiller/plant	12.21±0.8^a	15.37±1.2^a	15.21±1.1^a	17.52±1.1^b
Effective tiller/plant	12.27±0.12^a	14.43±0.1^a	14.75±0.1^a	15.21±0.12^b
Panicle length (cm)	20.56±2.4^a	23.14±1.6^a	23.32±1.5^b	26.17±1.0^c
Root length (25.45)	20.05±0.22^a	24.23±0.3^a	23.15±0.4^a	25.45±0.5^b
Root dry weight (g)	1.75±0.53^a	1.74±0.82^a	1.78±0.55^a	2.41±0.91^b
Root volume (cm^3)	21.25±0.5^a	25.11±0.2^a	24.05±0.3^a	28.25±0.4^b
Leaf area (cm^2)	246±11.4^a	248±10.9^a	255±10.2^b	285.26±11.5^c
Grain yield (g)	22.34±11.2^a	26.15±11.4^a	28.11±10.4^a	31.19±10.5^b
Filled grain/panicle	65.31±0.011^a	67.15±0.012^a	68.46±0.011^b	72.13±0.011^c
1000 grain weight (g)	20.93±0.011^a	24.22±0.011^a	24.71±0.011^a	26.54±0.011^b

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Bioaccumulation of Heavy Metals on the Fish: *Punctius ticto* from Kolab River, Koraput, Odisha, India

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Received: 28 Feb 2020 Revised: 02 Apr 2020 Accepted: 04 May 2020

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ABSTRACT

Koraput district is highland agro climatic zone of Eastern Ghatsof Odisha,India.It is known for its rich and various types of mineral deposits. Kolab river is situated at Sinkaran hills of Eastern Ghats in the district of Koraput. The Kolab River is mainly used for the purpose of irrigation and electricity power production. Kolab River is largely consumed by the people of undivided Koraput district. In this study, the impact of the heavy metal present in the fish *Punctius ticto* was investigated. This study is aimed to assess heavy metal concentration and their bioaccumulation status in commercially edible fishes. This is also aimed for the evaluation of human health risk for local consumers. The river Kolab has been one of the major recipients of industrial effluents in koraput district of Odisha state. The present study revealed the accumulation of heavy metals Cu, Mn, Fe, Zn, Ce, Eu in *Punctius ticto* and the presence of Mn, Fe, Sn, P in the river water. These output concentrations are higher than the standard limits. So, the presence of heavy metal in the river Kolab increases or produces in toxicity on different organs of fish or fishes. The presence of heavy metals i.e. Cu, Zn, Mn, Fe, Ce found in the commonly consumed fish *Punctius tictoand* in the water of river Kolab as well. Biotic organism of river ecosystem may come across with bioaccumulation of heavy metals that can put adverse consequences on human and livestock.

Keywords: Eastern Ghats, Heavy Metal, Bioaccumulation, *Punctius ticto*

INTRODUCTION

Environmental problem is the universal problem, most important pollutants are the heavy metals in aquatic network because of their accumulation, biomagnifications and toxicity by marine animals(1).The source of contamination for natural aquatic system are domestic, industrial anthropogenic activities(1,2).Environmental equilibrium and a

variety of aquatic entities face dreadful effects due to heavy metal pollution(3,4). Heavy metals are the pollutants that bioaccumulate in the food chain, causing antagonistic effects which also leads to even death. So, among other organisms' fishes are used to discover the health condition of the aquatic ecosystem (5,6). In fish, heavy metals mainly come from their diet and the bioaccumulations of heavy metals are more in fish that comes higher in foods chain (7). Heavy metals and metalloids occur or occurring in high concentrations, they become toxin for all the living organisms including human beings. Exposure to excessive amount of Hg, As, Cd, and Pb elements could be detrimental to living cells and can also lead to illness and even death (8).Inhibition of protein synthesis, microtubule disruption, disturbance of neurotransmitter function with increase in intracellular calcium are caused due to methyl mercury toxicity(9).Prolonged exposure to Cd even at low concentration cause prostatic proliferative lesions, lung cancer, bone fractures and kidney failure (10,11).

The Kolab River is one of the potential fish habitats and known to be an important breeding, feeding and nursery ground for many aquatic species. There has been no proper investigation carried out on the potential human risk due to heavy metal contamination in fish species harvested and consumed from the Kolab River. So, this is study aimed to determine the concentration of heavy metals in the fish *Punctius ticto* and the human health risk for local adult and children consumers.

MATERIALS AND METHODS

Punctius ticto and water samples were collected from the Kolab River. The fish which was preserved in the container was taken out and measured its length.The species is then identified taxonomically. The following fish *Punctius ticto* was then dried in the oven for about 100 ^{0}C for 3 days. The dried fishes are then crushed in to powder form by using Mortar and Pestle. The powdered samples were then ready for further detection of heavy metals. River water sample was also set for heavy metal detection. The study was conducted from 13thJanuary, 2020 to 11thFebruary, 2020 in the Department of Zoology, Centurion University, Bhubaneswar, Odisha, India.Fish sample *Punctius ticto* and river water sample was analyzed by undergoing XRF and water parameter experiments. The sampling site is inIntake Bridge, 2kms away from sunabeda town (18°45'21.4" N 82°49'23.7" E). The sample site is with high anthropogenic pressure like cattle and human beings.

Kolab River water has been tested with 6 parameters. They arepH, Temperature,Conductivity, Hardness of water, Dissolved Oxygen, Turbidity. Following parameters have been tested in the laboratory and successfully found out the result of each parameter. Statistical tool like mean, mean deviation were applied using Microsoft Excel.

RESULT AND DISCUSSION

Table 1 gives the concentration of different metals found in Fish: Punctius ticto and river water. Concentration of SiO_2–1.964%, P_2O_5 – 16.375%, SO_3 – 8.809%, K_2O – 0.398%, TiO_2 – 0.180%, CaO–70.371%, FE_2O_3 –1.016%, SrO–572.1ppm, ZrO_2 –41.7ppm, CeO_2 –420.9ppm, Eu_2O_3 –525.9ppm, CuO-345.6ppm, ZnO – 0.320%, MnO – 0.243% was found in fish and concentration of Si–0.243%, P–573.3ppm, S–198.3ppm, Cl–283.9ppm, K–258.2ppm, Ca–197.2ppm, Ti– 37.4ppm, Fe–272.3ppm, Mn–2.8ppm, Sn–33.5ppm was found in river water.

According to the XRF report of both the samples that is *Punctius ticto* and Kolab River water, all the compounds and water sample is converted into percentage and then their mean and standard deviation is calculated. For each and every compound with water sample, mean and standard deviation is calculated (Table – 1) and also represented graphically. After analysis of physico chemical parameters of the water, on the basis of experiments following results are found and calculated its mean value as well as standard deviation. The results are shown on the above-mentioned table as well as represented graphically for better understanding of the ratios

CONCLUSION

Fish has become main ingredient to be investigated as they are found to be the major victims of heavy metals contamination in an aquatic ecosystem. In this study, effects of heavy metals on fish were examined and literature review was performed. Heavy metals cause damage to the growth, development, reproduction, nourishment and survival of fish by affecting physiological, biochemical, and metabolic, systemic and genetic functions. As heavy metals are biologically indestructible for both humans and fish and both of them cannot metabolizeheavy metals, even if the fish do not exceed toxic concentrations in them, they may reach up to humans via consumption of fish and cause severe health problems. Thus, fish tissue contamination monitoring is an important function of sediment and water quality contamination. It shows a major effect on the next trophic level as they are consumed by different birds and animals that use fish as their food. It especially comes to the evaluation of human health risk because fish is a good source of food for human beings. From this it is concluded that domestic and anthropogenic activities should be limited or technologies should emphasize in finding out the solution for these activities and waste management programme.

ACKNOWLEDGEMENT

The authors would like to thank Dr. Siba Prasad Parida (Asst. Professor), Centurion University of Technology and Management, Bhubaneswar, Odisha, India.

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Table – 1 Sample report with mean and standard deviation

Compound → / Sample ↓	Si	P	S	Cl	K	Ca	Ti	Mn	Fe	Cu	Zn	Sn	Sr	Zr	Ce	Eu	Co2	H2O
Punctius ticto	1.96%	16.38%	8.81%	0.13%	0.40%	70.37%	0.18%	0.24%	1.02%	0.03%	0.32%	0	0.06%	0.00%	0.04%	0.05%	0	0
Kolab River water	0.24%	0.06%	0.02%	0.03%	0.03%	0.02%	0.00%	0.00%	0.03%	0	0.00%	0.00%	0	0	0	0	0	99.57%
Avg	1.10%	8.22%	4.41%	0.08%	0.21%	35.20%	0.09%	0.12%	0.52%	0.02%	0.16%	0.00%	0.03%	0.00%	0.02%	0.03%	0.00%	49.79%
Std Dev	0.008605	0.081589	0.043946	0.0005235	0.001861	0.3517565	0.000882	0.001214	0.004944	0.0001725	0.0016	0.00001675	0.000286	0.0000205	0.00021	0.000263	0	0.497855

Table – 2 Analysis of Parameters Kolab RiverWater

Water samples	pH	Conductivity	Temperature	Hardness	Dissolved Oxygen	Turbidity
sample 1	7.74	87.61	23.9	98.1	13	0.014
sample 2	7.41	88.5	24.6	98.2	11	0.011
sample 3	7.71	88.49	24.4	98.6	12	0.011
Mean	7.62	88.2	24.3	98.3	12	0.012
±SD	0.182483	0.51097945	0.360555128	0.2645751	1	0.001732

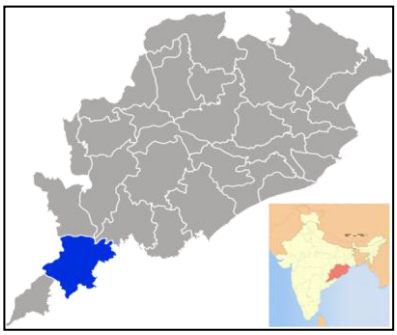

Fig.1.Map of Odisha, Blue highlighted is Koraput district, sample site

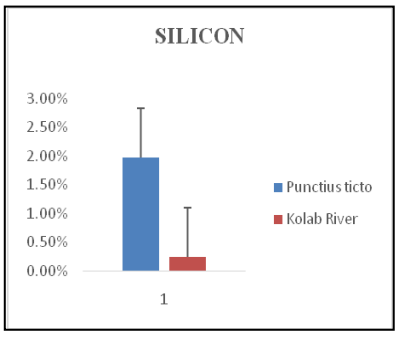

Fig.2.Mean Silicon

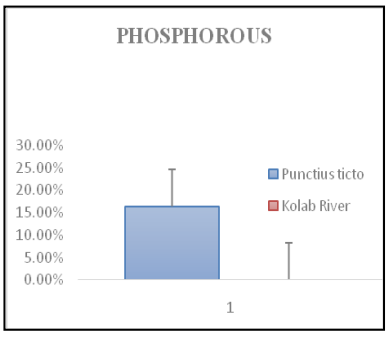

Fig.3.Mean Phosphorous

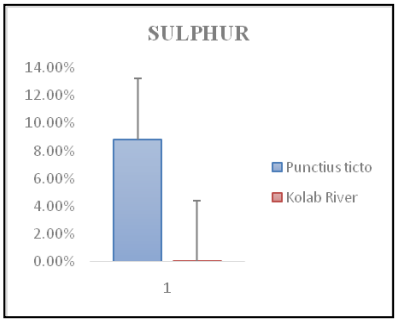

Fig.4. Mean Sulphur

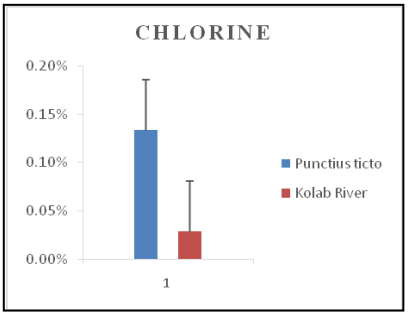

Fig.5.Mean Chlorine

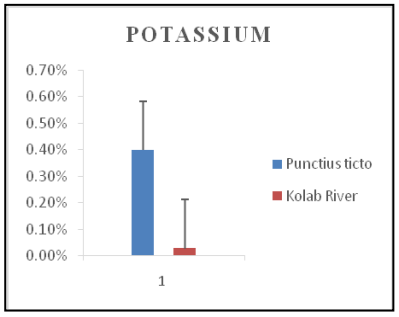

Fig.6.Mean Potassium

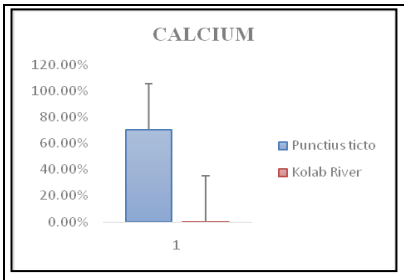

Fig.7. Mean Calcium

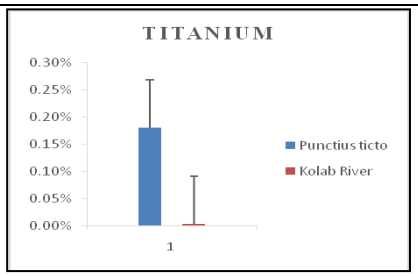

Fig.8.Mean Titanium

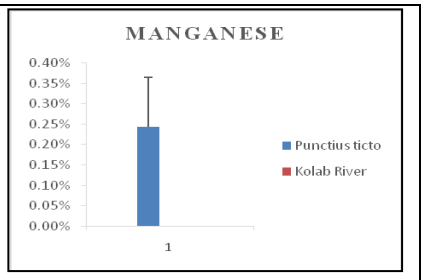

Fig.9.Mean Manganese

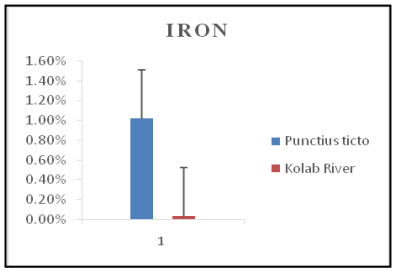

Fig.10. Mean Iron

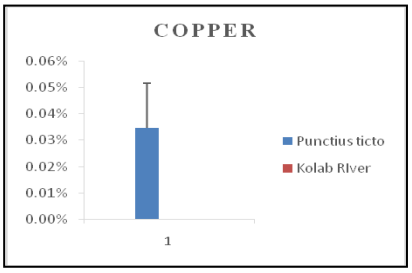

Fig.11. Mean Copper

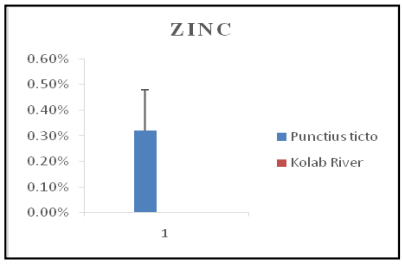

Fig.12.Mean Zinc

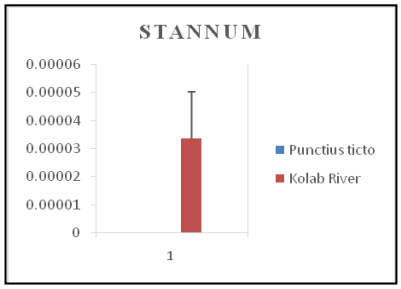

Fig.13. Mean Stannum

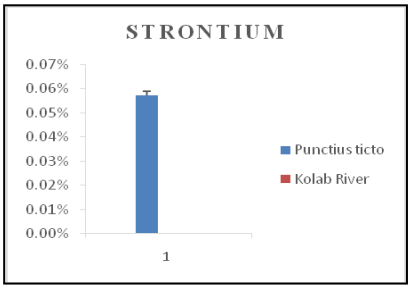

Fig.14. Mean Strontium

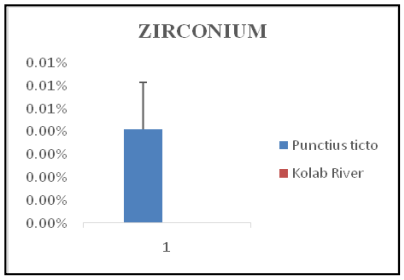

Fig.15. Mean Zirconium

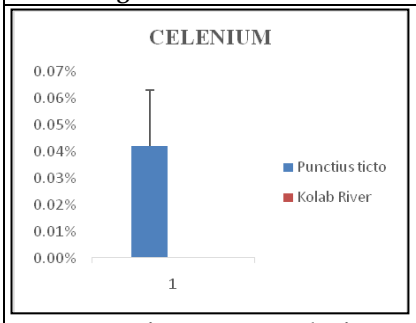

Fig.16. Mean Celenium

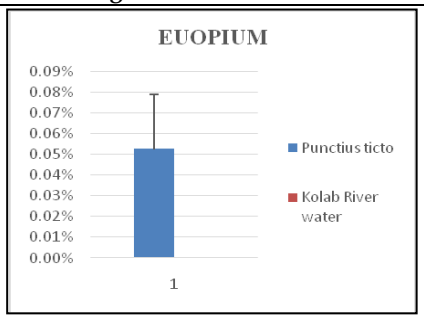

Fig.17. Mean Euopium

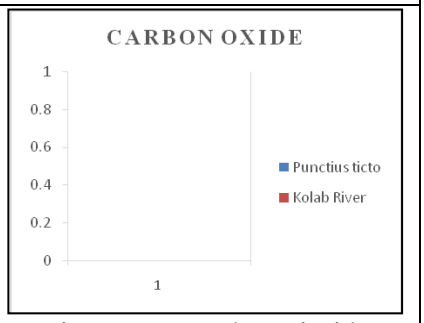

Fig.18. Mean Carbon Dioxide

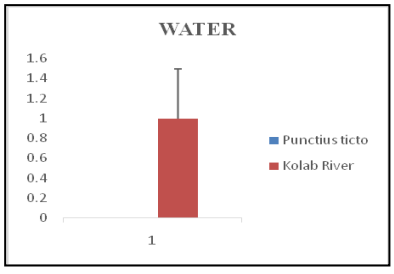

Fig.19. Mean Water

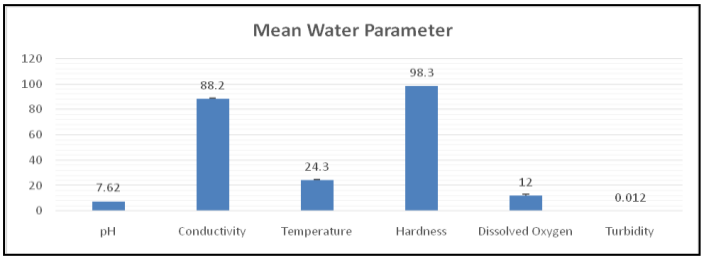

Fig.20. Graph of Water Analysis with mean and standard Deviation

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Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Work Place Stress at Women Police Constables

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Received: 26 Feb 2020 Revised: 01 Apr 2020 Accepted: 04 May 2020

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ABSTRACT

The review of literature has commonly highlighted a variety of workplace problems, such as family, working hours, shifts, as influences on police stress especially on female police. Lack of influence over work activities and bias against one's racial, gender. Interventions to redesign jobs to afford greater influence and to reduce within department bias are approaches that could reduce police stress.Police force is a constituted body of persons empowered by the state to enforce the law, protect property, and limit civil disorder. The present study is conducted to investigate the stress factors faced by the female police in their work. A survey is conducted with 78 numbers of female police constables with a structured questionnaire in Gajapathi and Nuapada Districts of Odisha state. A simple descriptive statistics, Independent T-Test has been used to analyse the data. The results have been discussed with proper bias in the findings.

Keywords: Female Constable Stress, Mental health, Work and Organizational domains, family

INTRODUCTION

Gender plays a major role in stress as female police personnel face more stress related problems than male police personnel. When women are overwhelmed by occupational stress they suffer from increased chronic stress, depression, heart disease, stomach disorders, alcohol and drug usage and even suicide attempts.Women are playing dynamic roles from homemaker to Political leader [1].In the 21st century, women enter the criminaljustice system as a response to social forces like societal violence, individual violent behaviour, social problems, child abuse,crime against women and children, and for better protection of women and juveniles. Women in policing have had animportant political, social, economic, and psychological impact [2].Stress is considered an integral part of modern life and itis the psychological or physiological reaction that occurs when an individual perceives an imbalance between the level ofdemand placed upon him and his capability for meeting that demand [3]. When compared to the other occupations, policejob is considered highly stressful and stress is an integral part of the life of a professional

police officers [4]**.** Police often encounter stressful situations in their daily work, and these stressors have cumulative effects [5]. Gender playsmajor role in stress as female police personnel face more stress related problems than their male counter part as they managethe family commitments and also working in law enforcement [6].Women in policing have had an important political, social, economic andpsychological impact. Stress is considered as an integral part of modern life and it is the psychological orphysiological reaction that occurs when an individual perceives an imbalance between the level of demandplaced upon him and his capability for meeting the demand. Gender plays a major role in stress as femalepolice personnel face more stress related problems than male police personnel. When women areoverwhelmed by occupational stress they suffer from increased chronic stress, depression, heart disease, stomach disorders, alcohol and drug usage and even suicide attempts. During festival timings, police oftenwork for more than 36 hours at a stretch. This may take a heavy charge on their health.

Literature Review

Studies have found the factors for stress among police were round-clock duty, no time for family, inadequate salaries, negative interaction with other police staff, poor equipment, no recognition, too many cases, insufficient staff and harassment [7]. Criticism by superiors, excess work, no rewards, inadequate value given to abilities and commitments and no satisfaction from work found the reasons for stress among police personnel [8].Mathur has found inadequate equipment, fear of severe injury, working conditions, anti-terrorist operations, lack of recognition, being killed on duty, work overload shooting someone in the line of duty, tackle with the public, lack of job satisfaction and police hierarchy were acted as stressors stress among Indian police personnel [9]. Political pressure, lack of time for family, negative public image and low salary were the primary causes of stress among police personnel [10].Shift work closely associated with occupational stress [11].

METHODOLOGY

In this descriptive study, 78 samples from the universal size of 258 taken from two Districts i.e. Gajapathi district (Border of Andhra Pradesh) and Nuapada district (Border of Chhattisgarh) in the state of Odisha. The respondents are only police constables from both the districts and not included any other officers. The data has been collected through a structured questionnaire with more 78 questions of different stress domain related. All the data has been analysed by SPSS 21 version to get the output. The details of two district samples are below

Objective

The objective of the study is to find out the stress related symptoms associated with Personal Domain variables, Work Domain Variables, Organizational domain Variables, Personal Domain Variables, Interpersonal Domain variables, Physical, Mental health Domain Variables, Social Domain Variables, Health and wellbeing Domain Variables and Coping strategy Variables.

Data Analysis and Interpretation

Table-1: Descriptive Statistics and P-Values of Personal Domainvariables

Personal domain variables	N	Mean	SD	t	df	t-value	p-value Sig. (2-tailed)
Insufficient personal time	78	3.077	1.2143	.495	107.880	.0742	.621
Burdened with unresolved issues of the past	78	2.9359	1.09710	-1.114	108.176	-.15101	.268
Working against will in the organization	78	2.3718	1.08243	.699	120.901	.09623	.486

Have to adapt to a new lifestyle	78	2.8846	1.12793	1.447	116.182	.20550	.150
Suffer from low self-esteem	78	2.5641	1.32498	2.064	107.258	.33706	.041
Worried about health	78	3.2821	1.26796	-.776	110.307	-.12217	.439
Motivated to take up challenges	78	3.4359	1.22304	.562	106.027	.08445	.575
Revision of personal habits	78	2.6667	1.12431	1.496	121.062	.21415	.137

For two district wise and personal domain variables, there is a significant difference in the mean opinion of respondents of two districts on burdened with unresolved issues of the past, working against will in the organization, have to adapt to a new lifestyle, suffer from low self-esteem and worried about health variables. For the other variables the mean opinion issame.

Table-2: Descriptive Statistics and P-Values of Work Domainvariables

Work domain variables	**N**	**Mean**	**SD**	**t**	**df**	**t-value**	**p-value Sig. (2-tailed)**
Performing tasks not related to the job description	78	2.5513	1.18044	.628	119.803	.09407	.531
Experience excessive work pressure	78	2.9615	1.37172	-2.323	109.235	-.39468	**.022**
Carrying lot of responsibilities and duties	78	2.8077	1.28990	-.323	111.280	-.05188	.747
No control over my work schedule (Working shifts)	78	3.1282	1.31286	-2.337	110.527	-.38111	**.021**
Working for long hours, on overtime and even on holidays.	78	3.0641	1.20970	-.549	120.362	-.08445	.584
Chance to do things for other people	78	3.4103	1.09824	-2.143	130.20	-.30561	.034
Freedom to use own judgement	78	2.9487	1.32799	1.117	118.888	.18791	.266
Feel insecure in the working environment	78	2.7179	1.32799	-.689	109.028	-.11330	.492
Not satisfied with day-to-day work	78	2.7051	1.18551	-.318	122.486	-.04815	.751
Difficulty with frequent jobs posting & transfer procedures	78	3.2436	1.40672	-1.557	111.461	-.27266	.122

For two district wise and work domain variables, there is a significant difference in the mean opinion of respondents of two districts on experience excessive work pressure, carrying lot of responsibilities and duties and freedom to use own judgement variables. For the other variables the mean opinion is the same.

Table-3: Descriptive Statistics and P-Values of Organizational Domainvariables

Organisational domain variables	**N**	**Mean**	**SD**	**t**	**df**	**t-value**	**p-value Sig. (2-tailed)**
Slow career progression	78	2.8846	1.24818	-1.491	109.341	-.23055	.139
Problems subject to complaints investigation	78	2.8974	.98811	.327	124.721	.04152	.744

Clear about duties and responsibilities	78	3.4231	1.37245	1.392	104.674	.23390	.167
Respect from higher authorities and colleagues	78	3.3846	1.40713	1.533	108.339	.26655	.128
I get supportive feedback on the work I do	78	3.2821	1.41327	1.647	104.433	.28481	.103
The opportunity for independent thought and action	78	2.9103	1.20805	2.906	118.554	.44439	**.004**
Positive organizational culture and climate	78	3.0385	1.30376	2.364	107.571	.38014	**.020**
Organization always supports the employees	78	3.2051	1.24169	.716	115.580	.11173	.476
Senior officer recognizes and rewards for outstanding performance	78	2.9615	1.35265	.604	114.493	.10242	.547

For two district wise and organisational domain variables, there is a significant difference in the mean opinion of respondents of two districts on get supportive feedback on the work done variable. For the other variables the mean opinion is thesame.

Table-4: Descriptive Statistics and P-Values of Interpersonal Domainvariables

Interpersonal domain variables	N	Mean	SD	t	df	t-value	p-value Sig. (2-tailed)
There is friction or anger between colleagues	78	2.4103	1.12164	-1.382	116.083	-.19514	.170
I am subject to personal harassment in the form of unkind words or behavior	78	2.2949	1.16339	-.894	110.407	-.12917	.373
I am subject to bullying at work	78	2.3974	1.09710	-.141	116.293	-.01953	.888
Struggle to get along with superiors, subordinates and peers	78	2.5256	1.24550	.268	108.354	.04122	.789
Difficult to communicate and share views	78	2.5897	1.16704	-.158	111.107	-.02288	.875
I am not able to satisfy the demands ofColleagues and public, since these are conflicting with one another.	78	2.7308	1.21327	-.960	115.867	-.14647	.339

For two district wise and interpersonal domain variables, there is a significant difference in the mean opinion of respondents of two districts on friction or anger between colleagues variable. For the other variables the mean opinion is same.

Table-5: Descriptive Statistics and P-Values of Social Domainvariables

Social domain variables	N	Mean	SD	t	df	t-value	p-value Sig. (2-tailed)
I spend so long at work that my outside relationships are suffering	78	2.9872	1.25350	-2.326	110.568	-.36218	.022

Loss of interest in social activities	78	2.3718	1.21793	.868	109.987	.13111	.387
Verbal & Physical aggression from the public	78	2.8077	1.26960	-1.365	109.279	-.21467	.175
Disturbs mood when witnessing accidents and domestic dispute	78	2.7308	1.25536	-1.128	109.760	-.17554	.262
I feel quiet and pleasant atmosphere in the society	78	2.9103	1.36929	-1.251	110.095	-.21258	.214
Job demands affect personal relationships (friends, relatives etc)	78	2.9231	1.23551	-1.256	111.852	-.19343	.212
Difficulties arising in communicating with multilinguistic public	78	2.6923	1.26191	-.096	109.707	-.01498	.924
Insufficient facilities to handle the society aspects	78	2.9231	1.15959	-1.134	113.158	-.16436	.259

For two district wise and social domain variables, there is a significant difference in the mean opinion of respondents of two districts on spend so long at work that outside relationships are suffering variable. For the other variables the mean opinion is the same.

Table-6: Descriptive Statistics and P-Values of Family Domainvariables

Family domain variables	N	Mean	SD	t	df	t-value	p-value Sig. (2-tailed)
Insufficient time to spend with family	78	2.6667	1.28595	.176	110.076	.02810	.861
Having family conflicts because of work issues	78	2.7692	1.21594	-.688	109.060	-.10353	.493
I am unable to take sufficient breaks for family chores	78	2.9615	1.11008	-1.167	113.662	-.16212	.246
I am away from family sometimes due to heavy duties	78	3.1026	1.19076	.053	115.165	.00790	.958
I receive good support from my family members	78	3.8333	1.25270	-1.892	120.601	-.30136	.061
Not able to attend relatives and family programmes	78	3.0897	1.28109	-.872	111.687	-.13916	.385
Difficult to fulfil my family financial needs	78	2.8590	1.28654	-.872	115.250	-.14095	.385

For two district wise and family domain variables, there is no significant difference in the mean opinion of respondents of two districts.

Table-7: Descriptive Statistics and P-Values of Health and wellbeing Domainvariables

Health and well Being Variables	N	Mean	SD	t	df	t-value	p-value Sig. (2-tailed)
Having sleeping troubles	78	2.6410	1.31894	-1.723	107.856	-.28056	.088
Threats of physical violence	78	2.5128	1.18150	-.126	115.152	-.01863	.900
Having poor concentration	78	2.3462	1.18241	1.109	117.414	.16547	.270
Want to be alone always from others	78	2.5256	1.22447	-.053	120.433	-.00820	.958

Feel out-of-control	78	2.6795	1.25350	-.087	117.040	-.01379	.931
Easily Frustrated	78	2.7949	1.24169	-.897	118.251	-.14080	.372
I work under a great deal of tension	78	2.7821	1.19139	-.115	122.541	-.01752	.909
I have worried after making a decision whether I did the right thing	78	2.3590	1.17301	1.156	121.994	.17300	.250
Marital problems	78	1.6667	1.57634	-.184	109.264	-.03585	.855

For two district wise and health and well-being domain variables, there is a significant difference in the mean opinion of respondents of two districts on having sleeping troubles, threats of physical violence, want to be alonealwaysfrom others and have worried after making a decision whether did the right thing variables. For the other variables the mean opinion is the same.

Table-8: Descriptive Statistics and P-Values of Physical, mental and other symptoms

Physical, mental and other symptoms	N	Mean	SD	t	df	t-value	p-value Sig. (2-tailed)
Headaches	78	2.3718	1.30044	-1.093	104.837	-.17412	.277
High Blood Pressure	78	2.1667	1.27327	1.025	109.568	.16182	.307
Depression	78	2.2179	1.05249	.369	121.023	.04949	.712
Change in appetite	78	2.5513	1.32555	-1.917	106.830	-.31291	.058
Smoking	78	1.1154	.35968	9.707	382.286	.71601	.000
Alcohol consumption	78	1.1154	.35968	10.536	373.972	.75962	.000
Feeling Physical weakness	78	2.5000	1.11367	-2.388	115.504	-.33430	.019
Nightmares	78	1.9359	1.08520	.629	123.475	.08736	.530
Suicidal thoughts		1.5128	.92222	3.099	156.979	.39125	.002

For two district wise and physical, mental and other symptoms variables, there is a significant difference in the mean opinion of respondents of two districts on smoking, alcohol consumption and feeling physical weakness variables. For the other variables the mean opinion is the same.

Table-9: Descriptive Statistics and P-Values of Coping Strategies

Coping Strategies	N	Mean	SD	t	df	t-value	p-value Sig. (2-tailed)
Exercise regularly	78	2.6026	1.27248	2.182	117.992	.35092	.031
I seek out emotional support from others	78	2.7051	.98177	1.484	125.538	.18731	.140
I try to spend more time unwinding with friends and/or loved ones.	78	2.8205	1.14805	1.256	118.165	.18239	.211
I remind myself to focus on the good things in my life instead of the bad.	78	3.4615	1.39286	.189	110.546	.03265	.851
I try to look at the issue from different perspectives	78	3.2821	1.19411	-.894	114.259	-.13380	.373
I readjust my existing goals to fit with the new situation	78	3.2179	1.28576	-.747	109.788	-.11911	.457
I take additional action to try to get rid of the problem	78	3.1667	1.22121	-.425	113.462	-.06492	.672
Organization providing employee assistance programmes	78	3.0385	1.32354	.547	108.482	.08945	.586

For two district wise and coping strategies domain variables, there is no significant difference in the mean opinion of respondents of two districts.

Findings

- In Gajapathi and Nuapada districts, women are 16.2 % and 24.4 %respectively
- In Gajapathi and Nuapada districts, 47.2 % and 43.7 % respondents are Graduates respectively
- In Gajapathi and Nuapada districts, 32.7% and 34.5 % of the respondent disagree that they are working against the will in the organization respectively
- In Gajapathi and Nuapada districts, 31.0% and 31.1 % of the respondents agree that they worry about their health respectively
- In Gajapathi and Nuapada districts, 30.4% and 28.6 % of the respondents disagree that they have to revise their personal habits respectively.
- In Gajapathi and Nuapada districts, 27.1% and 30.3 % of the respondents to a small extent agree that they are experience excessive work pressure respectively.
- In Gajapathi and Nuapada districts, 30.0% (neither great nor small extent agree) and 26.1% (to a small extent) of the respondents agree that they are working for long hours, overtime and even on holidays respectively.
- In Gajapathi and Nuapada districts, 26.7% (to a small extent) and 30.3% (Completely) of the respondents agree that there is freedom to use own judgement respectively.
- In Gajapathi and Nuapada districts, 25.1% and 26.1 % of the respondents Neither great nor small extent agree that they have difficulty with frequent jobs posting & transfer proceduresrespectively
- In Gajapathi and Nuapada districts, 37.0% and 31.9 % of the respondents sometimes agree that they have problems subject to complaints investigation respectively
- In Gajapathi and Nuapada districts, 29.4% (sometimes) and 37.0% (always) of the respondents agree that they get supportive feedback on the work done respectively
- In Gajapathi and Nuapada districts, 27.7% (sometimes) and 28.6% (always) of the respondents agree that the organization always supports the employees respectively.
- In Gajapathi and Nuapada districts, 33.7% and 36.1 % of the respondents very low agree that they are subjected to personal harassment in the form of unkind words or behavior respectively
- In Gajapathi and Nuapada districts, 34.0% and 32.8% of the respondents moderately agree that they are not able to satisfy the demands of colleagues and public, since these are conflicting with one another
- In Gajapathi and Nuapada districts, 34.3% (sometimes) and 34.5% (seldom) of the respondents agree that they face verbal & physical aggression from the public respectively.
- In Gajapathi and Nuapada districts, 29.4% (sometimes) and 33.6% (seldom) of the respondents agree that they have difficulties arising in communicating with multi-linguistic public respectively
- In Gajapathi and Nuapada districts, 31.7% (moderately) and 34.5% (a little bit) of the respondents agree that they are having family conflicts because of work issues respectively
- In Gajapathi and Nuapada districts, 32.0% (moderately) and 31.9% (a little bit) of the respondents agree that they are unable to take sufficient breaks for family chores respectively
- In Gajapathi and Nuapada districts, 27.1% (A few times feel this way) and 31.1% (Never feel this way) of the respondents agree that they have treats of physical violence respectively
- In Gajapathi and Nuapada districts, 29.0% (Sometimes feel this way) and 32.8% (Never feel this way) of the respondents agree that they are having poor concentration respectively
- In Gajapathi and Nuapada districts, 28.4% (Sometimes feel this way) and 37.8% (Never feel this way) of the respondents agree that they want to be alone always from othersrespectively
- In Gajapathi and Nuapada districts, 29.7% (Sometimes feel this way) and 28.6% (Never feel this way) of the respondents agree that they feel out-of- control respectively
- In Gajapathi and Nuapada districts, 32.0% and 31.1% of the respondents not at all agree that they have high blood pressure respectively
- In Gajapathi and Nuapada districts, 34.3% and 37.8% of the respondents a little bit agree that they have depression respectively

- In Gajapathi and Nuapada districts, 31.4% and 39.5% of the respondents not at all agree that they have change in appetite respectively
- In Gajapathi and Nuapada districts, 57.8% and 74.8% of the respondents not at all agree that they have habit of smoking respectively
- In Gajapathi and Nuapada districts, 34.3% and 28.6% of the respondents moderately agree that they seek out emotional support from others respectively
- In Gajapathi and Nuapada districts, 27.1% (moderately) and 16.8% (Just a little) of the respondents agree that they try to spend more time unwinding with friends and/or loved ones respectively
- In Gajapathi and Nuapada districts, 34.3% and 28.6% of the respondents moderately agree that they remind to focus on the good things in life instead of the bad respectively
- In Gajapathi and Nuapada districts, 31.7% and 31.9% of the respondents moderately agree that they try to look at the issue from different perspectives respectively

Suggestions

1. It is noted that both the Gajapathi and Nuapada districts police personnel are having insufficient personal time. The administration should consider that the duties allotted to them should be according to the workinghours.
2. The Nuapada district police personnel are carrying a lot of responsibilities and duties, work for long hours, overtime and even on holidays and feel in secure in the environment. As the staff is minimum to share the work, the administration should appoint the staff. It should also see that the area is free from terrorist and provide secured workplace.
3. Both the districts police personal are having slow career progress and problems complaint to investigation. Regarding these, the personal should plan their career and the higher authority should provide educational programme opportunities especially to the staff simultaneously with the job. Proper pre-departure training should be given in the process of investigation so that the staff can withstand with theproblems.
4. In Gajapathi district, it is noted that there is some personal harassment in the form of unkind words or behavior, bullying at work and struggle to get along with superiors, subordinates and peers. the higher authorities should treat the subordinates as the human capital for the organization so that to get positive feedback and development of theorganization
5. In Gajapathi the police personnel are in view that job demands affect personal relationships (friends, relatives etc). As discussed earlier, it is difficult to satisfy all the aspects of life but try to balance the quality of work-life by propertimetabling
6. In Gajapathi district the police personnel are facing insufficient time to spend with family, family conflicts because of work issues and unable to take sufficient breaks for family chores. Due to heavy duties, work overload, night shift system, long working hours etc it is difficult to spend with the family. It is suggested to make time for the family so that to reduce work stress. Make it off from work for familyissues.
7. In Gajapathi district the police personal are having sleeping troubles, threats of physical violence and feel out-of-control feelings. In police occupation, these are common symptoms seen. It is suggested to have emotional intelligence and make a strong mind to overcome these problems.
8. Provide assistance –An important aspect to improve the mental health of the police officials is organizing different support groups. Social support is importance on an individual's life when a person undergoes a stressful life event. It is proposed that engagement in positive support groups outside the workplace can help to ameliorate stress for policepersonnel.

CONCLUSION

Policing is considered as most stressful profession than other professions and they have the greater risk of stress. Especiallyfemale police personnel face more stress related problems than their male counterpart as they manage the

family commitments and also working in law enforcement. Studies have shown that working hours, education, work pressure, Superiors and sub ordinate relationship were the factors which led them to feel stressed. Hence the study was undertaken to find thestress among women police constables working in Gajapathi and Nuapada district in Odisha state. The results show that women police working in the areas where more and more obstacles may come to them because of the border districts. It was also found that age and designation of women police has not made any significant influence on stress. Stress among women constables was significantly varies in accordance with their educational qualification, marital status, years of experience. The study concludes with suggesting the department to regularly organise training programs and personal counselling for stress management of women police constables.

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Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.10 / Issue 60 / June / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

ANT Colony Optimization based Artificial Neural Network for Classification of Breast Cancer Dataset

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Received: 02 Mar 2020 Revised:04 Apr 2020 Accepted: 06 May 2020

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ABSTRACT

Breast cancer is one of the most commonly found and dangerous cancer among women which leads to a major research topic in medical science. In Medical science, the researchers or medical practitioners use vast data to get acquired facts, to predict the disease at an early stage, which can be used to enhance the curing ability. This study is conducted using data from the University of Wisconsin Hospitals, Madison from Dr. William H. Wolberg.In this research, two machine learning approaches i.e. ANN and ACO are used for comparative analysis. The goal is to find the best number of hidden nodes for ANN using Ant Colony Optimization. The performance is analyzed by using Sensitivity and Specificityto find out the best-suited model that can be used for classification. Ant Colony Optimization (ACO) is a bio-inspired technique formalized into a meta-heuristic for combinatorial optimization problems based upon which it will overcome the issue that has been represented in the work with a Root Mean Square Error (RMSE) of 0.1201 which is almost five times less as compared to conventional ANN.

Key words: Cancer, Artificial Neural Network (ANN), Ant Colony Optimization (ACO), Sensitivity, Specificity

INTRODUCTION

Cancer is a disease caused by abnormal cell growth. These cells exist because of the changes in gene expression, and then they will be developed into a population of cells that can attack specific tissues. This is very dangerous because it can cause death. Based on the Global Cancer (GLOBOCAN) statistics part of the International Agency of Research on Cancer (IARC) in 2018, there are 18.1 million cases of cancer in the world and 9.6 million of them have died [1]. In

the 18.1 million cases of cancer, while the most common cancer cases experienced by women are breast cancer cases. Until now, there has not been found a way to treat cancer efficiently and thoroughly.

In the vast research area of medical science, we have taken the classification of breast cancer using data mining techniques. Out of the two types of Breast Cancer, i.e. malignant and benign, the malignant tumor develops when cells in the breast tissue divided and grow without the normal controls on the cell death and cell division [2, 3]. With early diagnosis, 97% of the women survive for 5 years or more. In the healthcare industry, it is vital to understand the gradual developments of such a tumor. However, among all the classifiers, ANN has been chosen by the researchers most frequently and extensively. Our aim in this thesis is to evaluate the efficiency and effectiveness of the best combination of ANN and ACO (Ant Colony Optimization) on the Wisconsin Breast cancer (original) datasets. And the results obtained are measured using various performance metrics to compare among algorithms to find out the best-suited model for Breast Cancer Data Classification.

Literature Survey

A variety of classifications techniques were developed for breast cancer. The accuracy of many of them was evaluated using the dataset taken from the UCI machine learning repository. Previously, research regarding the classification and prediction of breast cancer has been carried out using several data mining techniques. Classification and agglomeration are 2 wide used ways in information mining.Neural networks and related techniques have a vast contribution when it comes to predicting breast cancer [1].Over the past few decades, Artificial Neural Networks have been employed increasingly by more and more researchers, and become an active research area. ANNs have afforded numerous successes with great progress in Breast Cancer classification and diagnosis in the very early stage.The technique, however, has somelimitations such as no guarantee to global optima, a lot of tuning parameters, and long training time. Single Hidden Layer Neural Networks (SHLN) was proposed by Huang and Babritoto tackle the mentioned problems with tree steps learning process called extreme learning machine (ELM) Standard and best-parameterized ELM model were proposed for BreastCancer early prediction [2, 3]. Results showed that it generally gave better accuracy, specificity, and sensitivity compared to BP-ANN. However, most existing works focus on prediction performance with limited attention with medical professionals as end-users and applicability aspects in real medical settings.

As of now various statistical and soft computing based classifiers have been proposed in the literature. The traditional statistical techniques like Euclidean Minimum Distance (EMD), Quadratic Minimum Distance (QMD), and K Nearest Neighbor (KNN) classifiers and Bayesians decision theory are used to build different classifiers. One of the drawbacks of the statistical method is that it depends on the correctness of the underlying assumption for its successful application [4]. Unlike the Soft Computing method andthe statistical method, the user needs to have a thorough grasp of the properties of the datasets for successful applications of the model.However, it is not always possible. As a result, statistical techniques based classifier generally gives less accuracy as compared to the soft computing based classifier. Goodman, Boggess, and Watkins, 2002 tried different methods that produced the following accuracies: Optimized Learning Vector Quantization (Optimized-LVQ) methods performance was 96.7%, big LVQ method reached 96.8%.Quinlan reached 94.74% classification accuracy using 10-fold cross-validation with the C4.5 decision tree method [5]. Abonyl and Szeifert used the Supervised Fuzzy clustering technique and obtained 95.57% accuracy.

To develop optimum or near optimum solutions, Meta-Heuristic algorithms are efficient supporters. Certain Bio-inspired algorithms, such as Bat Algorithm (BA), Swarm Optimization (PSO), Cuckoo Search (CS), Ant Colony Optimization (ACO) and Simulated Annealing (SA) [6]. R.F. TaveresNeto surveyed ACO and concluded that ACO is the most reliable approach for solving scheduling problems and determined some guidelines for implementing ACO [7, 8]. Although not all of the swarm intelligence algorithms are successful, a few techniques have proved to be very efficient and thus become prominent tools for solving real-world problems (Bank et al., 2007) some of the most

efficient and the most widely studied examples are Ant Colony Optimization (ACO) (Corne et al., 2012), Particle Swarm Optimization (PSO) (Fister et al., 2015; Corneet al., 2012;), Artificial Bee Colony (ABC) (Karaboga et al., 2012;), and recently proposed Firefly Algorithm (FA) and Cuckoo Search (CS) (Tavares Neto et al., 2013) [9, 10].

MATERIALS AND METHODS

Data

This Breast Cancer database was obtained from the University of Wisconsin Hospitals, Madison from Dr. William H. Wolberg. This data set (Table1) includes 699 number of instances and class distribution of Benign: 458(65.5%) and Malignant: 241(34.5%) of which 16 instances have missing attributes values removing that we have 683 instances of which 444 benign and 239 are malignant [4]. Description of the attributes of the WBC (Original) dataset:

Artificial Neural Network

An artificial neural network is a Supervised Learning Algorithm which means that we provide it the input data containing the independent variables and the output data that contains the dependent variable. For instance, in our example, our independent variables are X1, X2, and X3. The dependent variable is Y.In the beginning, the ANN makes some random predictions, these predictions are compared with the correct output and the error(the difference between the predicted values and the actual values) is calculated. The function that finds the difference between the actual value and the propagated values is called the cost function [4, 5]. The cost here refers to the error. Our objective is to minimize the cost function. Training a neural network refers to minimizing the cost function.A Neural Network executes in two phases: Feed Forward phase and Back Propagation phase.

Back Propagation

In the beginning, before we do any training, the Neural Network makes random predictions which are of course incorrect.We start by letting the network make random output predictions. We then compare the predicted output of the neural network with the actual output [6]. Next,we update the weights and the bias in such a manner that our predicted output comes closer to the actual output (Fig.1). Following steps involved:

Step 1: Calculate the cost: The first step in this phase is to find the cost of the predictions. The cost of the prediction can be calculated by finding the difference between the predicted output values and the actual output values. If the difference is large then cost will also be large.We will use the Mean Squared Error or MSE cost function. A cost function is a function that finds the cost of the given output predictions.

$$MSE = \frac{1}{n}\sum_{i=1}^{n} (\hat{Y}_i - Y_i) \quad (1)$$

Here, $Y_i$is the actual output value and $\hat{Y}_i$is the predicted output value and n is the number of Observations.

Step 2: Minimize the cost: Our ultimate goal is to fine-tune the weights of our neural network in such a way that the cost has minimized the minimum. And also we can only control the weights and the bias [7]. Everything else is beyond our control. We cannot control the inputs, we cannot control the dot products, and we cannot manipulate the Sigmoid Function.

The sigmoid function consists of 2 functions, logistic and tangential. The values of the logistic function range from 0 and 1 and -1 to +1 for tangential function. The sigmoid function returns 0.5 when the input is 0. It returns a value close to 1 if the input is a large positive number. In the case of negative input, the sigmoid function outputs a value close to zero [8, 9].It is a widely used activation function. This is a smooth function and is continuously differentiable. This

essentially means that when we have multiple neurons having the sigmoid function as their activation function the output is non-linear as well [10].Mathematically,

$$f(x) = \frac{1}{1+e^{-x}} \quad (2)$$

Given Figure 2 is the graphical representation of Sigmoid Function:

We have to find the dot product of the input features (matrix of independent variables) with the weights. Next, pass the summation of dot products through an activation function. The result of the activation function is the predicted output for the input features.To minimize the cost, we need to find the weight and bias values for which the cost function returns the smallest value possible. The smaller the cost, the more correct our predictions are.To find the minima of a function, we can use the gradient descent algorithm [11]. The gradient descent can be mathematically represented as:

$$*W_x = W_x - a\left(\frac{\partial Error}{\partial Wx}\right) \quad (3)$$

$\partial Error$ is the cost function. The above equation tells us to find the partial derivative of the cost function for each weight and bias and subtract the result from the existing weights to get new weights.The derivative of a function gives us its slope at any given point [12]. To find if the cost increases or decreases, given the weight value, we can find the derivative of the function at that particular weight value. If the cost increases with the increase in weight, the derivative will return a positive value which will then be subtracted from the existing value.On the other hand, if the cost is decreasing with an increase in weight, a negative value will be returned, which will be added to the existing weight value since negative into negative is positive.

In the above equation, a is called the Learning Rate, which is multiplied by the derivative. The learning rate decides how fast our algorithm learns.At last, we have the Total Error, which needs to be minimized.To get an improvised version of this here we take Gradient Descent which is combined with every algorithm and is easy to understand and implement [13].

Ant Colony Optimization

From Biology to Algorithms

Ant colony optimization was inspired by the observation of the behavior of real ants. In this section, we present several observations made in experiments with real ants, and then we show how these observations inspired the design ofthe ACO meta-heuristic [14].

Ants

One of the first researchers to investigate the social behavior of insects wasthe Frenchentomologist Pierre-Paul Grasse. In the 1940s and 1950s, he wasobserving the behavior of termites, in particular, the Bellicositermesnatalensisand Cubitermesspecies[15]. He discovered that these insects are capable toreact to what he called“significant stimuli”, signals that activate a geneticallyencoded reaction. He observed that the effects of these reactions can actas new significant stimuli for both the insect that produced them and for theother insects in the colony. Grasse used the term stigmergyto describe thisparticular type of indirect communication in which “the workers are stimulatedby the performance they have achieved” [16, 17].Artificial ants live in a discrete world they move sequentially through a finite set of problem states.The pheromone update (i.e., pheromone depositing and evaporation) is notaccomplished in the same way by artificial ants as by real ones.Sometimes the pheromone update

is done only by some of the artificial ants, and often onlyaftera solution has been constructed.Some implementations of artificial ants use additional mechanisms that do not exist in the case of real ants [18]. Examples include look-ahead, local search, backtracking, etc.

Probability of an ant to chose any path:

$$P_{ij}^{k}(t) = \frac{\{[\tau_{ij}(t)]^{\alpha}.[n_{ij}(t)]^{\beta}\}}{\{\sum_{l \epsilon N_i^k}[\tau_{il}(t)]^{\alpha}.[n_{il}(t)]^{\beta}\}} \quad (4)$$

$k = k^{th}$ *ant*
P_{ij}^{k} *= probability of selecting ij path by* k^{th}*ant*
τij *= pheromone concentration*
n_{ij} *= constants (A heuristic indicator)*
α *=exponent factor control*
β *= the behavior of* τ *and n*
Pheromone update for each path:

$$\tau ij\,(t+1) = (1-).\ \tau ij\,(t) + \sum_{k=1}^{m} \Delta \tau i^{k}(t)$$

ρ *= evaporation rate (0~1)*
$\Delta \tau i^{k}$ *=change in pheromone by* k^{th}*ant in ij path*

$$\Delta \tau i^{k}(t) = \begin{cases} 1/L(t), & \text{if (i,j) path is used by } k^{th} \text{ ant} \\ 0, & \text{otherwise} \end{cases}$$

Proposed algorithm

Parameters

```
%% ACO Parameters
MaxIt =20;      % Maximum Number of Iterations
nAnt =10;       % Number of Ants (Population Size)
Q=1;
tau0=10*Q/(nVar*mean(model.D(:)));  % Initial Pheromone
alpha =1;       % Pheromone Exponential Weight
beta =1;        % Heuristic Exponential Weight
rho =0.05;      % Evaporation Rate
```

Simulation Result

The next step after applying implementing machine learning models is to seek out how effective is that the model, i.e. how the models performed on the datasets. This is carried out by running the models on the test dataset which was set earlier. The test dataset comprised 35% of the dataset for Breast Cancer classification. To determine and compare the performances of the different algorithms, several metrics have been used.

Confusion Matrix

Summarization the performance of a classification algorithm is based on a technique which is known as a confusion matrix. It is arguably the easiest way to regulate the performance of a classification model by comparing how many positive instances are correctly/incorrectly classified and how many negative instances are correctly/incorrectly classified [19]. In a confusion matrix, as shown here, the rows represent the actual labels while the columns represent the predicted labels(Table 2).

True Positives (TP): These are the occurrences where both the predictive and actual class is true (1), i.e. when the patient has complications (breast cancer in this case) and is also classified by the model to have complications.True Negatives (TN): True negatives are the occurrences where both the predicted class and actual class is False (0), i.e. when a patient does not have complications and is also classified by the model as not having complications.

False positive (FP): Normal/ Malignant behavior that is wrongly classified as malignant by the system. This value represents incorrect classification decisions where the normal classified as malignant, the increasing of FP value increases the computation time but; on the other hand, it is considered as less than harmful of FN value increasing.
False Negative (FN): These are occurrences where the predicted class is False (0) but the actual class isTrue (1).

ANN Result
After calculating the ANN Result, we get this which is mentioned below Table 3.

ACO Results
After calculating ACO result, we get this which is mentioned below Table 4.

Performance Metrics

Several performance metrics have been used to figure out the performance of the proposed algorithms. As the paper sincerely deals with classification problems, performance metrics relating to classifications are discussed here. For Breast CancerPrediction, if the target variable is 1(malignant), then it is a positive instance, meaning the patient has Breast cancer. And if the target variable is 0 (benign), then it is a negative instance, stating that the patient does not have cancer [20] (Table 5&6).

Specificity

The Classifier's performance to spot negative results is related by Specificity. It is considered as the TNR (True Negative Rate). It is a measure of the number of patients who are classified as not having complications among those who did not have complications. Specificity identifies accurately classified negative samples to total samples. If Specificity becomes high like sensitivity and their difference is very less (<1%), then the classifier is a better one.
Specificity = (TN/TN+FP)

Sensitivity

Sensitivity is also regarded as TPR (True Positive Rate). Sensitivity evaluates how well a model can recognize abnormal records. Higher the sensitivity is better the classifier becomes. It always identifies the proportion of accurately classified samples to total samples. The high value of TP means a high value of sensitivity leading to high accuracy.
Sensitivity = (TP/TP+FN)
We calculate the performance matrix which is described below:
Similarly, we calculate the performance matrixwhich is described below:

Convergence curve

From the convergence curve, it is clear that the RMSE has been highly reduced from a value of 0.5 to 0.1201 with just a 20 number of iteration(Fig.4).

Comparison of Result

The statistical measure, comparison plot represents in the figures 5&6. where the results in all cases are improved and specifically in case of sensitivity results is very much convincing.The confusion matrix shows the improvement in the negative class of the considered data which is improved from a value of 10 to 187 .

CONCLUSION

ANN has been chosen by the researchers most frequently and extensively. Our aim in this thesis is to evaluate the efficiency and effectiveness of the improvised version of ANN by applying ACO (Ant Colony Optimization) on the Wisconsin Breast cancer (original) datasets. However, among all the classifiers, the conventional ANN algorithm highly encouraged to be used in classification but in the case of a dataset having fewer attributes ANN fails, and the error somewhat come around 0.5. So the implementation of ACO to decide the correct number of hidden nodes that will overcome the issue has been represented in the work with a root mean square error of 0.1201 which is almost five times less as compared to conventional ANN. And similarly, the comparison plots in the results section represent the enhanced result of the proposed algorithm is compared to the conventional algorithm. So in the future, we will try to analyze the code to add more decision variables and also therecent optimization techniques, that will return more accurate results in comparatively less time.

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Table 1.Wisconsin Breast Cancer (Original) dataset

No.	ATTRIBUTES	DOMAIN
1	Sample code number	1-10
2	Clump thickness	1-10
3	Uniformity of cell size	1-10
4	Uniformity of cell shape	1-10
5	Marginal adhesion	1-10
6	Single epithelial cell size	1-10
7	Bare nuclei	1-10
8	Bland chromatin	1-10
9	Normal nucleoli	1-10
10	Mitoses	1-10
11	Class	2 for benign,4 for malignant

Table 2.Confusion Matrix

	Predicted Negative	Predicted Positive
Actual Negative	TN	FP
Actual Positive	FN	TP

Table 3. Confusion Matrix of ANN

True positive (TP)	430
False positive (FP)	25
True negative (TN)	10
False negative (FN)	231

Table 4. Confusion Matrix of ACO

True positive (TP	428
False positive (FP)	54
True negative (TN)	187
False negative (FN)	30

Table 5. Performance Matrix of ANN

RMSE-ep	0.6065
MSE-ep	0.3678
SENSITIVITY	0.65052
SPECIFICITY	0.6159

Table 6.Performance Matrix of ACO

RMSE-ep	0.1201
MSE-ep	0.0600
SENSITIVITY	0.9345
SPECIFICITY	0.7759

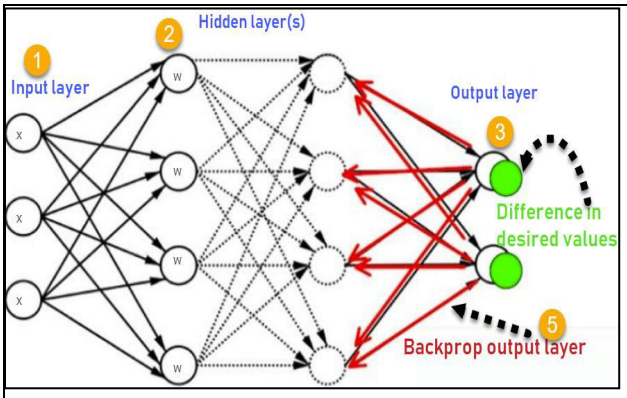

Figure 1. Back Propagation

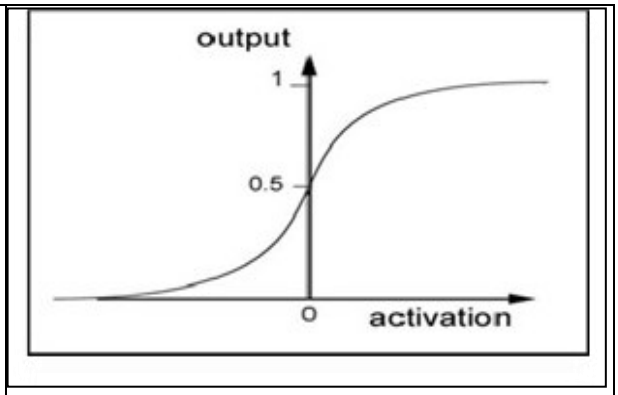

Figure 2. Sigmoid Function

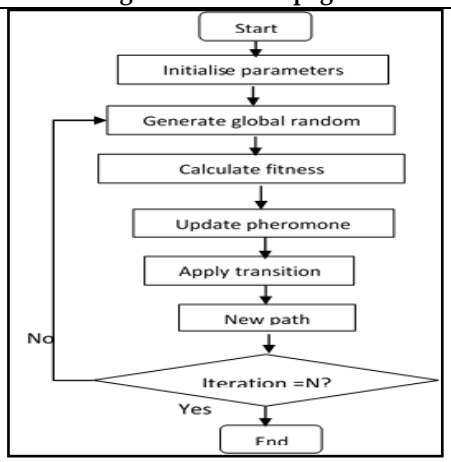

Figure 3: Flow Chart of Ant Colony System

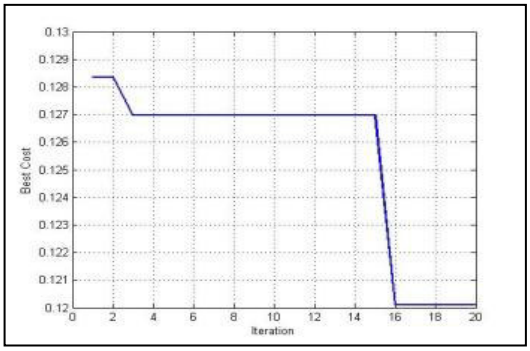

Figure 4: Convergence Curve for Optimized ANN

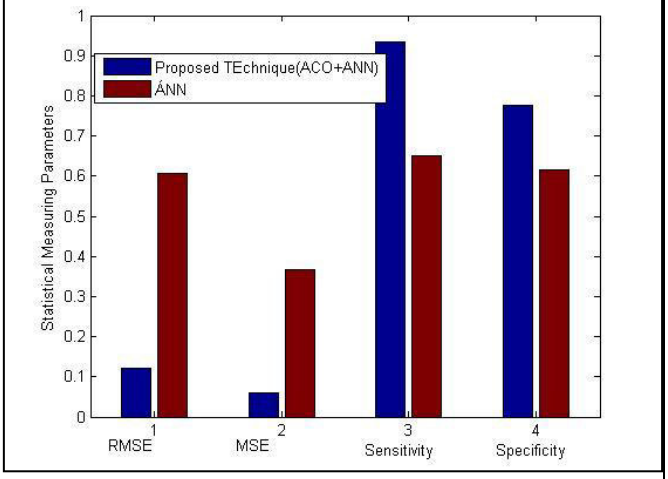

Figure 5.Statistical Measure Comparison (1)

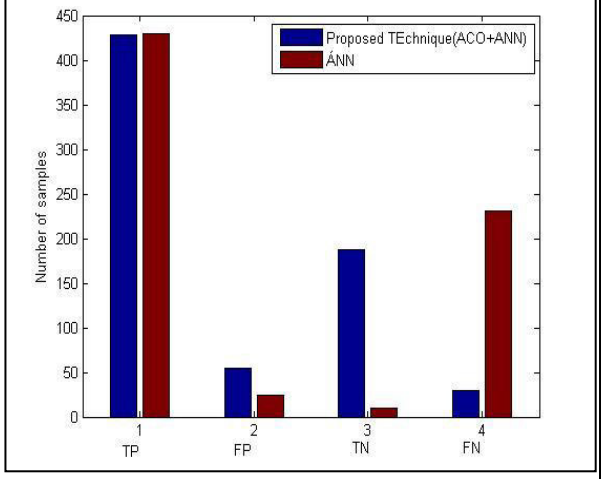

Figure 6.Statistical Measure Comparison (2)