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RESEARCH ARTICLE

Physical and Nutritional Evaluation of Freeze Dried Jamun Pulp Powder Stored at Different Package and Storage Conditions

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ABSTRACT

Jamun *Syzygium cumini* is a seasonable fruit which is widely distributed in forest tree in India and other tropical region. It is consumed as fresh for its nutritive value. The fruit is rich in anthocyanin, tannins, Gallic acid and many Phytonutrients. High fluctuation in market price on season times is mainly due to wastage. Drying is done to enhance the storage stability, reduce transport weight and retains its nutritive values. Freeze drying was conducted at -40°C the dried powder was packed at two different packaging material Metalized Polyester Pouches (MPE) and Low Density polyethylene Pouches (LDPE). Under three various storage conditions were S1 (Ambient temperature), S2 (ambient temperature in Dark), S3 (low temperature 4°C). On comparing the results of storage study it was found that Moisture content 3.15 %, Bulk density 0.60, solubility 69.85 and flow ability 31.47% was found at the end of six month in S3 condition packed in MPE. The nutritional properties like ascorbic acid 12.29 mg/g, total phenols 14.38 mg/g; total Anthocyanin 102.10 mg/g and Flavonoids 6.68 mg/g have significant effect with respect to packaging material and storage conditions. From the study it was concluded that MPE is suitable packaging material in Low temperature for Jamun pulp powder stored for six month.

Keywords : Phytonutrient, Drying, Metalized polyethylene, Anthocyanin.

INTRODUCTION

Syzygium cumini is, commonly known as black plum or "Jamun" is an important medicinal plant in various traditional systems of medicine. Other common names are Jambul, Black Plum, Java Plum, Indian Blackberry, Jamun etc. Today these trees are found growing throughout the Asian subcontinent, Eastern Africa and South America [1]. *Syzygium cumini* is an ever green tropical tree, which can grow above 30 m height. The fruit is astringent and taste varies from light acidic to sweet. Tree fruits once in a year and the berries are sweetish sour to taste. The ripe fruits are used for health drinks, making preserves, squashes, jellies and wine. Different parts of the jambolan were also reported for its antioxidant, anti-inflammatory, neuropsycho-pharmacological, anti-microbial, anti-bacterial, anti-HIV, antileishmanial and antifugal, nitric oxide scavenging, free radical scavenging, anti-diarrheal, antifertility, anorexigenic, gastroprotective and anti-ulcerogenic and redioprotective activities. A little quantity of fruit syrup is very useful for curing diarrhoea [2]. A mixture of jamun juice and mango juice in equal quantity is very useful for quenching thirst for diabetic patient. Jamun is used for preparation of wine particularly in Goa. The vinegar prepared from juice extracted from slightly ripe fruits is stomachic, carminative and diuretic, apart from having cooling and digestive properties. Almost every part of the tree is used for one purpose or other. A tree made of tender leaves and alcoholic extracts of leaves and fruit is used in South America for curing the stomach disorder.

Almost all parts of the tree are used for various purposes. Ripe fruits are very juicy, almost odorless, with a pleasant, slightly bitter, astringent taste. The fruit pulp is used to make jams, jellies, juice, vinegar and puddings. Fruits are also used to make wine in vast quantities in the Philippines. These fruits are not yet totally exploited and organised cultivation of these fruits and awareness about the importance are immediate needs [3]. These crops are easier to grow and hardy in nature, producing even under adverse conditions. Moreover, these fruits are playing a vital role in nutrition and livelihood use of rural and tribal masses for employment and income generation. Apart from nutritive value, these fruits are particularly more important for medicinal properties and famous for the retentive value in Ayurvedic medicine. Most people are familiar with the medicinal properties of locally available fruits.The research findings of the drying of some exotic fruits and comments on the type of dryers and drying methods for the drying of exotic fruits. In dried cranberries 20 volatile compounds were detected [4].

Freeze-drying is a process recommended for drying of heat-sensitive products. Some advantages of this technique are that it allows the shrinkage and the degrading reactions in the material, which are common in conventional drying operations, to be minimized. The knowledge of drying kinetics is essential for modeling and optimization of a freeze-drying process. Freeze-dried products are believed to have the same characteristics as those of fresh ones. As such, preservation and retention of the attributes such as shape, appearance, taste, nutrients, porosity, color, flavor, texture and biological activity of the fresh samples makes this technique one of the most fascinating and applicable process for drying food materials. Nevertheless, longer drying time is required due to the freeze-dryer's lower vapor pressure driving force as compared with that of conventional drying methods Marques *et al.* [5].

MATERIALS AND METHODS

Sample preparation

Jambola mature fruits were directly obtained from producers in the region of Pollachi. The fruits were sorted by its maturity and the fully ripped fruits were washed in normal tap water. The free water in the fruit was removed using hair dryer and wiped out with tissue papers. Pre weighed 100 g of the Jamun fruit was packed in each PP zip lock bag and kept in deep freezer at -30°C for further use. The stored Jamun fruits were taken from the deep freezer and kept in room temperature to reach its normal state. Jamun pulp was extracted manually by separating the pulp from the seed. The extracted pulp was dried in Freeze dryer at -40°C. After drying these samples were store in Metalized

polyethylene package and in LDPE. These packages were stored in three different storage conditions. The dried pulp powder is shown in Fig.1.

Storage conditions

Freeze dried samples were stored immediately after processing in sterile LDPE and MPE packages The storing conditions were S1 (Ambient temperature), S2 (Ambient temperature in Dark), S3 (Low temperature 4°C). Samples were tested for 6 months, on 30 days of interval the samples were tested for analysis.

Physical Characteristics

Moisture content

Moisture content was determined using an automatic electronic moisture analyser (A & D make, model MX-50) which registers loss of mass as moisture percent. The sample is heated by a halogen lamp. The drying temperature for was set at 80 °C and moisture percent accuracy at 0.01 percent. About 2.0 g of jamun pulp powder was taken to determine moisture content.

Bulk density

Bulk density was determined by adding 2.0 g of powder to a 5.0 ml graduated cylinder and holding the cylinder tape it for continues 50 times. The bulk density was calculated by dividing mass of the powders by the volume occupied in the cylinder. This method is done given by Singhanat Phoungchandang and Anong Sertwasana [6].

Solubility

Solubility test was done by Germano *et al.* [7]. A small sample of dry powders of 1.0 g was added to 10 ml of water at 30 °C in a 50 ml beaker. The mixture was stirred using a magnetic stirrer at 7 rpm. Solubility was measured as the time taken for the powders to completely soluble in water. ISM was determined by dissolving 2.0 g powder in 20 ml water and the solution was centrifuged at 3000 rpm for 5 min. An aliquot of 10 ml was used for moisture content determination using automatic electronic moisture analyser set at 105 ºC.

Flowability

Flowability was analyzed by the static angle of repose method Teunou and Fitzpatrick [8]. The height of the "Funnel" through which the powder passes was fixed relative to the base. The base is fit with a graph to find out the diameter of the cone. The cone pile has to touch the tip of the funnel end. The height remains constant and the diameter varies according to the property of the powder.

Nutritional Characteristics

Ascorbic Acid

A Sample of 2 g was extracted with metaphosphoric acid (3%) and the total AA was determined by titrating a known weight of sample against 2,6-dichlorophenol-indophenol dye. Result was expressed in mg/100 g of fresh fruit [9].

Total phenol determination

Total phenolic content of the extracts was determined by Folin Ciocalteu reagent method with some modifications. Powder extract (1 ml) was mixed with Folin Ciocalteu reagent (0.1 ml, 1 N), and allowed to stand for 15 min. Then 5 ml of saturated Na_2CO_3 was added. The mixtures were allowed to stand for 30 min at room temperature and the total phenols were determined spectrophotometrically at 760 nm. The calibration curve was prepared by preparing Gallic acid (10–100 μg/ml) solution in distilled water. Total phenol values are expressed in terms of Gallic acid equivalent (mg/g of extracted compound) [10]

Flavonoids Determination

Aluminium chloride colorimetric method Chang *et al.*[11] with some modifications was used to determine flavonoids content. Plant extract (1 ml) in methanol was mixed with 1 ml of methanol, 0.5 ml Aluminium chloride (1.2%) and 0.5 ml potassium acetate (120 mM). The mixture was allowed to stand for 30 min at room temperature; the absorbance of the reaction mixture was measured at 415 nm. The calibration curve was prepared by preparing quercetin (5-60 μg/ml) solution in methanol. Flavonoids content is expressed in terms of quercetin equivalent (mg/g of extracted compound).

Total Anthocyanin

Total anthocyanin content was determined by the spectrophotometric method Cinquanta *et al.* [12]. About 10 mg of powder were extracted two times with 10 ml of a HCl/ water/ethanol solution (1/29/70). The extract was centrifuged for 10 min at 10,000g and recorded in a Beckman DU-640 spectrophotometer (Beckman Coulter, Fullerton, USA). Total anthocyanin content was expressed as cyanidin-3-rutinoside. The molar absortivity (Emolar) of cyanidin-3-rutinoside used was 32,800 at k max absorbance (About 534 nm), in HCl/ water/ethanol solution (1/29/70) at 20 °C. Analyses were performed in duplicate and the results were expressed as mg/100 g of dried matter.

Statistical Analysis

All data obtained were expressed as mean ± standard deviation. Data was analysed by using one-way Analysis of Variance (ANOVA). The statistical analysis was performed using GraphPad Prism 5.0 for Windows. Trends were considered significant when the means of the compared sets differed at $P < 0.05$.

RESULTS AND DISCUSSION

Influence of Storage conditions and Packaging materials on Physical parameters

The results shows the moisture content, flow ability, bulk density and solubility of Jamun Pulp dried in Freeze drier at -40°C and stored at two different packaging materials with Metalized Polyester Film Pouches (MPF) and Low Density Polyethylene Pouches (LDPE) on various storage conditions such as Room Temperature (ST1), Room Temperature Dark (ST2) and Low Temperature or Refrigerated condition (ST3). The result for storage study is shown in Table1. The moisture content of the sample is significantly different with respect to storage conditions and packaging materiasl. The highest moisture content was found at 180th day (6th Month) 3.26 % which was stored in LDPE package in Normal Room temperature condition (ST1).

The Lowest increase in moisture content was evaluated after 180th day 3.08 % when stored in MPF in Low temperature condition. Whereas in case of flow ability of Jamun pulp powder stored after room temperature has significantly different with respect to storage conditions. Due to moisture absorption and because of crystalline formation in pulp powder inside the packaging material the flow of the powder varies. Physical properties of a material are also highly dependent on the material's moisture content, and each material will behave differently [13] the flowability of confectionary sugar and detergent at two moisture contents. As the moisture content increased, the internal angle of friction of both the sugar and detergent decreased. As expected, the more cohesive powders have a higher angle of repose. The poorest flow ability was found in LDPE packaging material with stored in room temperature with 43.77 at the end of six month , but the powder stored in low temperature in both LDPE and MPF showed a good flow ability as 31.47, 31.86.

The flow property of freeze dried powder was maintained throughout the six month of storage period. In case of bulk density powder stored in LDPE and in MPE under room condition significantly different on comparing with the low temperature storage. Bulk density of food powders has also been observed to decrease with an increase in the particle size as well as with an increase in equilibrium relative humidity [14]. The last physical property is the

solubility; it is a widely used test for all food grade powder to check the solubility in water. Temperature also has a substantial effect on bulk solid flow ability. The most drastic temperature effect is the freezing of the moisture contained within the granular materials and on particle surfaces [15]. The result shows that the least solubility was found in powder store in normal room condition in LDPE. The results found that there is gradually decrease in solubility from 3rd month of storage period. Whereas the freeze dried powder stored in Low temperature in refrigerated and in Dark condition in room temperature is no significant differ with respect to solubility.

Influence of Storage conditions and Packaging materials on Nutritional parameters

The nutritional parameter of Jamun Pulp Powder was analyzed during each month of storage period (Six month). The results were tabulated in table 2. Jamun is a medicinal fruit rich in ascorbic acid, Phenol, Anthocyanin and Flavonoids. Jamun appears to be the only berry that contains the five anthocyanidins present in blueberry/bilberry along with significant amounts of ellagic acid /ellagitannins. This study reveals the storage property and the type of packaging material suitable for Freeze dried Jamun Pulp Powder. Ascorbic acid content of freeze dried powder stored in MPF and LDPE was 10.52 mg/g on the initial day of storage. Whereas there is a dramatic change in ascorbic acid content stored in normal room temperature which exposed to light as 6.77 mg/g and 9.62 mg/g this is stored in LDPE and MPF.

But during the dark storage conditions and Low temperature conditions the AA content was increased gradually with less reduction in Ascorbic acid 11.79 mg/g and 11.07 mg/g on 180th day of storage in Dark condition and 12.29 mg/g and 12.10 mg/g on 180th day of storage in low temperature condition. This mainly due to the packaging material used, on comparing both the packaging material LDPE did not completely prevent the access of moisture and light, contributed to the extensive deteriorating in the levels of ascorbic acid. In another study, freeze-drying was found to retain the maximum amount of vitamin C (Ascorbic acid) in papaya However, it is generally accepted that retention of vitamins also depends on the nature of foods reported by Hawlader *et al.*[16] and Jayaraman *et al.* [17].Next nutritional parameter is phenol and flavonoids which is highly present jamun pulp. During freeze drying at -40 °C these main phytonutrients were retained. To store these phytonutrients enriched powder for a long time two packaging material were selected based on its properties. Flavonoids and phenol content was significantly different on packaging material. Lowest phenol content was observed in LDPE packaging material 13.5mg/g on 180th day of storage and the MPE packed powder gave a very less reduction 14.38 mg/g on the 180th day of storage in Low Temperature condition.

According to another study, onions subjected to freezing showed reduced levels of flavonols, however, those subjected to freeze-drying offered increased amount of flavonols [18]. But there is fluctuation during the storage period this is due to the chemical reactions resulting in pigment degradation. Small Oscillation was found in flavonoids content during the storage period on all the three conditions. In LDPE packed powder contain 101.79 mg/g which stored in room temperature. The powder stored in MPE is 102.10mg/g on the 180th day of storage in Low temperature. Changes in nutrient content during storage of different foods are not clear; the actual amount may increase, decrease or remain unchanged [19]. The anthocyanin content was also determined and it was found to be significantly differ on packaging and storage conditions. Gradual change was found from 7.25 mg/g in the initial storage period to 6.53mg/g in LDPE and 6.68 mg/g in MPE stored in low temperature. Also, the thermal Processing destroyed some anthocyanins. A study about the evolution of anthocyanins in raspberries during jam making, in which heat was used, showed that 17%–40% of anthocyanins were lost [20].

CONCLUSION

The Jamun pulp powder stored in two different packaging materials shows a small decrease in Ascorbic acid, Phenols, Anthocyanin and high change in moisture content, solubility, bulk density and flow ability. The results of

the present study reveal that freeze-drying can be explored as a viable method for processing tropical fruits retaining the maximum amount of their naturally occurring Phyto nutrient. The changes that occur in physical parameter are due to retention in moisture. The decrease in solubility during the storage period on all the three conditions was mainly due to increase in moisture content. Therefore it can be concluded that the permeable packaging material was MPE Metalized Polyethylene Pouches and store condition at Low temperature influence less in the nutritional and physical parameters. This study reveals that these storage conditions are suitable for Freeze Dried Jamun Pulp powder for about six month with all the phytonutrient properties. The dried powder remained stable during these storage conditions tested and it can be used as a food supplement.

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Table 1. Physical Parameters during Six Month Storage of Freeze dried Jamun Pulp Powder

Storage		Moisture content %		Flow Ability		Bulk Density		Solubility %	
Temp	Days	MPF	LDPE	MPF	LDPE	MPF	LDPE	MPF	LDPE
ST1	0	2.95±0.20	2.95±0.20	29.01±0.12	29.0±0.10	0.68±0.11	0.68±0.11	71.85±0.85	71.85±0.85
	30	2.97 ±0.06	3.01 ±0.02	29.33±0.58	30.57±0.51	0.67±0.04	0.67±0.03	71.86±0.85	72.33±0.58
	60	3.04±0.02	3.05±0.02	30.63±0.40	31.40±0.40	0.64±0.03	0.63±0.02	70.51±0.45	70.41±0.37
	90	3.10±0.04	3.12±0.01	30.71±0.07	34.07±0.38	0.61±0.01	0.62±0.01	69.65±0.41	69.86±0.23
	120	3.12±0.01	3.18±0.03	30.99±0.13	35.51±0.89	0.62±0.01	0.63±0.02	69.66±0.10	69.41±0.06
	150	3.14±0.02	3.19±0.01	31.03±0.05	37.50±0.46	0.62±0.02	0.61±0.01	69.60±0.14	69.21±0.01
	180	3.17±0.03	3.26±0.03	31.47±0.31	43.77±0.49	0.61±0.02	0.60±0.03	69.37±0.45	69.17±0.08
ST2	0	2.95±0.20	2.95±0.20	29.01±0.12	29.01±0.10	0.68±0.11	0.68±0.11	71.85±0.85	71.85±0.85
	30	3.03±0.12	2.97 ±0.06	29.33±0.58	29.33±0.58	0.69±0.01	0.69±0.01	72.90±0.85	72.57±0.60
	60	3.03±0.03	3.04±0.05	30.13±0.21	30.57±0.21	0.68±0.01	0.67±0.01	71.16±0.26	71.19±0.18
	90	3.05±0.04	3.06±0.01	30.79±0.33	30.79±0.33	0.65±0.03	0.64±0.01	70.42±0.36	69.87±0.45
	120	3.07±0.02	3.12±0.02	31.57±0.25	35.40±0.53	0.63±0.02	0.64±0.01	69.78±0.38	69.32±0.09
	150	3.10±0.02	3.16±0.03	31.34±0.40	35.47±0.93	0.61±0.02	0.62±0.01	69.77±0.03	69.52±0.06
	180	3.12±0.01	3.19±0.02	31.53±0.11	38.50±0.50	0.62±0.01	0.60±0.02	69.53±0.15	69.41±0.16
ST3	0	2.95±0.20	2.95±0.20	29.01±0.12	29.0±0.10	0.68±0.11	0.68±0.11	71.85±0.85	71.85±0.85
	30	2.95±0.06	2.93±0.06	29.01±0.01	29.01±0.02	0.70±0.01	0.69±0.01	73.33±0.42	73.30±0.20
	60	2.95±0.05	3.01±0.09	29.86±0.28	29.92±0.29	0.68±0.01	0.67±0.01	72.57±0.49	72.30±0.44
	90	3.01±0.03	3.04±0.03	29.89±0.29	30.27±0.17	0.65±0.01	0.63±0.01	71.59±0.26	71.77±0.15
	120	3.04±0.02	3.07±0.01	30.03±0.05	30.29±0.18	0.65±0.02	0.63±0.01	70.92±0.38	71.10±0.13
	150	3.12±0.03	3.17±0.03	30.38±0.58	31.33±0.15	0.64±0.01	0.62±0.01	70.15±0.31	69.75±0.61
	180	3.15±0.02	3.18±0.02	31.47±0.12	31.86±0.15	0.60±0.01	0.61±0.01	69.85±0.25	69.69±0.27

Table 2. Nutritional Parameters during Six Month Storage of Freeze dried Jamun Pulp Powder

Storage		Ascorbic Acid (mg/g)		Total Phenol (mg/g)		Total flavonoids (mg/g)		Total Anthocyanin (mg/g)	
Temp	Days	MPF	LDPE	MPF	LDPE	MPF	LDPE	MPF	LDPE
ST 1	0	10.52± 0.12	10.52±0.11	13.72±0.15	13.72±0.15	105.77±0.34	105.77±0.34	7.25±0.15	7.25±0.15
	30	10.49±0.03	9.42±0.03	14.37±0.08	13.94±0.04	103.30±0.34	100.14±0.08	6.70±0.02	7.42±0.03
	60	10.41±0.09	9.23±0.04	15.05±0.12	13.99±0.09	104.03±0.09	100.22±0.13	6.53±0.18	6.06±0.07
	90	9.20±0.11	8.16±0.07	15.07±0.07	13.95±0.12	104.12±0.13	101.42±0.19	6.44±0.11	5.93±0.07
	120	9.91±0.05	8.11±0.03	14.75±0.05	13.72±0.04	104.29±0.15	101.35±0.11	6.36±0.08	5.87±0.11
	150	8.84±0.05	7.7±0.09	14.7±0.09	13.5±0.04	104.2±0.09	101.1±0.10	6.3±0.08	5.8±0.10
	180	9.62±0.13	6.77±0.09	14.77±0.11	13.79±0.04	104.80±0.16	101.79±0.08	6.66±0.30	4.73±0.46
ST 2	0	10.52± 0.12	10.52±0.11	13.72±0.15	13.72±0.15	105.77±0.34	105.77±0.34	7.25±0.15	7.25±0.15
	30	12.05±0.02	11.43±0.02	15.63±0.04	15.35±0.14	104.31±0.04	103.95±0.07	6.98±0.02	6.6±0.02
	60	13.25±0.14	12.73±0.12	16.76±0.10	16.48±0.08	104.04±0.06	103.70±0.02	6.63±0.03	6.70±0.05
	90	12.4±0.07	11.95±0.07	15.88±0.10	15.63±0.16	104.72±0.05	103.93±0.02	7.16±0.06	7.03±0.05
	120	12±0.09	11.69±0.06	15.03±0.08	14.93±0.06	104.94±0.03	104.07±0.06	7.20±0.06	7.13±0.03
	150	11.67±0.10	11.42±0.14	14.94±0.05	14.69±0.04	105.20±0.05	105.01±0.03	7.34±0.05	7.25±0.06
	180	11.70±0.04	11.07±0.06	14.93±0.06	14.85±0.03	105.28±0.07	105.14±0.06	7.33±0.04	7.25±0.05
ST 3	0	10.52± 0.12	10.52±0.11	13.72±0.15	13.72±0.15	105.77±0.34	105.77±0.34	7.25±0.15	7.25±0.15
	30	13.54±0.12	12.74±0.15	16.45±0.08	15.83±0.15	104.52±0.12	102.67±0.10	7.25±0.04	6.95±0.09
	60	13.67±0.08	13.09±0.10	16.21±0.20	15.99±0.09	103.21±0.09	102.34±0.33	7.25±0.22	6.96±0.07
	90	12.70±0.05	12.25±0.10	15.74±0.05	15.31±.0.03	103.86±0.16	102.88±0.11	7.25±0.07	7.12±0.08
	120	12.81±0.17	12.58±0.16	15.64±0.17	15.52±0.08	103.86±0.03	102.66±0.10	7.19±0.02	7.01±0.05
	150	12.69±0.13	12.43±0.13	14.48±0.14	14.27±0.15	102.54±0.19	101.89±0.12	7.12±0.12	6.81±0.11
	180	12.29±0.11	12.10±0.10	14.38±0.10	14.01±0.12	102.10±0.11	101.49±0.09	6.68±0.06	6.53±0.14

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RESEARCH ARTICLE

Comparative Efficiency of Irrigation Methods with Different Irrigation Schedules and Fertigation for Groundnut

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ABSTRACT

Performance evaluation of irrigation schedule and fertigationis an important part of research aimed at improving agriculture production and water management. Here we describe the results of a field experiment conducted at Wu Daogou hydrology and water resources experimental station for two consecutive seasons 2014-2015. Using a randomize block design we compared two irrigation methods, a sprinkler and traditional check basin and furrow method. By this approach we aimed to investigate the effect of micro-sprinkler irrigation with fertigation under different schedules and land formations on groundnut pod yield and water use efficiency, as compared to the traditional method. Fertilizer was applied to the soil by either surface irrigation (0.80 IW/CPE (IW – irrigation water, CPE – cumulative pan evaporation) or fertigation (micro-sprinkler irrigation), under two types of land configuration (check basin or furrow). Micro-sprinkler irrigation was scheduledfor once every three days with two level of irrigation (100%and 60%ETc).Both micro-sprinkler regimes were more water efficient than the traditional approach, with total water saving of 30.07-31.5% (100% ETc) and 33.5-39.1% (60% ETc). For both the crops tested, the highest yield (3,796-3,864kg/ha) and best water use efficiency (8.72-9.22 kg/ha/mm) were achived by micro-sprinkler irrigation at 100 % ETc with fertigation under furrows. Thus, our data indicate that the micro-sprinkler irrigation method at 100% ETc may be optimal groundnut crop production.

Keywords: Micro-sprinkler, schedule of irrigation, Fertigation, Land Configuration, Water Use Efficiency Groundnut.

INTRODUCTION

Production agriculture is facing a series of major challenges in providing sufficient food, fiber, and fuel to support a growing global population while our water resources, environmental health, and available arable land decline and climate changes. In agricultural ecosystems, physical and biological processes such as cycling of carbon, water, and nutrients are linked with social and economic processes. To achieve sustainability, it is essential that we understand how these processes interact, and their impacts on the environment through space and time. Innovative approaches will be needed to address these issues and maintain a safe and abundant food supply as the agronomic, economic and social problems facing production agriculture gain in complexity.

Water productivity is an important driver in projecting future water demands (Amarasinghe et al. 2004; Kijne et al. 2003). Efficient irrigation technologies help establish greater control over water delivery (water control) to the crop roots, reduce the non-beneficial evaporation from field and non-recoverable percolation, and return flows into "sinks" and often increases the beneficial ET, though the first component could be very low for field crops. Water productivity improves with reduction in depleted fraction and yield enhancement. Since at the theoretical level, water productivity improvements in irrigated agriculture can result in saving of water used for crop production, any technological interventions which improve the crop yields are also, in effect, water saving technologies. Hence, water saving technologies in agriculture can be broadly classified into three: water saving crop technologies; water saving and yield enhancing irrigation technologies; and, yield improving crop technologies. As well asefficient irrigation management helps not only maintain farm profitability in a scenario of limited, higher cost water supplies but also result in water saving to meet future water requirements. The potential to conserve water depends on the capabilities of the irrigation system and the commitment of the operator to implement timely water saving practices and technologies (Schütze and Schmitz 2009).

Micro-irrigation includes drip and micro-sprinklers. It is a flexible set of technologies that can potentially be used on almost every crop, soil type and climatic zone if justified economically. It is characterized by the applications of water in small amounts using frequent irrigations (i.e. daily). It is also sometimes referred to as localized irrigation and can provide numerous crop production and water conservation benefits that address many of the water quality and supply challenges facing modern irrigated agriculture. Micro-irrigation acreage is increasing steadily worldwide and is fully expected to continue its rapid growth in the foreseeable future (Lamm et al. 2007).

In this research we focused on the comparative efficiency of Micro-sprinkler irrigation over check basin.Micro-sprinkleris the pressurized and low volume irrigation system, which is recognized as an efficient irrigation technology to get more crop yield per drop (Krishnamurthi et al. 2003). It has an added advantage of applying fertilizers through irrigation water. Fertigation is an appropriate method of fertilizer application from the fertilizer use efficiency angle (Shinde et al. 2002). Similarly, furrow is the best land formation to improve soil physical conditions suited for pod development (Nikam and Firake 2002). Meanwhile in irrigation scheduling, a climatological approach based on IW/CPE ratio (IW- irrigation water, CPE- cumulative pan evaporation) has been found most appropriate. This approach integrates all the weather parameters that determine water use by the crop and is likely to increase production by at least 15–20% (destine NG, 1972). Optimum scheduling of Irrigation led to increase in pod yield and water use efficiency (WUE) (taha, m and gulati,j.m.l2001). The concept of scheduling of irrigation at IW/CPE ratios assumes importance in optimizing the water requirement with various methods of irrigations adopted.

The purpose of the study is to consolidate the existing knowledge on input management potential in groundnut and examine the cumulative effects of efficient irrigation technology (micro-sprinkler irrigation), apt method of fertilizer application (fertigation) anddesigned the efficiency of irrigation methods at different irrigation schedules for groundnut.

MATERIALS AND METHODS

Experimental site

The experiment was conducted at the research field of Wu Daogou hydrology and water resources experimental station Bengbu, Anhui agricultural university Hefei during two consecutive seasons June- September of 2014 and 2015. It is located at latitude 33^{0}16'75.70" N and longitude 117^{0}29'83.46" E at an elevation of about 25m above mean sea level (MSL) (fig.1). The related soil and water test were conducted in laboratory of agricultural water resources, faculty of engineering, Anhui agricultural university Hefei.The land at experimental site was uncultivated for about one year.

Therefore, it was ploughed twice in both season by disk plough followed by rotavator and cultivator and then leveled. The total area (8 × 18m) was divided into two plots. The plot of (8 × 8m) as occupied by micro-sprinkler irrigation method under check basin and furrows, while another plot of (8 × 8m) was occupied by check basin and furrow irrigation method. In order to avoid the seepage of water from micro-sprinkler to check basin water application practices plot a polyethylene sheet up to depth of 2ft was provided. Data were recorded on following parameters i.e., Soil characteristics (soil texture, dry bulk density, infiltration rate, welting point, filed capacity)ph and Ec Water saving and increase in yield. Soil Characteristics like soil texture, dry bulk density, infiltration rate, welting point, filed capacity of the experimental site for the depth of 0-15, 15-30, 30-45, 45-60 cm are present in (Table 1).

Micro sprinkler irrigation system was installed in the experimental field while this system comprised 63 mm PVC main pipes and 50 mm PVC sub main pipes were used to deliver the water from the source (tube well) to the field. The laterals with 12 mm LDPE pipes were placed at a distance of 3 m to a length of 8 m on either side of the sub main. The micro-sprinklers were placed along the laterals at an interval of 2 m in order to have 100 per cent wetting area. Daily pan evaporation was recorded from the open pan evaporimeter available at the meteorological observatory of Wu Daogou experimental station. The irrigation water given was measured with the help of the water meter fixed to the pipeline and the irrigation was scheduled accordingly as per the sub treatments The 2 kg/cm^2 operating pressure of micro-sprinklers was maintained at the time of each irrigation operation. The discharge rate of micro-sprinklers was 60 lph.

The experiments were conducted with ten treatments and laid out in a randomized block design with three replications. The treatments consisted of soil application of 100 % recommended dose of fertilizer in surface irrigation at 0.80 IW/CPE ratio with 6 cm depth under check basin irrigation and micro-sprinkler irrigation scheduled once in three days with two levels of irrigation (100 % ETc and 65 % ETc) and two methods of fertilizer application with 100 % recommended dose of fertilizer namely 17 : 34 : 54 kg NPK/ha (soil application and fertigation) under two types of land formations (check basin and furrow).

Fertilizer application

Fertigation is the application of soluble nutrients with via the irrigation water; fertilizer was applied through fertigation applicator withinterval of 8 splits at 8 days starting from 15 days after sowing to 75 days after sowing. Phosphorous in the form of single super phosphate was applied before sowing as basal dose through soil application for all the treatments under study. Nitrogen in the form of urea and potash in the form of muriate of potash were given at sowing as basal dose through soil application for treatments. The required quantity of N and K fertilizers were dissolved separately in ten litres of water and supplied through ventury unit. The dates of sowing and harvest are given below Table 2.

The sowing and life irrigation were given uniformly to all the plots irrespective of the treatment schedule and subsequent irrigations were given as per the treatments based on the evaporation values. Irrigation was given through field channels for the surface plots by using a parshall flume. The micro-sprinkler irrigation was scheduled based on Evapo-transpiration of crop (ETc) (01) as well as also calculated the ET◦ using the FAO–PM equation (02).

$$ET\ c = E\ p \times Kp \times K\ c \qquad (01)$$

Where, ET c = Evapo-transpiration of crop (mm)

E p = Pan evaporation (mm)

K p = Pan factor (0.80) K c = Crop Coefficient

Kc (%) is the crop coefficient for groundnut, the crop growth stages were, respectively, 25, 25, 30 and 25 days for initial stage, development stage, mid-season stage, and late season stage. According to FAO (2014), in this work the crop coefficients equal to 0.40, 0.40-1.15, and 0.6 were used for initial, mid, and late season stage, respectively.

Where the FAO–PM equation,

$$ET = \frac{0.408\Delta(Rn - G) + \frac{900\gamma}{T + 273}\, u2(es - ea)}{\Delta + \gamma(1 + 0.34u_2)}\ \{mm.\ day^{-1}\} \qquad (02)$$

where Δ (kPa/◦C) is the slope of saturation vapor pressure curve at air temperature, (Rn^2 MJ/m/day) is the net radiation at the crop surface, G (MJ/m/day) is the solar heat flux density, γ =0 .665×10^{-3}P·kPa/◦C is the psychometric constant, u_2 [m/s] is the wind speed at a 2m height from ground surface, and e_s and e_a (kPa) are the saturation and the actual vapor pressure, respectively, and T (◦C) is the mean daily air temperature at a 2-mm height from ground surface. In this work, an average ETo was estimated using the monthly mean weather data for a 20-year period (june1994–September 2014) recorded at wudaogou experimental station of hydrology. The monthly average ET_o calculated was 500.8 for June, 511.12 for July, 513.05 for august, and 498.9 for September.

RESULTS AND DISCUSSION

Utilization of water

It was observed that crop A during the year 2014 consumed more irrigation water than crop B during the year 2015 irrespective of the irrigation treatments. Regarding irrigation methods, in both crops, surface irrigation required higher quantity of irrigation water as compared to micro-sprinkler irrigation. Among the micro-sprinkler irrigation treatments, irrigation scheduled at 100 % ETc registered higher irrigation water use than irrigation scheduling at 60 % ETc. Consequently, it was found that the micro-sprinkler irrigation at 100 % ETc saved irrigation water up to 32.1per cent in Crop A and 29.07 per cent in Crop B as compared to surface irrigation at 0.80 IW/CPE ratios with 6 cm depth. Micro-sprinkler irrigation at 60 % ETc saved more irrigation water up to 44.70 per cent and 40.71 percent in Crop A and Crop B respectively. It was obvious that micro-sprinkler irrigation at 60 % ETc saved 11.01 per cent more irrigation water than micro-sprinkler irrigation at 100 % ETc.Consumptive use of water was assessed to find out the quantum of water used by the crop. It was computed using quantity of irrigation water applied and effective rainfall. The results are presented in (Table 3).

The data on effective rainfall revealed that Crop A recorded higher effective rainfall as compared to Crop B. Surface irrigation plots received higher effective rainfall than micro-sprinkler irrigation. However, micro-sprinkler irrigation at 60 % ETc experienced higher effective rainfall than micro-sprinkler irrigation at 100 % ETc during Crop A. In Crop B, the difference between micro-sprinkler irrigation at 60 % ETc and micro-sprinkler irrigation at 100 % ETc was almost negligible.Consumptive use was observed to be higher for surface irrigation (612 mm – 628.50 mm) than micro-sprinkler irrigation. Within the micro-sprinkler irrigation treatments, it was observed that micro-sprinkler irrigation at 100 % ETc registered higher consumptive use (418.82 mm – 435.16 mm) than 60 % ETc (406.96 mm –

383.1 mm) in both crops. Micro-sprinkler irrigation at 100 ETc recorded total water saving of 31.5 per cent in Crop A and 30.07 per cent in Crop B while micro-sprinkler irrigation at 60ETc registered total water saving of 33.5 per cent in Crop A and 39.1 per cent in Crop B as compared to surface irrigation at 0.8 IW/CPE ratio with 6 cm depth. These results corroborate the findings of Rama Praba Nalini (1999).

Total yield of pod

It revealed that the yieldfrom both crops, which wasrecorded high pod yield in micro-sprinkler irrigation as compared to surface irrigation (Table 4).The increase in pod yield in micro-sprinkler irrigation was mainly due to high frequency irrigation which in turn maintained the soil moisture content in the active root zone at adequate level throughout the crop period. Within micro-sprinkler irrigation treatments, there was an increase in pod yield with micro-sprinkler irrigation scheduling at 100 % ETc (70 - 350 kg/ha) over micro-sprinkler irrigation scheduling at 60 % ETc, depending on the method of fertilizer application. Fertigation through micro-sprinkler irrigation recorded significantly higher pod yield than soil application under micro-sprinkler irrigation or surface irrigation irrespective of the effects of land configuration.

Further, fertigation through micro-sprinkler irrigation indicated that high frequency of fertigation leads to efficient utilization of applied fertilizer by the crop. This resulted in higher growth and yield attributes leading to a considerable yield increase in groundnut. The result confirms the findings of Prabhakaran (2000). It was further noted that furrow irrigation methodalso recorded higher pod yield compare to check basin methodeven it recorded high with fertilizer application methods. The analysis of the combined effects of irrigation method with scheduling, fertilizer application method and different land arrangement revealed that micro-sprinkler irrigation at 100 % ETc with fertigation under furrow (T_7) resulted in significantly higher pod yield of 3864 kg/ha in Crop A and 3796 kg/ha in Crop B.

Water use efficiency data (Table 3) shows that micro-sprinkler irrigation recorded high water use efficiency as compared to surface irrigation. It was mainly due to high pod yield and maximum saving in irrigation water. As result of low water use efficiency recoded in surface irrigation might be in high amount of water losses and less yield production. Within the micro-sprinkler irrigation treatments, micro-sprinkler irrigation at 100 % ETc registered higher water use efficiency than 60 % ETc in Crop A and vice versa in Crop B, regardless of the effects of fertilizer application method and land arrangement. Fertigation through modern irrigation technology has recorded higher water use efficiency as compare to soil application under surface irrigation or micro-sprinkler irrigation. The beneficial effects of combining fertigation with micro-sprinkler irrigation on pod yield, and hence on water use efficiency, might perhaps largely stem from the constant soil moisture content at field capacity leading to proper proportion of water and air in the active root zone and also reduction of nutrient leaching losses due to the restriction of wetting area to active root zone.

Deolankar and Firake (1999) and Tumbare and Bhoite (2002) reported similar findings in chilli, the analysis of the combined effects of irrigation method with scheduling, fertilizer application method and land management revealed that micro-sprinkler irrigation at 100 % ETc with fertigation under furrow (T_7) registered significantly higher water use efficiency of 9.22 kg/ha/mm in Crop A and 8.72 kg/ha/mm in Crop B. Surface irrigation with soil application of fertilizers under check basin (T_1) recorded the least water use efficiency of 2.58 kg/ha/mm in Crop A and 2.43 kg/ha/mm in Crop B. It could be concluded that NK fertigation with 100 % recommended dose of fertilizer in 8 splits at 8 days interval through micro-sprinkler irrigation at 100 % ETc furrow recorded higher water use efficiency than soil application of 100 % recommended dose of fertilizers with either furrow or check basin under surface irrigation at 0.8 IW/CPE ratio (6 cm depth). The study therefore concludes that micro-sprinkler irrigation system is suited for improving water use efficiency in field crops through water saving and yield augmentation.

CONCLUSION

Overall, experiment design to identifying the best irrigation schedule of modern irrigation technology management and optimum fertilizer application and frequency, water use efficiency, land management and crop water productivity for better production of groundnut. Results obtained on all these dimensions, the micro-sprinkler irrigation at 100 % ETc under furrows provedto be the most effective practice and suitable irrigation schedule for groundnut to obtain maximum yield (3864 kg/ha) compere to irrigation schedule of 60% (3222 kg/ha) and traditional technology (1929 kg/ha). Consumption of water under sprinkler irrigation method (425.65 mm) less than under traditional irrigation methods (600 mm), with water saving percentage (30.07%) and water use efficiency through traditional and sprinkler irrigation at 100 % ETc(2.58) and (9.22) respectively, the most productive output from the interval of fertigation with 100 % dose in 8 equal splits at 8 days interval from 15 days after sowing to 75 days after sowing and furrow is the adequate land condition for groundnut. Based on our results, it can be concluded that micro-sprinkler irrigation at 100 % ETc with 100% fertilizer dose is beneficial the best over schedule 60% and check basin in irrigating groundnut.

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Table 1: measurement of Soil physical properties of the field

S.No.	Parameter	Adopt Method	Reference	Soil characteristics
1	Soil Texture	Bouyoucos Hydrometer	Bouyoucos (1962)	Sandy clay loam
2	soil bulk density	Core method	McIntyre andLoveday (1974)	1.46g/cm3
3	Field capacity	Veihmeyer and Hendrickson	Veihmeyer, F.J. and Hendrickson, A.H. (1931)	22.01%
4	EC (dS/m)	1:2 Soil Water extract	Rowell(1994)	1.32 dS/m
5	pH	1:2 Soil Water extract	Rowell(1994)	7.6
6	Infiltration Rate	Double Ring Infiltration	Bouwer(1986)	26 mm/hr
7	Permanent welting point	Veihmeyer and Hendrickson	Veihmeyer, F.J. and Hendrickson, A.H. (1928)	14.30%

Table 2: The dates of sowing and harvest

Details	Date of sowing	Date of harvest		Field duration (days)	
		MSI	SI	MSI	SI
First crop A	15,06,2014	21.09.2014	30.09.2014	97	106
Second crop B	14.06.2015	15.09.2015	24.09.2015	91	100

Table 3.Water consumption of irrigation method and irrigation schedule

Treatments	Irrigation Water (mm)		Water Saving (per cent)		Effective Rainfall (mm)		Consumptive Use (mm)		Total Water Saving (per cent)	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Surface Irrigation at 0.8 IW/CPE	480.0	600.0			132.0	28.50	612.0	628.50		
Micro sprinkler Irrigation at 100 % ETc	325.65	425.56	32.01	29.07	93.17	27.60	418.82	435.16	31.5	30.07
Micro sprinkler Irrigation at 60 % ETc	265.32	355.71	44.70	40.71	131.62	27.60	406.96	383.31	33.5	39.1

Table 4.Pod yield with different schedule, formation and fertigation

Treatment	Pod yield kg/ha		Water use efficiency kg/ha/mm	
	2014	2015	2014	2015
T_1=CB+SI+SA	1583	1533	2.58	2.43
T_2 –FM + SI + SA	1929	1887	3.15	3.00
T_3=CB+MSI AT 100%ETC+SA	2191	2071	5.23	4.75
T_4=CB+MSI AT 100%ETC +F	3450	3407	8.23	7.82
T_5– CB + MSI at 60 % ETc +SA	2087	2029	5.12	5.29
T_6 – CB +MSI at 60 % ETc +F	3073	2919	7.55	7.61
T_7 – FM +MSI at 100 % ETc + F	3864	3796	9.22	8.72
T_8 – FM +MSI at 60 % ETc + F	3222	3148	7.91	8.21
CD (P = 0.05)	225	234	0.53	0.55

CB – Check Basin, SA – Soil Application of Fertilizer, MSI – Micro-sprinkler Irrigation, F – Fertigation, SI – Surface Irrigation, FM -- furrow method

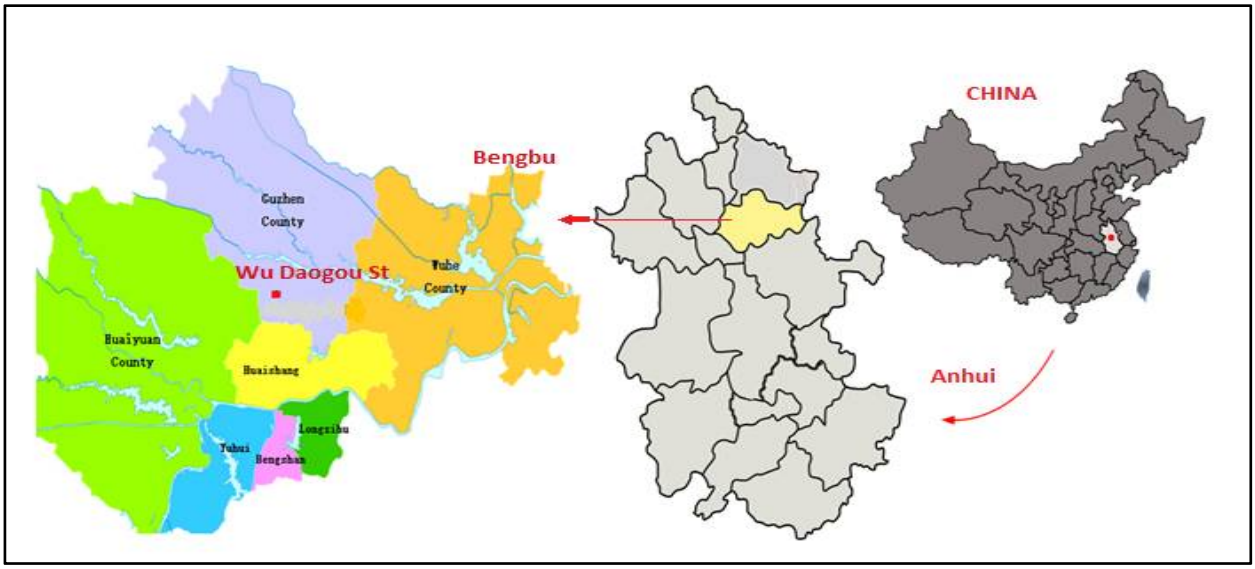

Fig 1: Location of experimental station of Wu Daogou Bengbu

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RESEARCH ARTICLE

Effectiveness of Varmam and Thokkanam Therapy in *Azhal Keel Vayu* (Osteoarthritis)

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ABSTRACT

Azhal Keel Vayu (Osteo arthritis) is a most common disease of joints mainly in elderly people. Mostly knee, lower back and neck are involved. Even though OA is common in elderly people, one in two adults develops symptoms of OA during their lifetime.Short and immediate response is achieved in Siddha system of medicine (one of the Indian traditional system of medicine) through various types of the external therapies like Varmam therapy (Stimulating certain points in the body by pressing manually) and Thokkanam (massage therapy) combined with Indian siddha traditional medicines.Varmam and Thokkanam – This technique is one of the specialities of Siddha system of Medicine.It is the stimulation and the Manipulation method of the vital points in the body. Marutham Siddha Varma Hospital is rendering medical service to OA patients with these specialised therapies.This study was carried out in patients who came to Hospital OPD during the period of Dec 2014 to Jul 2015. 30 OA patients were studied in this trial. The prognosis of those patients with respect decrease in symptoms like pain, stiffness and improvement of physical function was studied using Osteo Arthritis Index.

Keywords : *Azhal Keel Vayu*, OA, Varmam, Thokkanam, Siddhamedicine, OA Index.

INTRODUCTION

Azhal Keel Vayu is a disorder of the Joints due to aggravated Vatham and pitham in joint Space. Major Symptoms are crepitation, Pain,swelling and Tenderness.In Siddha literature charecteristics of Azhal keel vaayu is represented as,

"Pithakkeel VaayvuThannar
Piranku keel muttu vinki".

Azhal keel Vayu is characterised by the breakdown of cartilages (the tissue that cushions the ends of the bones between joints), bony changes of the joints, deterioration of tendons and ligaments and various degrees of inflammation of the joint surface. The goal of OA Treatment is to reduce pain andto improve the joint function.People turn their focus on Traditional system of medicines for better management. Siddha system of medicine approaches the OA Patients with Oral Anti-Vatha, Analgesic siddha medicines in addition with Varma (Stimulation of Vital points) and Thokkanam (Manipulation Therapy).In Marutham Siddha Hospital, The OA patients are treated with internal Siddha medicines in combination with Varmam and Thokkanam Therapy.

Aims and Objectives

To ensure the efficacy of Varmam, Thokkanam in OA.

MATERIALS AND METHODS

The OA patients who visited the Marutham Hospital for treatment were enrolled in the study. 30 OA patient who visited our OPD between Dec 2014 to Jul 2015 were included in this study.Patients were divided into 2 groups, each group 15patients.One group of patients treated with both Siddha internal medicines and Varmam Therapy. Another group of patients only treated with Siddha internal medicines.

Internal Siddha medicines

1. AmukkaraChooranam – 1 Gms BD with Honey. Mix with Arumuga chendooram – 100 mgs BD with Honey.
2. Rasagandhi Mezhugu Capsule. – 1 caps BD with Buttermilk.

Varma Points

Patients were given following Varma points Therapy.
1. Moto Varmam- center point of base of patella
2. Asaivuthirikannu Varmam- middle of medial and lateral border of patella
3. NaaithalaiVarmam- apex of patella

Oseto arthritis Questionnaire was developed and analysed for 1st day, 15th day and after 1 month with the following index.

Osteoarthritis Index

Name:_____ Date:_____

Instructions: Please rate the activities in each category according to the following
Scale of difficulty: 0 = None, 1 = Slight, 2 = Moderate, 3 = Very, 4 = Extremely
Circle **one number** for each activity_____

Pain

1. Walking 0 1 2 3 4
2. Stair Climbing 0 1 2 3 4
3. Nocturnal 0 1 2 3 4
4. Rest 0 1 2 3 4
5. Weight bearing 0 1 2 3 4

_____

Stiffness

1. Morning stiffness 0 1 2 3 4
2. Stiffness occurring later in the day 0 1 2 3 4

_____

Physical Function

1. Descending stairs 0 1 2 3 4
2. Ascending stairs 0 1 2 3 4
3. Rising from sitting 0 1 2 3 4
4. Standing 0 1 2 3 4
5. Bending to floor 0 1 2 3 4
6. Walking on flat surface 0 1 2 3 4
7. Getting in / out of car 0 1 2 3 4
8. Going shopping 0 1 2 3 4
9. Putting on socks 0 1 2 3 4
10. Lying in bed 0 1 2 3 4
11. Taking off socks 0 1 2 3 4
12. Rising from bed 0 1 2 3 4
13. Getting in/out of bath 0 1 2 3 4
14. Sitting 0 1 2 3 4
15. Getting on/off toilet 0 1 2 3 4
16. Heavy domestic duties 0 1 2 3 4

RESULTS AND DISCUSSION

Patients taking internal medicine along with Varma treatment (Fig.1)

After 1st visit, 15thDay visit and After 1 month OA Index Score were analysed.
At the time of 1st visit, patients OA index Score for pain, stiffness and physical function was found to be 4, 3, 4.
At the time of 2nd visit (15th Day Visit), patients OA index Score for pain, stiffness and physical function averagely fall of 2, 2, 3.
At the time of Final visit, patients OA index Score for pain, stiffness and physical function averagely fall to 1, 1, 2.

Patients taking internal medicine along with out Varma treatment (Fig.2)

After 1st visit, 15thDay visit and After 1 month OA Index Score were analyzed.
At the time of 1st visit, patients OA index Score for pain, stiffness and physical function was found to be 4, 3, 4.
At the time of 2nd visit (15th Day Visit), patients OA index Score for pain, stiffness and physical function averagely fall of 3, 3, 3.
At the time of Final visit, patients OA index Score for pain, stiffness and physical function averagely fall of 2, 2, 3.

Summary

OA patients undergoing Varma treatment along with internal medicines showed better improvements than patients taking internal medicines without Varmam therapy.

CONCLUSION

From this study, it is concluded that the siddha medical Treatment with Varmam and Thokkanam shows very good improvement in OA Patients, Particularly in reducing the symptoms like Pain, Stiffness and Physical action. From the above study It is finally concluded that Varmam and Thokkanam Therapies along with internal Siddha medicines effective in management of OA.

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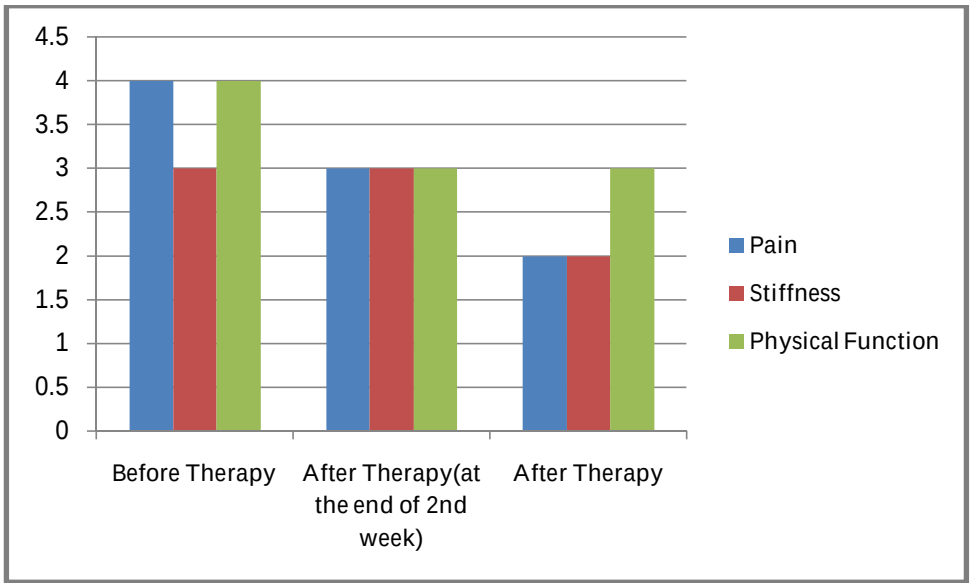
Osteo arthritis Index Score Tables

Table 1.Patients taking internal medicine along with Varma treatment

Symptoms OA	OA Index Score - Before Treatment	OA Index Score – After 4 week Treatment	OA Index Score – After 4 week Treatment
Pain	4	2	1
Stiffness	3	2	1
Physical Function	4	3	2

Table 2. Patients taking internal medicine without Varma treatment

Symptoms OA	OA Index Score – Before Treatment	OA Index Score – After 2 week Treatment	OA Index Score – After 4 week Treatment
Pain	4	3	2
Stiffness	3	3	2
Physical Function	4	3	3



Osteo arthritis Index Score diagrams
Fig. 1.Patients taking internal medicine along with Varma treatment

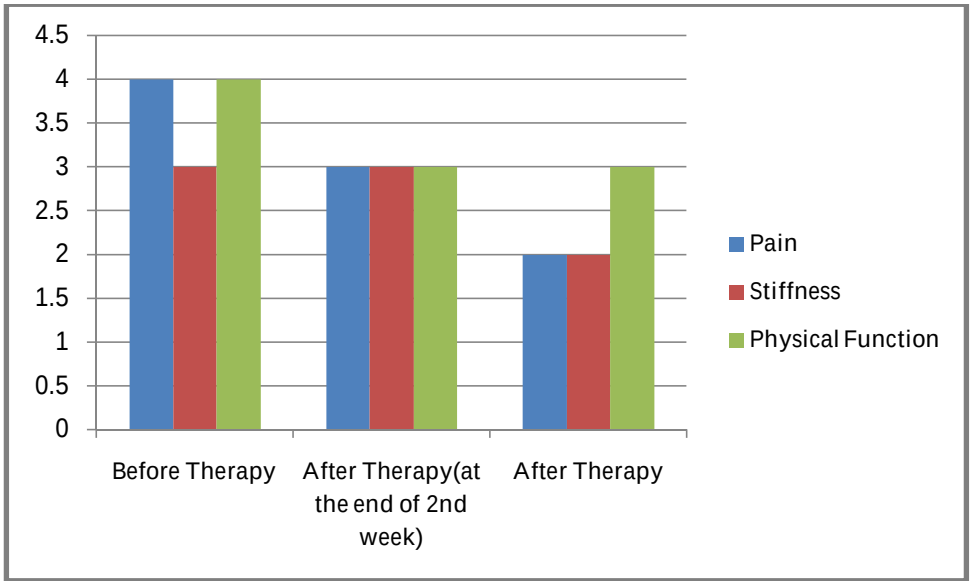


Fig.2.Patients taking internal medicine without Varma treatment

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RESEARCH ARTICLE

Survival Analysis among Oral Cancer Patients at a Tertiary Hospital in East Coast of Peninsular Malaysia

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ABSTRACT

Oral cancer is an uncontrollable growth of cells in the oral cavity includes lips, tongue, cheeks, floor of the mouth, hard and soft palate, sinuses and pharynx. It usually presented as asymptomatic ulcer that persisting for more than three weeks.For prognosis of oral cancer patients on differences concerning gender and race, the survival analysis was done using Kaplan-Meier estimator, log-rank test and Cox's regression methods. Survival analysis is a collection of statistical procedures for data analysis where the outcome variable of interest is time until an event occurs.In this study, mortality presented a higher increase among males (mean±SEM: 131.410±13.482; 95% confidence interval: 104.985-157.835) than in females (mean±SEM: 144.000±11.225; 95% confidence interval: 121.999-166.001). Oral cancer frequencies in males (62.10%) are higher than in females (37.90%) during the study period. Squamous cell carcinoma was most prevalence (55.17%) among our population regardless of gender and race (Malays, Chinese and Indians). Most of the cases were detected among Malays (75.90%), followed by Chinese (13.80%) and Indians (10.30%).Age and gender are the risk factors for oral cancer. Most of the cases were occurs at age above 50 years with male predominantly. Survival analysis showed no significant evidence of the different survival time on differences concerning gender and race.

Keywords : Survival analysis, Oral Cancer, Kaplan-Meier estimator, Log-rank test and Cox's Regression

INTRODUCTION

Oral cancer is the eleventh most common cancer in the world [1]. In Malaysia, oral cancer incidence varies by gender and ethnic group with the highest prevalence among Indians and indigenous groups [2-3]. It is preventable and the early detection of the malignancy greatly increases the survival rates as the mouth is easily accessible for self or clinical examination [4-5]. The prognosis of oral cancer is poor with the lowest survival rate of less than 50 percent, within a five-year period [6]. Usually the malignancies originated from floor of the mouth, lips, base of the tongue or surrounding tissues from oropharynx. The oral cancer can be either epithelial or connective tissue in origins. Oral squamous cell carcinoma is the most common diagnosed malignancy in the oral region. Despite the important role played by genetic factors in carcinogenesis, oral cancer is overwhelmingly influenced by environmental and life style risk factors such as heavy use of tobacco, excess alcohol consumption, paan or betel nut chewing and poor oral hygiene [7-9].

Survival analysis is a branch of statistics used for analyzing the data involves the time and a specific event of interest. The survival time is the time started from a defined point to the occurrence of a specific event. This analysis was done using three statistical methods such Kaplan-Meier estimator; log-rank test; and Cox's regression hazard [10-12]. The Kaplan-Meier estimator or product limit estimator was used to estimate the survival function from lifetime data. It computes the probabilities of the outcome or specific event at a certain point of time. The Kaplan-Meier survival curve was used to define the survival probability in a specific period of time while considering time in many small intervals. The two survival curves can be compared statistically by testing the null hypothesis using log-rank test and Cox's regression. It has fewer assumptions compared to parametric models [13-16]. Log-rank test compares the observed and expected number of events for each study group. Cox's regression or proportional hazard regression was used to analyze the survival time data to get the hazard rate models. It studies the risk factors by time. These prognostic tools were widely used to find out the survival probability in breast cancer, lung cancer, prostate cancer, colorectal cancer, malignant glioma, pancreatic cancer, neuroendocrine tumor liver metastases, and head and neck squamous cell carcinoma [17-24]. Thus, this paper applies the Kaplan-Meier estimator, log-rank test and Cox's regression for survival analysis among oral cancer patients on differences concerning gender and race. The main goal of this study is to describe factors associated with trends over time in the incidence and survival rates among oral cancer patients.

MATERIALS AND METHODS

This is a survival time study. The data were collected from all oral cancer patients who admitted to Hospital Universiti Sains Malaysia (USM) for this study. There were only 29 patients who diagnosed of having oral cancers started from 2010 to 2016 years. The frequency analysis was done to demonstrate the demographic profile of all oral cancer patients. All the procedures were in accordance with the ethical guideline of Human Research Ethics Committee of USM (JEPeM). Two methods were applied in this study as follows:

i. Frequency Analysis
ii. Survival Analysis
    a) Kaplan-Meier Estimator
    b) Log-rank test
    c) Cox's regression

Survival data relate to the time taken for an individual to reach a certain event such as death and failure in organ systems function. However, not everyone will have experienced the end point of the study such death and organ failure. Censoring means that an individual has not experienced oral cancers by the end of the study (i.e. they withdrew from the study or died from an unrelated event). The survival analysis was done in analyzing the expected duration of time among oral cancer patients until death happens with regards to gender and races. The survival plot

displays survival probabilities (cumulative probability of an individual remaining alive at any time after baseline). The cumulative survival probability is the product of the survival probabilities up to that point in time.

Probability of survival on month *i*:

$$\frac{n_i - d_i}{n_i} = \frac{\text{No. alive the month before - No. dying on month } i}{\text{No. alive the month before}} \quad \text{(i)}$$

Survival until month *i*:

$$S(t_i) = \frac{n_i - d_i}{n_i} \times S(t_{i-1}) = \frac{n_1 - d_1}{n_1} \times \frac{n_2 - d_2}{n_2} \ldots\ldots\ldots \times \frac{n_i - d_i}{n_i} \quad \text{(ii)}$$

These probabilities are calculated for months when the event (i.e. death) happens in this study. When observations are censored, the probability of survival remains the same. The log-rank test tests the hypothesis that there is no difference in survival times between groups studied at all time points in the study. The log-rank test compares the observed and expected number of events for each group using the same statistic test as the Chi-squared test although the calculations for the expected frequencies are different. The assumptions for the test are that the survival times are continuous or ordinal and that the ratio of the risk of the event happening in group 1 compared to the risk in group 2 remains constant (proportional hazards assumption). Statistic test for comparing two groups *A* and *B*:

$$x^2 \text{ logrank} = \frac{(O_A - E_A)^2}{E_A} + \frac{(O_B - E_B)^2}{E_B} \quad \text{(iii)}$$

The calculation of the expected values is a bit tricky and time consuming:

$$E_A = \Sigma \frac{d_i n_{Ai}}{n_i}$$

With,

d_i = no. of events at time t_i,

n_{Ai} = no. of people at risk at time *i* in group *A*,

n_i = total no. of people at risk

The degrees of freedom are: number of groups − 1, significant level of 5% and 1 df, the critical value is $x^2_{0.05,1} = 3.84$. If the statistical test result was more than 3.84, reject the H_0 .Compares the hazards (as ratios) of the two treatment groups and allow several variables to be taken into account. The hazard is the risks (probability) of reaching the endpoint (i.e. death) at time point *i*, given that the individual has not reached it up to that point. For standard multiple regressions the model produced is of the form:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_k x_k \quad \text{(iv)}$$

For modelling the hazard function, the model is:

$$\lambda_i(t) = \lambda_0(t) \exp(\beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_k x_k \quad \text{(v)}$$

$\lambda_i(t) =$ Hazard function at time point *t* for individual *i*, $\lambda_0(t) =$ baseline hazard function (hazard function when all explanatory variables are set to 0)

RESULTS AND DISCUSSION

A total of 29 oral cancer patients aged from 20 to 99 years were enrolled into this study. Most patients were detected to have oral cancer aged between 70 to 79 years with 10 (34.48%), 7 (24.14%) aged between 50 to 59 years, 4 (13.80%) aged between 40 to 49 years and 60 to 69 years respectively. While, no patients were detected at age between 30 to 39 years and only 1 (3.44%) patient detected at ate between 20 to 29 years Table 1. Current age was calculated until 2016

and few cases were reaching an end point such death with organs failure and metastases. The oral cancer detected was higher among males, 18 (62.10%) compared to females, 11 (37.90%). Most of the patients were Malays with 22 (75.90%), 4 (13.80%) Chinese and 3 (10.30%) Indians respectively. Squamous cell carcinoma was the most prevalence detected among our population with 16 (55.17%), followed by 4 (13.80%) buccal mucosa carcinoma, 3 (10.34%) carcinoma mouth and floor, 2 (6.90%) adenoid cystic carcinoma and only 1 (3.44%) detected of having a carcinoma oral cavity, malignant fibrous histiocytoma buccal mucosa, oral papillary adenocarcinoma and rhabdomyosarcoma respectively.

The estimated mean time until death is 131.410 months for males and 144.000 months for females Table 2. Thus, females have an increased chance of survival than males. The plot showed the survival probability is lower for males at all time points so they are less likely to survive Figure 1.Table 3 shows the results of the Overall Comparisons using Log Rank (Mantel-Cox). Test of equality of survival distributions for the different levels of gender. Suitability of the model used Table 3, the model was fit. The *p*-value is the probability of getting a statistic test of at least 0.884 if there really is no difference in survival times for males and females. As the *p*-value (0.347) is more than 0.05, it can be concluded that there is no significant evidence of a difference in survival times for males and females. While, refer to the plot Figure 1 , comparison of survival probability between males and females was between 0.8 and 1 can be seen with naked eyes, however, it was too small until statistical test define it was no different ($p = 0.347$).

The model reasonably fits well. Proportional hazard assumption is met Table 4. There are no interaction and multicollinearity problem. Not significant event with different race, the death probability was the same. There is no evidence of a difference death risk between Chinese and Malay ($p = 0.496$, Exp (β) = 0.032) in either sex. While, there is also no evidence of a difference death risk among Indian compared to Malay ($p = 0.628$, Exp (β) = 0.031) in either sex.

Oral cancer most commonly occurs in middle-aged and older individuals [25]. However, oral malignancies are also reported in young adults in recent years [25-28]. In this study Table 1, most of the cases were detected at age above 50 years and squamous cell carcinoma encompasses at least 55% of all oral malignancies in our population. The median age of diagnosis of tongue's cancer is 61 years. Only approximately 2% of patients are diagnosed before the age of 35 and another 7% before the age of 45 [29]. Squamous cell carcinoma is the most common malignancy (90% to 95%) affecting the oral cavity beginning as inflammatory lesions such as leukoplakia, erythroplasia and erythroleukoplakia [30]. It generally affects men aged over 50 years and accompanied with high tobacco and alcohol consumption. It rarely occurs in the young age, under 40 years [30-34]. The strong association between oral cancers with tobacco use is well established [10-11]. In our population, most of the smoker was male, female smoker was rare. It is reasonable when most of the cases were detected in males than in females. In addition, intraoral and oropharyngeal tumors are more common among male than female, with a male: female ratio of over 2:1 [33].

For survival analysis among oral cancer patients, as the *p*-value greater than 0.05, it can be concluded that there is no significant evidence of different in survival time for males and females. The estimated mean time until death for males is 131.410±13.482 months, 95% confidence interval (CI: 104.985-157.835) Table 2. While, the estimated mean time until death for females is 144.000±11.225 months, 95% confidence interval (CI: 121.999-166.001) Table 2. This paper described the basics of Kaplan-Meier survival curves where two very small comparison groups was easily seen through the plot and appeared to be a great difference Figure 1. However, the log rank test showed the two curves were not significantly different ($p = 0.347$) Table 3. These hypothetical data illustrate a crucially important point of the Kaplan-Meier method, the main focus is on the entire curve of mortality rather than on the traditional clinical concern with rates at fixed periodic intervals. Moreover, simple Cox's regression model showed that there is no difference of death risk among Malays, Chinese and Indians Table 4. Mean survival time for oral cancer patients before reach an end point which is death accompanied with organ failure and metastases was approximately 4 to 5

years. This is in concordance where approximately 60 per cent of people diagnosed with oral cancer will survive only up to five years [19].

CONCLUSION

In this study, survival analysis showed no significant evidence of the different survival time on differences concerning gender and race. Age and gender are among the risk factors for the oral cancer. Indeed, most of the cases were occurs at age above 50 years with male predominantly. The demographic factor and individual dietary habits may play a role in the oral cancer cases.

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Conflict of Interest

The authors declare there is no conflict of interest regarding the publication of this paper.

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Table 1: Profile demographic of oral cancer patients

Profile demographics	Category	Frequency n (%)
Gender	Male	18(62.10%)
	Female	11(37.90%)
Detection age	20-29 years' old	1(3.44%)
	30-39 years old	0(0.00%)
	40-49 years old	4(13.80%)
	50-59 years old	7(24.14%)
	60-69 years old	4(13.80%)
	70-79 years old	10(34.48%)
	80-89 years old	3(10.34%)
	90-99 years old	0(0.00%)
Current age	20-29 years old	0(0.00%)
	30-39 years old	1(3.44%)
	40-49 years old	2 (6.9%)
	50-59 years old*	5(17.24%)
	60-69 years old*	5(17.24%)
	70-79 years old*	7(24.14%)
	80-89 years old*	7(24.14%)
	90-99 years old*	2(6.90%)
Race	Malay	22(75.90%)
	Chinese	4(13.80%)
	Indian	3(10.30%)
Types of oral cancer	Adenoid Cystic Carcinoma	2(6.90%)
	Buccal Mucosa Carcinoma	4(13.80%)
	Carcinoma Oral Cavity	1(3.44%)
	Carcinoma Floor of the Mouth	3(10.34%)
	Malignant Fibrous Histiocytoma Buccal Mucosa	1(3.44%)
	Oral Papillary Adenocarcinoma	1(3.44%)
	Rhabdomyosarcoma	1(3.44%)
	Squamous Cell Carcinoma	16(55.17%)

* One or more cases have reached an end point such death with organs failure and metastases.

Table 2: Means for Survival Time

Gender	Mean[a]			
	Estimate	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Male	131.410	13.482	104.985	157.835
Female	144.000	11.225	121.999	166.001
Overall	138.774	10.301	118.585	158.963

Table 3: Overall Comparisons

	Chi-Square(d.f)	Sig.
Log Rank (Mantel-Cox)	0.884(1)	0.347

Table 4: Simple Cox Regression Hazard

	Variable	Crude HR (95% CI)	Wald statistics (df)	ρ-value
Race	Malay (0)			
	Chinese (1)	.032	.463 (1)	.496
	Indian (2)	.031	.235 (1)	.628

Reference category: Malay

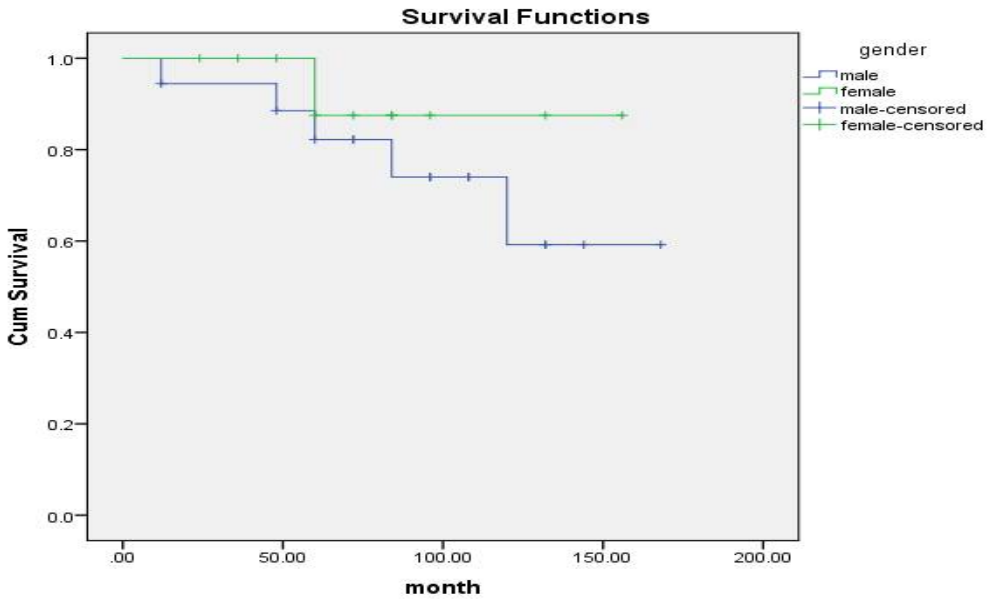

Figure 1: Survival probability plot

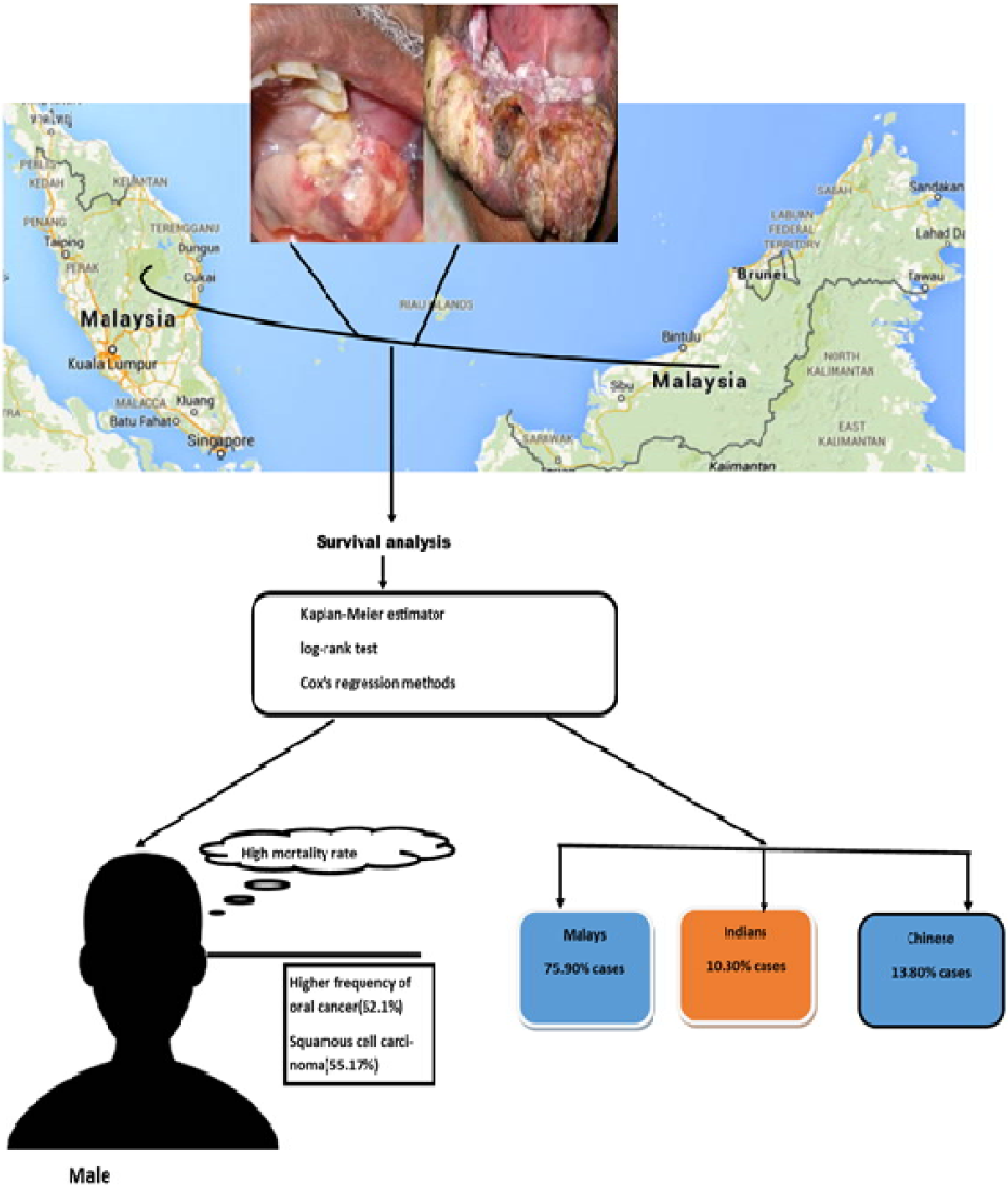

Figure 2: Graphical abstract of survival analysis of oral cancer patients

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RESEARCH ARTICLE

Influence of Storage Period and Temperature on Respiration Rate of Table Grapes ('Bangalore Blue syn or Isabella' 'Bhokri')

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ABSTRACT

This research gives the impact of different four temperature (5, 10, 15 °C and 28 ˚C) and storage periods at 4 days on the respiration reaction rate (RO_2 and RCO_2) of fresh grape variety ('Bangalore Blue syn or Isabella' 'Bhokri'). This research revealed the parameter such as time and temperature are very important factor on the respiration rate of grapes and important in the develop correct modified atmosphere packaging. It needs an known previously studied model for other fruits or vegetable to predict the respiration of grapes also depends on time of storage and temperature.RO_2 and RCO_2 are found to be in rage of 0.33 to 17.22 ml/kg h for black grapes and1.62 to16.09 ml/kg h, for white grapes, and 3.25 to 26.97 CO_2 ml/kg h for black grapes and 0.32 to 23.07 CO_2 ml/kg h for white grapes respectively for both variety. RO_2 and RCO_2 were found to be increased with increased in temperature from 5°C to Room temperature. Decreasing storage temperature of grapes from Room temperature to 5 °C decreased RO_2 and RCO_2 Respectively. Temperature is one of the major parameter which has the main impact on respiration and the synergy between time and temperature also consequential effected on RO_2 and RCO_2.The paper gives an idea that respiration rate of grape will be defined in well descriptive manner that environmental factor, temperature and time with a combination of an calculating the activation energy produced at time of RO_2 and RCO_2 of fresh grapes.

Keywords : Fresh grapes, respiration model, shelf life.

INTRODUCTION

Grapes are non-climacteric fruits. The shelf life of grapes is very less. Increasing the storage life of grapes is beneficial for farmers in order to store the grapes long time. Modified atmosphere packaging technique was studied by so many researchers for different fruit and vegetables but past few years it become one of the important technology in preservation. Modified atmosphere packaging technology increases the self life and keeps the freshness and quality of fruits and vegetables by slow down the respiration rate and decreases the chance of microbial spoilage by preventing microbial growth (Artés and others 2000).Modified atmosphere packaging can be used for fresh fruits also, grapes are produced in, with major producing state as Maharashtra, Karnataka, Andra Pradesh, Tamilnadu and some part in north India. In India grapes were packed without using any new advance techniques such as grape were packed in cartoon boxes, perforated plastic bags, thermacool boxes, paper etc. studied related measurement of grapes will help to understand the RR and through these its help to design MAP for grape.

The mathematical modeling for grapes carried out with the model used by Fonseca et al., 2002.There is no corrective model for respiration of fresh grapes to explain it properly. Therefore, the present research deals with the experimental study were to examine the influence of storage period and temperature on respiration of fresh produced table grapes and to find out a corrective model related respiration rate. At the end they can provide basic information related to the development of modified atmosphere packaging for table grapes.

MATERIALS AND METHODS

Raw material

Black grapes (variety.'Bangalore Blue syn or Isabella') produced by organic farming and harvested from a commercial vineyard located in madhampatty, tamil nadu (india) at commercial maturity during the season. And white grapes (variety "Bhokri") were harvested from a commercial vineyard located in theni district at commercial maturity during the season. And white grape were brought in perforated boxes to the Process and R & D laboratory, karunya University, Coimbatore. The time taken to reach sample is 12 hr. Samples of were weighed approximately 500 g each sample and each sample was placed inside a glass chamber of about 4350 ml, and equilibrated at the desired storage temperature (5, 10 ,15 °C and 28°C).

Experimental setup and measurement of respiratory gases

The rate of respired gases of the fresh grapes was measured using the closed system method Fonseca and others 2002. A closed chamber was constructed by using glass material with dimension 20×15×15 cm grapes were placed in chamber and initial concentration of O_2 and CO_2 measured. The procedure followed for both the variety over the time respectively. Were used to store grape samples at the different temperatures of 5, 10, 15 °C and 28°C. To ensure hermetic seal, taflon tape was used into the gap between lid and the glass chamber. The grapes are placed inside the chamber and the respiratory gases are measured using PBI-Checkmate II Dansenser for 24 hr interval. And the rate of oxygen and car-di-oxide can be measured by using following equations.

RO_2 and RCO_2 were evaluated by putting readings in Enq 1 and Enq 2.

$$Ro_2 = \frac{(yo_2^{ti} - yo_2^{tf}) \times V}{100 \times M \times (tf - ti)} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad 1$$

$$Rco_2 = \frac{(yco_2^{tf} - yco_2^{ti}) \times V}{100 \times M \times (tf - ti)} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad 2$$

Where, yo_2 is the concentration percentage at the t_i initial time in hours(h), t_f is the concentration percentage at final time, same way yco_2 is the concentration percentage at the t_i initial time in (h), t_f is concentration percentage at final

time, M is the weight of sample in (kg) and V is equal to free volume inside the chamber measured by total volume of chamber4350ml minus by the volume occupied by grapes. The volume occupied by the grapes calculated from the weight of grape bunch over density (0.45 g/cm^3) followed by pouring water inside chamber,then to evaluating the impact of storage period on respiration rate of grape. The grapes are placed inside the chamber and the respiratory gases are measured using PBI-Checkmate II Dansenser for 24 hr interval. The glass chamber lid was slightly opened to minimize anaerobic respiration. Following overnight storage time the chamber were closed hermetically and concentration of oxygen and carbon-di-oxide measured. This process repeated over a 4 day storage period and slightly fungal growth and stem color changed was observed over this period. Using Eqn. 1 and 2 the gases eluted and consumed were measured.

Statistical analyses

The pareto chart were used with two elements that was for different temperature and time. Analysis of variance carried out, the data obtained from experiment were measured with one –way ANOVA at 95%confidance levels.

RESULTS AND DISCUSSION

Effect of time and temperature on the respiration rate

It was revealed from the results in Figure 2 RO_2 and RCO_2 values ranged from 6 to 14.46 ml CO_2/ kg hr for black grapes and in white grapes it found small difference from 5 to 15°C it was 3.25 to 6.81 ml CO_2/ kg hr but at 28°C it was found in decrease it may be have effect of anaerobic process or white grape respire differently than black grape. The results shows it black grape respire more than white grape and the rate still increase as temperature increases and it evident from pareto chart the reducing storage temperature of grapes from 28 to 5 °C decreased RO_2 and RCO_2 by about more than 95% up to nearby 40%, respectively. Oxygen and carbon dioxide levels were measured over time and the respiratory quotient (RQ) of grapes was estimated at 1.90 mg kg^{-1}h^{-1} and 1.60 mg kg^{-1}h^{-1} for black grapes and white grapes respectively. The effect of lowering temperature was reported by Torrieri et al 2010 for processed broccoli, they found 88 and 84% slow the respiration as temperature decreases from 20 to3°CThe slightly slower percentage decrease in RR of fresh white table grapes found in the present study compared black grape as well as to other types of fresh produce.

Mathematical modeling

Arrhenius-type model used to explaine the function of temperature on resipration Torrieri et al., 2010 for both R_{O2} and R_{CO2} were added to Eqn. 3 and 4:

$$R_{O_2} = R^i_{O_{2,ref}} \times e^{\left[\frac{-E_{a,O_2}}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right]} \quad \text{..... 3}$$

$$R_{CO_2} = R^i_{CO_{2,ref}} \times e^{\left[\frac{-E_{a,CO_2}}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right]} \quad \text{..... 4}$$

Where, R_{O2} and R_{CO2} are respiration rate at temperature at T. $R^i_{O2\ ref}$ and $R^i_{CO2\ ref}$ are initial respiration rate at reference temperature T_{ref} and R is the universal gas constant. $E_{a\,O2}$ and $E_{a\,CO2}$ are activation energy (KJ/mol) T is the storage temperature. Now substituting R_{O2} and R_{CO2} in Eqn. 1 and 2, with Eqn. 3 and 4, respectively, where, y^i_{O2} = 20.6 %,is atmosphere concentration of oxygen and y^i_{CO2} = 0.2 % is carbon-di-oxide concentration. t is duration take place in respiration process and parameter estimates of $R^i_{O2,ref}$, $R^i_{CO2,ref}$, $E_{a,O2}$ and $E_{a,\,CO2}$ were estimated using calculating equation 1&2 with Microsoft Excel (Microsoft Office 2003).In more details to understand it, effect of time and temperature power function equation used in Eqn. 5 and 6 Uye & Yashiro 1988 was combined with Arrhenius-type model, which describes temperature effect on respiration rate Eqn. 7 and 8

$$R_{O_2} = at^b \quad \text{..... 5}$$

$$R_{CO_2} = at^b \quad \text{..... 6}$$

Combine the above equations 5 and 6 to Arrhenius-type model, respectively to describe both the influence of temperature and time on the respiration rate of grapes.

$$R_{O_2} = R^i_{O_{2,ref}} \times e^{\left[\frac{-E_{a,O_2}}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right]} + at^b \quad \text{..... 7}$$

$$R_{CO_2} = R^i_{CO_{2,ref}} \times e^{\left[\frac{-E_{a,CO_2}}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right]} + at^b \quad \text{..... 8}$$

A secondary model was built by substituting RO_2 and RCO_2 in eqn. 1 and 2 respectively,

$$y^{tf}_{O_2} = y^{ti}_{O_2} - \left[R^i_{O_{2,ref}} \times e^{\left[\frac{-E_{a,O_2}}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right]} + at^b\right] \times \frac{M}{V}\left(t_f - t_i\right) \times 100 \quad \text{..... 9}$$

$$y^{tf}_{CO_2} = y^{ti}_{CO_2} - \left[R^i_{CO_{2,ref}} \times e^{\left[\frac{-E_{a,O_2}}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right]} + at^b\right] \times \frac{M}{V}\left(t_f - t_i\right) \times 100 \quad \text{..... 10}$$

where, parameter evaluate of $R^i_{O2,ref}$, $R^i_{CO2,ref}$, $E_{a,O2}$ and $E_{a,CO2}$ previously obtained from Eqn. 3 and 4 were incorporated into Eqn. 9 and 10. all data gained at all variation of temperature, so that RR can be predicted at any temperature. Data was further analyzed using SPSS software.

CONCLUSION

Temperature is the major functional element in the post harvest period of table grape because of the change on rate of biological reaction such as respiration. Increases in temperature have direct relationship with increase in respiration rate of any fruits. Temperature increases from 5°C to 28°C i.e room temperature. The Arrehenius relationship describes the influence of storage period and temperature on physiological reaction. The findings of this research paper to know the modeling of stored fruits and the importance of maintaining most favorable cold storage condition for grapes as well as the fresh produces along with supply chain. The power function equations combined with Arrehnius type equation predict the influence of time and temperature on freshly produced two grape varieties. And the data further analyzed used in design proper Modified atmosphere packages for fresh grapes.

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Table No.: 1. r^2 Values of respiration rate with different temperature

Storage Temperature° C	Black grapes r^2 Value		White grapes r^2 Value	
5	RO_2 = .499	RCO_2 = .667	RO_2 = 1	RCO_2 = .979
10	RO_2 = .809	RCO_2 = .849	RO_2 = 1	RCO_2 = .885
15	RO_2 = .881	RCO_2 = .887	RO_2 = 1	RCO_2 = .988
28	RO_2 = .959	RCO_2 = .897	RO_2 = 1	RCO_2 = .915

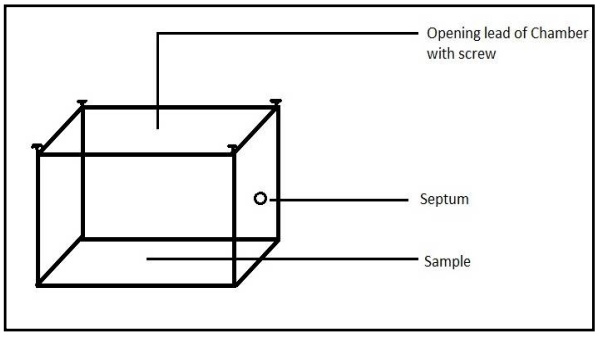

Fig.1 Experimental setup and measurement of respiratory gases

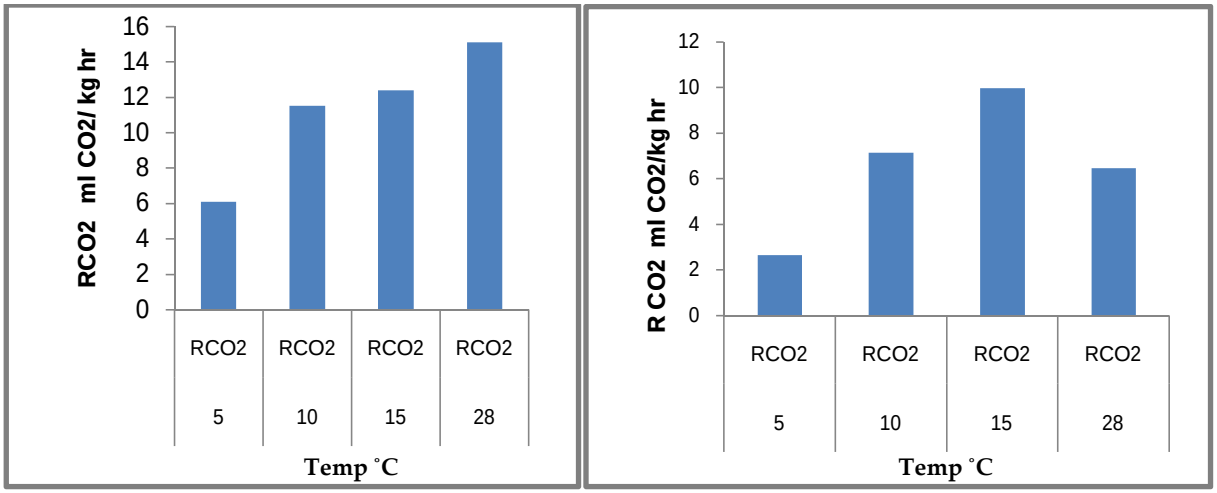

Fig.2.RCO₂ of black Grapes and white grapes at different Temperature

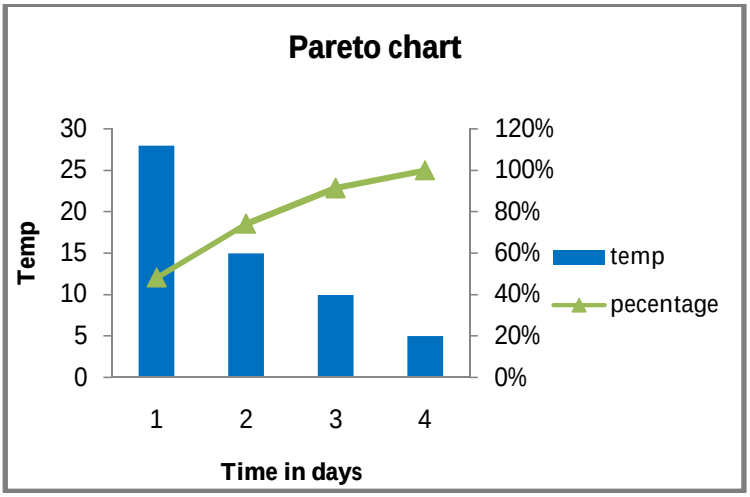

Fig.3.Pareto chart showing the effect of time, temperature (Temp) and their interaction on the respiration rate of grapes at 95 % significance level, indicated as a line

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RESEARCH ARTICLE

Induction of Hepatocelluar Carcinoma using N-Nitrosodiethylamine in Wistar Rats

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ABSTRACT

To study the chemical induction of hepatocellular carcinoma in rats.To establish the rat model of hepatocellular carcinoma.Eighty two male wistar rats were divided randomly into 10 different groups.Group I was Normal Control, Group III was Vehicle Control, VIII was Aqueous extract Control and Group IX was Alcoholic extract Control. Hepatocellular carcinoma was induced in group II, IV, V, VI, VII and X using single intraperitoneal injection of NDEA @ 200mg/kg body weight in saline followed by 0.05% phenobarbital in Distilled Water (DW) for 16 weeks for the promotion. Haematobiochemical parameters, serum alpha2 macroglobulin and ultrasonography was studied in rats after 16 weeks of induction of hepatocellular carcinoma.It is observed that the liver specific enzymes like ALT, AST, ALP, GGT and LDH were significantly increased in the groups where hepatic cancer was induced. Administration of NDEA to rats increased the level of alpha2 macroglobulin in the serum after the completion of promotion period. These changes were supported by the ultrasonographical examination.These findings suggested that NDEA is a potential compound in the induction of hepatocellular carcinoma.

Keywords : Hepatocellular carcinoma, NDEA, Wistar rats.

INTRODUCTION

Hepatocellular carcinoma (Hepatoma)[1] is the incidence of primary liver cancer in liver parenchyma cells or hepatocytes. The liver is known as a favourable site for malignant seed second only to the skin, presumbly because of its rich blood supply [2]. Primary malignant hepatic tumors may arise from any constituent cells of the liver, but the only two common liver cell cancers are hepatocellular carcinoma and carcinoma of the biliary epithelium (cholangiocarcinoma).Hepatocellular carcinoma (HCC) is one of the burning issue among human beings and HCC has been reported in various species of animals including cattle[3], dogs[4], sheep[5], horse[6] and fowl[7]. The most common primary liver tumor in dogs (second most common primary liver tumor in cats) is hepatocellular carcinoma (HCC). Hepatocellular carcinoma (HCC) is the most common hepatic neoplasm in dogs corresponding to more than 50 % of all malignant tumors[8].The hepatic cancer has become the 5th commonest malignancy worldwide and the 3rd leading cause of cancer-related mortality, accounting for 5.6% of all human malignant tumors[9].

In Great Britain, HCC comprises of 20% of all tumours found in slaughtered sheep[10]. There are relatively few reports on bovine hepatocellular carcinoma. Incidence data and age distribution of HCC in cattle is not known because most cases were obtained at the time of slaughter.To understand the underlying mechanism in the process of development of the disease and its progress, it is important to develop the strategy to combat the dreadful disease. The need for the development of various in vivo cancer models has been in demand. Experimental liver cancer in animals is thus developed for studying the progress of the disease scientifically, minutely in vivo and to develop therapeutic and other combating strategies to fight against it.

There are various in vivo animal models already available for the purpose. Generally, virus-induced, radiation-induced, neoplastic cell-transplanted and chemical-induced liver cancer animal models have been widely studied.Manyhepatocarcinogens such as aflatoxins, acetylaminofluorene, diethylnitrosamine (DEN) have been successfully used to develop hepatocarcinogenesis in animals. Diethylnitrosamine is a powerful hepatocarcinogen in rats that were being studied for chronic toxicity.N-nitrosodiethlyamine (NDEA), also known as diethlynitrosamine or DEN, is slightly yellow liquid with a boiling point of 175-177°C and a specific gravity of 0.94. It is soluble in water, ethanol, diethyl ether, and organic solvents. It is a representative chemical of a family of carcinogenic n-nitroso compounds[11]. Diethylnitrosamine (DEN) is a well known liver carcinogen in rats, forming DNA adducts in the liver and inducing hepatocellular carcinoma without cirrhosis through the development of putative preneoplastic enzyme-altered focal lesions.Several biochemical markers have been suggested for biomonitoring the actions of anticancer agents.

Serum α fetoprotein (AFP) is a useful tumour marker for the detection and monitoring of liver cancer development although the false negative rate with AFP level alone may be as high as 40% for patients with small size tumour[12,13]. Recently, α 2-macro-globulin (α 2M), a homotetrameric major acute-phase glycolprotein has been suggested as a novel cytochemical marker characterizing preneoplastic and neoplastic rat liver lesions negative for hitherto established cyto-chemical markers[14].The purpose of the present study is to successfully develop the chemical rat model of hepatocellular carcinoma.

MATERIALS AND METHODS

Animals

The study was conducted on 82 healthy adult male Wistar rats. Healthy adult rats of 6 - 8 weeks of age were procured from Animal Research Facility, Zydus Research Center, Moraiya, Ahemdabad, Gujarat, India and were maintained under standard management conditions. All the protocols as per the Committee for the Purpose of

Control and Supervision of Experiments on Animals (CPCSEA) guidelines on the Care and Use of Laboratory animals were followed. Rats were kept under constant observation during entire period of study.

Housing and Environmental condition

All the rats were housed in polypropylene cages at Laboratory Animal House Facility in an environmentally controlled room with 22 ± 3 ºC temperature and 30-70% humidity. Light/dark cycles of 12/12 hours were maintained throughout the experimental period. All necessary managemental procedures were adopted to keep the rats free from stress.

Chemicals

N- nitrosodiethylamine (NDEA) (Sigma Chemical Company, St. Louis, MO, USA) was used to induce hepatocellular carcinoma . All other chemicals used in the study were of analytical grade.

Induction of Hepatocellular Carcinoma

Hepatocellular carcinoma was induced in Group II, IV, V, VI, VII and X rats by administering single intra peritoneal injection of NDEA at a dose concentration of 200 mg/kg body weight mixed in saline. Two weeks after administration of NDEA, 0.05% of Phenobarbitone was administered by dissolving in drinking water up to 16 weeks to promote hepatocellular carcinoma[15].

Experimental Design

Rats were selected randomly and divided in to 10 groups (Group – I, II, III, IV, V, VI, VII, VIII, IX and X) each group comprised of eight animals except group II which contained 10 animals. All the rats were numbered group wiseand individually. Group I served as normal control consisted of healthy animals. Hepatocellular carcinoma was induced in rats of group II, IV, V, VI, VII and X using*N*-nitrosodiethylamine as inducing agent followed by Phenobarbitone as promoter.

Biochemical Analysis

Serum samples were collected for biochemical analysis. The activities of Alanine aminotransferase (SGPT) (IU/L), Aspartate aminotransferase (SGOT) (IU/L), Alkaline Phosphatase (AKP) (IU/L), γ-Glutamyltransferase (GGT) (IU/L), Lactate dehydrogenase (LDH) (IU/L) were analysed using commercial diagnostic kits procured Coral kits (Coral Clinical Systems, Goa, India) with the help of fully Chemistry analyser (BS-120, Mindray).

Estimation of Alpha 2 Macroglobulin in serum samples

Concentration of Alpha 2-Macroglobulin in serum of all animals was estimated using ELISA kit (Immunology Consultants Laboratory, Inc., Newberg, USA).

Hepatic Ultrasound

An US examination was performed using a multifrequency linear transducer (7.5 to 10 MHz) and esaote MyLab40 VET (Esaote Europe B.V., Philipsweg 1, 6227 AJ Maastricht, The Netherlands) ultrasonography machine at Department of Veterinary Surgery and Radiology, Anand Agricultural University, Anand. All imaging was performed in fundamental brightness mode (B-mode) Two dimensional B mode image plans were acquired with optimization of the gain and the time gain compensation settings, which were kept constant throughout the experiment. The animals were examined in supine position to assess the liver. The liver was assessed by placing the transducer just distal to the last right costal cartilages and angling its beam cranially, obtaining multiple transversal and longitudinal scans. Ultrasonographic findings were analysed to avail qualitative information associated with hepatocellular carcinoma.

Statistical Analysis

The statistical analysis of data generated on various parameters was subjected to statistical analysis using completely randomized design (CRD). All the data have been presented as mean ± SE.

RESULTS AND DISCUSSION

Effect of induction of hepatocellular carcinoma on liver enzymes in different groups of Wistar rats are given in Table 1. The activity of enzymes specific for liver function and the concentration of alpha-2 macroglobulin in serum were in normal range in group I, group III, group VII and group VIII rats.

Alpha-2 Macroglobulin

After 16 weeks of promotion period, alpha-2 macroglobulin concentration was significantly ($P < 0.05$) increased in serum (13.86±1.95ng/ml) of HCC induced animals compared to normal animals (1.25±0.85ng/ml).It is given in Table 2.

Hepatic Ultrasound

On 126th day, the characteristic ultrasonographic changes were observed in liver of rats in which hepatocellular carcinoma was induced. It showed increase in the size of the lobes. Small focal HCC appeared hypoechoic (figure 2) and larger lesions were hyperechoic with heterogenous appearance. It also showed heterogenous liver parenchyma with indistinct (irregular) hepatic border as compared to homogeneous liver parenchyma, with medium level echogenicity and a regular hepatic surface in liver of group I rat (Normal Control) (Figure 1).Cancer is a slow multi-stage, multi-step process which involves the appearance of discrete cell populations at different stages in the process[16].The hepatic cancer has become the 5th commonest malignancy worldwide and the 3rd leading cause of cancer-related mortality, accounting for 5.6% of all human malignant tumors. N-nitrosodiethlyamine (NDEA), also known as diethlynitrosamine or DEN, is a representative chemical of a family of carcinogenic n-nitroso compounds.It forms DNA carcinogen adducts in the liver and induce hepatic cancer[17]. On metabolic biotransformation,NDEA produces promutagenic products, O6 ethyldeoxyguanosine and O4 and O6-ethyldeoxythymidine in liver which are responsible for carcinogenic effects. Phenobarbitone acts as a tumour promoter when administered subsequent to an initiating carcinogen like N-nitrosodiethylamine.

Tissue damage is the sensitive feature in the cancerous conditions. So any deterioration or destruction of the membrane can lead to the leakage of these enzymes from the tissues. Hence, elevation of these liver specific enzymes observed in liver cancer condition may be due to the progression of tumor growth[18] with increased activities of these aminotransferases in plasma and serum of cancer bearing rats. Increased ALP activity in liver cancer condition may be due to cell necrosis, organ dysfunction and cellular injury in tumour bearing rats[19]. Elevated levels of ALP indicates the presence of HCC in group II. Elevation in the levels of GGT in the present study due to the hepatic injury caused by DEN leading to the instability of liver metabolism which in turn leads to distinctive changes in the serum enzyme activities like GGT which is an intracellular enzyme reflects progress of carcinogenesis. Increased activity of this enzyme noted in group II animals indicates the basic tumour burden. LDH is a familiar sensitive marker of solid neoplasm[20] and many studies revealed increased LDH activity in various types of tumor. The discharge of LDH reflects a nonspecific alteration in the plasma membrane integrity and/or permeability.

Tumour markers comprise a wide spectrum of biomacromolecules synthesised in excess concentration by a wide variety of neoplastic cells. Alpha-2 macroglobulin (A2M) is a homotetrameric major acute-phase glycoprotein and novel cytochemical marker characterising preneoplastic and neoplastic rat liver lesions negative for hitherto established cytochemical markers. It is tightly linked to the rat hepatocarcinogenesis from the initial stage to tumour progression even in conditions, which are undetectable, by established cytochemical markers such as placental

glutathione-Stransferase (GST-P) and gamma -glutamyltransferase (gamma - GT) positive lesions. A2M functions as a carrier protein and regulator for various growth factors and cytokines such as transforming growth factor-α (known to be involved in the onset of hepatocyte apoptosis) [21]. Furthermore, A2M partially counteracts the inhibitory effects of transforming growth factor-α on proliferation of neoplastic hepatocytes, suggesting that under some conditions, A2M can promote hepatocarcinogenesis by perturbing transforming growth factor-α-induced apoptosis [22].Increased concentration of this tumour marker in serum indicates the induction or progression of hepatocellular carcinoma.Ultrasound is usually the first imaging modality in the evaluation of liver disease because it is easy to perform, widely available, relatively inexpensive and is cost effective.The findings of present study avouched the findings reported by [23].Larger lesions are heterogeneous due to fibrosis, fatty change, necrosis and calcification ,a peripheral hollow of hypoechogenicity may be seen with focal fatty sparing as explained by [24]which corroborates with the findings of present study. The fibrotic changes seen in many rats may be attributed to necrosis which causes collapse of the parenchymal framework of the liver [25].

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Table: 1. Activity of Liver Specific Enzymes After the Induction Period

Name of the group	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	GGT (IU/L)	LDH (IU/L)
(I)NC	87.82±21.58	157.66±10.75	108.67±25.47	43.11±0.31	148.81±7.16
(II)HCC Control	126.48**±9.70	250.59*±29.34	232.08*±44.12	72.77*±0.72	333.93*±76.21
(III)VC	87.11±4.34	156.89±11.23	109.45±25.67	43.23±0.43	147.34±7.45
(IV)Aq Ext (200)	125.15**±7.05	209.66±41.39	242.48±34.70	71.99±0.39	370.91±72.14
(V)Aq Ext(400)	131.48±8.74	223.60±41.09	271.61±41.72	72.64±0.52	400.54**±66.50
(VI)Al Ext(200)	113.60±8.82	252.77*±34.93	285.85**±34.13	72.49*±0.50	355.53±74.62
(VII)Al Ext(400)	114.00±3.78	238.69±24.08	257.05±30.23	72.53*±0.48	335.34±19.51
(VIII)Aq EC	83.93±8.25	153.29±7.62	109.50±7.77	43.01±0.32	147.93±8.20
(IX)Al EC	89.14±4.45	157.74±12.50	110.84±8.41	42.28±0.30	146.39±9.76
(X)Reference drug	111.41*±8.96	200.48±46.64	218.75±44.47	71.91±0.55	340.15±75.77

* Indicates P <0.05 , ** Indicates P < 0.01

NC- Normal Control, HCC- Hepatocellular, VC-Vehicle Control, Aq Ext (200)- Aqueous extract 200 mg/kg, Aq Ext (400)- Aqueous extract 400 mg/kg, Al Ext (200)- Aqueous extract 200 mg/kg, Al Ext (400)- Aqueous extract 400 mg/kg, Aq EC- Aqueous Extract Control, Al EC- Alcoholic Extract Control.

Table: 2. Activity of Alpha-2 Macroglobulin After the Induction Period

Group	Name of the group	A2M (ng/ml)
I	Normal Control	1.27 ± 0.25
II	HCC Control	11.83±2.01
III	Vehicle Control	1.21±0.45
IV	Aqueous plant Extract-200mg/kg	9.5±0.51
V	Aqueous plant Extract-400mg/kg	9.95±3.35
VI	Alcoholic plant Extract-200mg/kg	9.14±0.61
VII	Alcoholic plant extract -400mg/kg	16.8±1.04
VIII	Aqueous plant Extract Control	1.19±0.56
IX	Alcoholic plant Extract Control	1.20±0.47
X	Reference drug	12.02±0.82

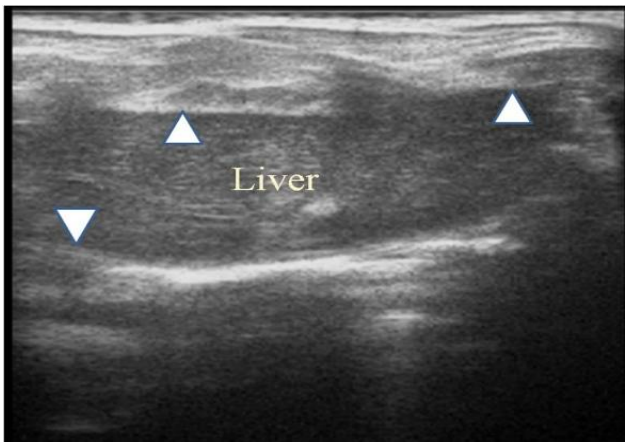

Figure 1: Ultrasonographic image of liver of Group I rat showing homogeneous liver parenchyma, with medium level echogenicity and a regular (straight) hepatic surface (Arrow head)

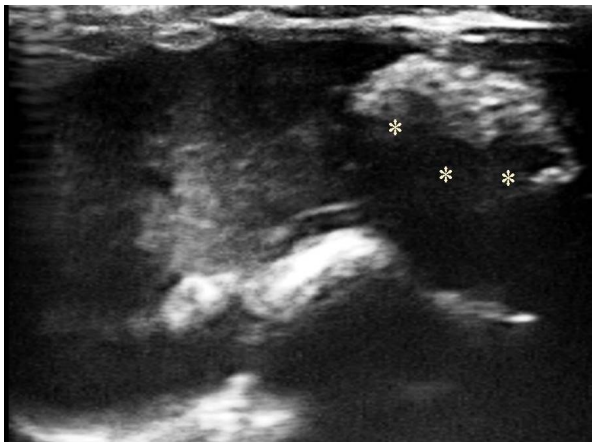

Figure 2: Ultrasonographic image liver of Group II rat showing hypoechoic small focal HCC represented using star

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RESEARCH ARTICLE

Assessment of Effect of Curcumin Supplementation on Sperm Functional Membrane Integrity and Acrosome Integrity of Malabari Buck Spermatozoa using Hos-G Procedure

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ABSTRACT

The study was conducted to determine the effect of curcumin on post thaw functional membrane integrity and acrosome integrity of Malabari buck semen. Curcumin as an antioxidant used in the study to preserve sperms from cryo damages by reactive oxygen species (ROS). The disruption of sperm plasma membrane and acrosome integrity induced by cryopreservation were detected by a combined hypo osmotic spermswelling-giemsastaining procedure (HOS-G). Using artificial vagina, ejaculates from five adult Malabari bucks were collected and evaluated microscopically at 37ºC. Semen samples were pooled after the removal of seminal plasmaand extended with Tris based extender containing 0.5 *mM*and 2.5 *mM* curcumin. Equilibrated the samples were subjected for HOS-G procedure for pre freeze evaluation. Semen samples were cryopreserved and post thaw evaluation for plasma membrane and acrosome integrity. Both concentration of curcumin yielded significantly ($p<0.01$) higher percentage of

pre freeze and post thaw functional membrane integrity than that of control. However, significantly ($p<0.01$) higher post thaw functional membrane integrity was observed with 2.5 *mM*curcumin supplementation. The percentage of spermatozoa having both functional membrane integrity and acrosome integrity were significantly ($p<0.01$) higher in 2.5 *mM* curcumin supplemented group during pre freeze and post thaw stage.Percentage of sperms with intact acrosomes were significantly ($p<0.01$) higher in curcumin supplemented group than that of control whereas no significant difference noted between two concentration of curcumin in both pre freeze and post thaw evaluations.

Keywords : curcumin, cryo damages, HOS-G, functional membrane integrity, acrosome integrity.

INTRODUCTION

In Kerala, majority of goat population belongs to Malabari breed or its crossbreds which plays a significant role in the livestock economy of the state. The breed possess high adaptability to the climatic conditions of the state with good prolificacy and known for its good quality milk and meat.The propagation of its germplasm is essential as, the number of bucks are very scarce due to their slaughter at the young ages. Semen cryopreservation and artificial insemination allows a wide spread distribution of valuable genetic material.However, the low fertility rate limit the use of frozen semenin goat.Hence, an appreciable increase in the recovery of good quality spermatozoa following semen cryopreservation is necessary to achieve reasonable fertility rates (Gacitua and Arav, 2005).During cryopreservation of semen sperms are subjected to various cryo-injuries. The main causes of these cryo-damages are attributed to exposure of spermatozoa to various stressors of osmotic, oxidative and thermal origin. The exposure of spermatozoa to various environmental factors results in cold shock and generation of ROS. The seminal ejaculate is equipped with antioxidants within the sperm cytoplasm and in the seminal plasma to counteract the ROS attack. However, the loss of cytoplasm during the epididymal maturation period and the presence of high amount poly unsaturated fatty acids (PUFA) in the mammalian sperm membranes makes them susceptible to the oxidative damage.The increase in oxidative stress accompanied with reduced endogenous antioxidant defence mechanism can lead to impaired sperm function and fertility.

The addition of exogenous antioxidants with extender will reduce the oxidative damages induced during cryopreservation. Curcumin is a phenolic chain-breaking antioxidant, donating hydrogen atoms from the phenolic groups (Barclay *et al*., 2000). Prior studies on curcumin revealed its pharmacological and biological properties such as anti-tumour, anti-inflammatory, anti-infective and antioxidant activity. Many researchers used curcumin as an antioxidant in the cryopreservation of semen at different concentrations in a wide range of species (Bucak *et al*., 2010; Bucak *et al*., 2012; Soleimanzadeh and saberivand, 2013; Omur and Coyan, 2016).Functional membrane integrity and acrosomal integrity are two essential pre requisites for fertilization which are highly correlated with fertility. Hypo osmotic swelling – Giemsa staining procedure is a combined procedure to assess both the parameters (Selvaraju*et al*., 2008).Hence the present study is envisaged to study the effect of curcumin on post thaw functional membrane integrity and acrosome integrity of Malabari buck semen.

MATERIALS AND METHODS

Animals and semen collection

Five adult healthy Malabari bucks aged between two to five years, maintained at Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, Pookode were used for the study. A total of sixty ejaculates were collected from these bucks twice weekly using Danish type artificial vagina

maintained at 42- 45ºC under adequate pressure. All the collected ejaculates were incubated in a water bath maintained at 37ºC for further evaluation and processing.

Semen processing, freezing and thawing

The volume of ejaculates were measured from the graduated collection vial and sperm concentration was determined using ovine photometer (IMV technologies, France).Sperm motility parameters were recorded using bright field microscope with biotherm facility kept at 37ºC under optimum magnification (10X and 40X).Ejaculates with minimum volume of 0.5ml, progressive sperm motilitymore than 80 per cent and sperm concentration greater than 2500 million/ml were selected for the study. The qualified samples diluted with Tris buffer were centrifuged (700g for 5 minutes) for the removal of seminal plasma. Sperm pellets were washed twice and resuspended in Trisbuffer. Sperm suspensions were pooled to reduce individual variability.

The pooled ejaculate was divided into three aliquots and were diluted with Tris based extender (Tris-3.028g, citric acid-1.675g, fructose-1.25g, egg yolk-5ml, glycerol-6ml, benzyl penicillin- 1000 IU/ml, streptomycin-1000 µg/ml and triple distilled water up to 100ml) containing curcuminat 0.5 mM (C-1) and 2.5 mM (C-2). Tris extender without curcumin was kept as control.The pooled semen samples were extended (400 million sperms/ml) and packed in 0.25 ml French mini straws. The packed semen samples in each groups were equilibrated at 5ºC for 2hr and were subjected to freezing in static liquid nitrogen (LN_2) vapours manually(5 cm above LN_2 level for 10 minutes) and stored in cryocans for a period of 3 weeks.

Assessment of functional membrane integrity and acrosome integrity using HOS-G procedure

HOS-G procedure was carried out to assess the functional membrane and acrosome integrity of spermatozoa as per Selvaraju*et al*. (2008). The osmolarity of hypo osmoticand control solutions were adjusted to 100mOsm/L and 300 mOsm/L,respectively. Hypo osmotic solution and control solution (500µl each) were taken separately into two microcentrifuge tubesand incubated at 37ºC. To each of these solutions, 50µl of pre freeze semen samples were added and incubated for 30min in water bath kept at 37ºC.After incubation, 10µl of buffered formal saline (BFS) was added into both the tubes and mixed properly. Smears were prepared from both the solutions in two different microscopic glass slides and air dried.

The slides were immersed in BFS for 30 min, washed in running tap water and were allowed to dry. The slides were kept in giemsastain (4%) overnight, washed in distilled water and air dried. A minimum of 200 spermatozoa were counted in smears prepared from both hypo osmotic and control solutions under a bright field microscope (1000 magnification). The spermatozoa with hair pin bend at the principal piece of the tailwere considered as hypo osmotic swelling positive (HOS positive) and those with evenly distributed giemsa stain in the acrosome region were considered to have intact acrosome (acrosome positive).
Based on the reaction to HOS-G test, the sperm cells were classified into four subpopulations:

- HOS positive and acrosome positive (HPAP)
- HOS positive and acrosome negative (HPAN)
- HOS negative and acrosome positive (HNAP)
- HOS negative and acrosome negative (HNAN)

The HPAP and HPAN subpopulations were added to calculate HOS positive cells, whereas HPAP and HNAP subpopulations were added together to calculate acrosome positive cells. The percentage of sperms with coiled tail in the control (300 mOsm) was subtracted from the percentage of sperms reactive to the HOS (100 mOsm) to obtain the actual proportion of sperms positive for the hypo osmotic swelling test. The per cent of sperms belong to HP,

HPAP and AP were calculated as per the formula mentioned below.

- HPAP = HPAP (100mOsm) – HPAP (300mOsm)
- HP = HPAP+HPAN (100mOsm) – HPAP+HPAN (300mOsm)
- AP =HPAP(300mOsm) + HNAP (300mOsm)

Statistical analysis

The data were statistically analysed using SPSS (Statistical package for social studies) software version 21st,2010.Percentage data were transformed using Arcsine prior to analysis. Treatment means were compared using one way ANOVA. Wherever the treatments were found to be different, Duncan's multiple range test (DMRT) was used for comparing the treatment means.

RESULTS AND DISCUSSION

The effect of semen extenders with varying concentrations of curcumin on pre-freeze and post-thaw sperm functional membrane integrity and acrosome integrity per cent (Mean ± SE) of Malabari buck spermatozoa are presented in Table.1 and 2, respectively.

Pre-freeze functional membrane integrity and acrosome integrity

The per cent of spermatozoa withpre-freeze functional membrane integrity was significantly ($p<0.01$) higher with 0.5*mM*(group C-1) and 2.5 *mM*(group C-2) curcumin supplementation than that of control, however the values did not vary significantly ($p>0.05$) between C-1 and C-2. The pre freeze HOS-G response was significantly higher ($p<0.01$) with 2.5 *mM* curcumin supplementation than that of control, however there was no significant difference between 0.5 *mM* and 2.5 *mM* curcumin concentrations. Pre freeze acrosome integrity per cent of spermatozoa in curcumin supplemented groupsand control vary nonsignificantly ($P>0.05$).

Post-thaw functional membrane integrity and acrosome integrity

The post thaw HOS and HOS-G positive response of0.5*mM*and2.5 *mM* curcumin supplemented groups (C-1 and C-2, respectively) were significantly ($p<0.01$) higher than that of control. While comparing the two curcumin supplemented groups, C-2 showed significantly ($p<0.01$) higher HOS and HOS-G positive response than that of C-1 group. The acrosomal integrity in C-2 was significantly ($p<0.01$) higher than that of C-1 and control, however no significant ($P>0.05$) difference was observed between C-1 and C-2 group**.**These findings are in agreement with the previous observations by Bucak *et al*. (2012) in bull semen and Omur and Coyan (2016) in ram semen. The postthaw functional membrane integrity integrityobserved with 1*mM*curcumin supplementation was significantly ($p<0.01$) higher than that of 2.0 *mM* concentration and the control(Omur and Coyan, 2016).However in bull semen supplementation of 0.5*mM*curcuminresulted in significantly ($p<0.01$)higher postthaw functional membrane integrity than curcumin 2.0 *mM*and control group. The supplementation of curcumin at different concentration significantly improved the post thaw acrosome integrity in buck and ram spermatozoa (Bucak *et al*., 2010 and Omur and Coyan, 2016). In contrast, in bull semen supplemented with curcumin did not showed any significant reduction in post thaw acrosome abnormalities compared to control (Bucak *et al*., 2012).

Cryopreservation was extensively used for the preservation of the gametes which maintained cell metabolism to a minimum level and thereby prolonged the life of the cells. However, all the procedures associated with freeze-thawing make the cells susceptible to oxidative stress and cryo- injuries. The cryopreservation induced cold shock and osmotic stress will damage the sperm plasma membrane, which in turn accelerates the lipid peroxidation (Yousef *et al.,* 2003).The reduced cytoplasmic antioxidant system and presence of PUFA in the plasma membrane make the spermatozoa more susceptible to lipid peroxidation (Agarwal *et al.*, 2005). Several antioxidants have been

used with varying mechanism of action and results to alleviate the cryo-injuries during cryopreservation with promising results in different species (Tuncer*et al.,* 2010, Khalili*et al.,* 2010;Saraswat*et al*., 2012).The functional membrane integrity of spermatozoa influences sperm metabolism including sperm motility, capacitation and fertilization.

A significant positive correlation was observed between the percentage of spermatozoa with swelling response in HOST and sperm capacitation and also with ovum penetration (Jeyendran*et al.*, 1984). In addition, sperm acrosome integrity is essential for sperm capacitation and fertilization of ova. As the freeze thawing process damages the acrosome, assessment of acrosome integrity is an integral part of sperm quality tests(Watson, 1975).During cryopreservation, the sperm plasma membrane becomes rigid as its normal liquid crystalline nature is transformed to gel state and the junction between the gel fraction and other lipid and protein becomes leaky and ruptures (Hammerstedt*et al.*, 1990). This process of membrane rupture may be get accelerated by the formation of ROS which causes lipid peroxidation of PUFA (Alvarez and Storey, 1984).

The role of curcumin as a chain breaking antioxidant was exploited in many toxicity mitigation studies (Sahoo*et al*., 2008; El-Wakf*et al*., 2011; Snehaand Ramtej, 2014) and for semen cryopreservation in various species (Bucak *et al*., 2010; Bucak *et al*., 2012; Soleimanzadeh and Saberivand, 2013; Omur and Coyan, 2016). The free radical trapping ability of curcumin could protect the sperm plasma membrane from lipid peroxidation (Bucak *et al*., 2010). The protective effect of curcumin on functional membrane integrity could be due to the prevention of oxidative damage of cell membrane through detoxification of free radicals such as hydroxyl, peroxyl and superoxide radicals.The altered membrane state and impaired functioning of ion channels in the acrosomal region induced by cryopreservation facilitates calcium ion influx resulting in cryocapacitation, which reduces the survivability of sperms in the female reproductive tract (Bailey *et al.*, 2000). The plasma membrane protective action of curcumin might have prevented the acrosomal damages during freezing and thawing process.Further studies are required to elucidate the exact protective mechanism of action of curcuminas an antioxidant in various cells particularly for the sperm cryopreservation. In addition, standardization of the concentration of curcumin in various semen extenders for the cryopreservation of spermatozoa from various species is warranted for its use in the field level.

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Table 1. The effect of semen extenders with varying concentrations of curcumin on prefreeze sperm functional membrane integrity and acrosome integrity per cent(Mean ± SE) of Malabari buck spermatozoa

Groups (n=12)	Pre freeze		
	HOS positive#	HOS-G positive	Acrosome positive#
C-1	62.33 ± 1.90^{b}	44.92 ± 1.42^{bc}	82.17 ± 1.89
C-2	65.17 ± 1.22^{ab}	47.71 ± 1.19^{b}	83.17 ± 1.22
Control	61.89 ± 1.40^{c}	41.92 ± 1.26^{c}	81.96 ± 1.41
F-value	9.25**	10.77**	6.51^{ns}
p-value	0.001	0.001	0.001

** Significant at 0.01 level (p<0.01); # Angular transformation was used for analysis;Values with different superscripts within column differ significantly; ns nonsignificant ;C-1 - supplemented with curcumin 2.5 *mM*; C-2 - supplemented with curcumin 0.5 *mM*; HOS-positive- hypoosmotic swelling positive; HOS-G positive- hypoosmotic swelling positive and acrosome positive.

Table 2. The effect of semen extenders with varying concentrations of curcumin on postthaw sperm functional membrane integrity and acrosome integrity per cent(Mean ± SE) of Malabari buck spermatozoa

Groups (n=12)	Post thaw		
	HOS-positive#	HOS-G positive#	Acrosome positive#
C-1	37.58 ± 1.51^{b}	27.17 ± 1.66^{b}	70.00 ± 0.88^{b}
C-2	43.50 ± 1.57^{a}	32.79 ± 1.75^{a}	72.08 ± 0.86^{b}
Control	31.79 ± 1.76^{c}	19.00 ± 1.35^{c}	66.04 ± 0.87^{c}
F-value	16.73**	20.91**	18.17**
p-value	0.001	0.001	0.001

** Significant at 0.01 level (p<0.01); # Angular transformation was used for analysis;Values with different superscripts within column differ significantly;C-1 - supplemented with curcumin 2.5 *mM*; C-2 - supplemented with curcumin 0.5 *mM*; HOS-positive- hypoosmotic swelling positive; HOS-G positive- hypoosmotic swelling positive and acrosome positive.

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RESEARCH ARTICLE

Antibiotic Sensitivity Studies of Intrauterine Pathogens in Postpartum Cows with Subclinical Endometritis

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ABSTRACT

Antibiotic sensitivity of intrauterine pathogens in postpartum cows with subclinical endometritis was studied to assess the efficacies of commonly used intrauterine antibiotic preparations under field conditions. A total of 24 postpartum cows between 60-90 days in milk (DIM), positive for subclinical endometritis as diagnosed by presence of fluidity in uterus (FIU) on transrectal ultrasonography (TRUS) and presence of polymorphonuclear leucocytes (PMNLs) in endometrial cytology were selected. The endometrial samples were cultured under laboratory conditions for the isolation of intrauterine pathogens. After 24 h of bacteriological incubationon Nutrient Agar at 37°C, the growth was identified as either Gram positive or Gram negative. Antibiotic sensitivity test was done by Kirby-Bauer or the disc diffusion method on Mueller-Hinton Agar. Out of the total samples cultured, 75 per cent (18/24) of them were evident for bacterial growth with Gram positive cocci being predominant. The intrauterine pathoegens showed 83.33 per cent sensitivity to Cephapirin Benzathine and Levofloxacin, 100 per cent to sensitivity to Cephalexin, 88.89 per cent to Oxytetracycline. However, there was no statistical significance in the sensitivity of intrauterine pathogens to the intrauterine antibiotic preparations.

Keywords : Subclinical endometritis, intrauterine pathogen/s isolation, antibiotic sensitivity.

INTRODUCTION

Postpartum bacterial complication is a frequent finding in dairy cows. Imbalance between postpartum bacterial complications and uterine self-defence mechanisms leads to uterine infections like puerperal metritis, pyometra, clinical endometritis and subclinical endometritis. (Sheldon *et al*., 2006). Subclinical endometritis is a chronic, inapparent form of uterine infection, without any clinical signs and systemic illness. The uterine environment becomes unsuitable for nourishing the early pre-implantation embryo causing its death and results in repeat breeding. Subclinical endometritis, also referred to as cytological endometritis, is defined as the presence of increased number of polymorphonuclear leucocytes (PMNLs) in the endometrial cytology.Diagnosis of endometritis is based on history, clinical symptoms in the form of uterine changes and nature of discharges and can be confirmed by bacteriological changes and histopathological examinations (Agarwal *et al*., 2005).

A wide variety of mixed bacterial population can be isolated from postpartum uterus.Studies by Sheldon and Dobson (2004),Singla *et al.* (2004), Zilaitis *et al.* (2004) and Drillich (2006) showed that postpartum uterus was contaminated with mixed bacterial population with *Staphylococcus aureus,Escherichia coli*, *Arcanobacterium pyogenes*, *Proteus spp* and other obligate anaerobic organisms being predominant ones.The primary objective of this research work was to study the prevalence of bacterial growth in endometrial samples collected by modified cytobrush technique, isolation of bacterial organism/s and to assess the antibiotic sensitivity of the intrauterine pathogens to the commonly used intrauterine antibiotic preparations used under field conditions.

MATERIALS AND METHODS

Postpartum cows between 60-90 DIM which were clinically normal, and without any postpartum complications, were randomly selected and screened for subclinical endometritis by transrectal ultrasonography (TRUS), modified cytobrush technique and bacterial culture. On TRUS, presence of fluidity in uterus (FIU) was assessed. Endometrial cytology was used to determine the per cent of polymorphonuclear leucocytes (PMNLs) by modified cytobrush technique. The endometrial sample collected using cytobrush, after preparation of endometrial cytological slides were transferred aseptically to a sterile screw capped vial containing nutrient broth (HiMedia) and was transferred to the microbiology laboratory in a cold chain within 2 h of sample collection for bacteriological culture and antibiotic sensitivity test (ABST).

Harvesting of endometrial samples

Endometrial samples were collected using modified cytobrush technique. Sterile human endocervical brush (SteriUNO ®) was modified for use in bovines to harvest the endometrial samples. The assembly was prepared under strict aseptic conditions in a sterile laminar air-flow.Cows were properly restrained in a trevice, the perineum and vulva was cleaned and wiped with paper towel. Per-rectally, by gloved lubricated hand, the cervix was manipulated and the modified cytobrush assembly was introduced into the uterus. The endometrial sample was harvested by gently rotating the cytobrush on the endometrium. After collection, the AI sheath was drawn forward to avoid vaginal contamination.The endometrial slides were prepared by rolling the cytobrush onto sterile microscopic slides and immediately transferred the cytobrush to a sterile screw capped vial containing sterile nutrient broth and was then transported to the Department of Veterinary Microbiology within 2 h of sample collection in a cold chain.

Microbiological evaluation

Microbiological culture for isolation of organism

The cytobrush, after sample collection was immersed in sterile nutrient broth kept in sterile screw capped vial for microbiological culture. The sample was processed within 2 h of collection. Under aseptic conditions, the cytobrush

was streaked onto Brain Heart Infusion Agar (BHIA) (HiMedia) plate and incubated for 24-48 h at 37°C for isolation of bacteria. After the incubation period, bacterial colonies were stained using Gram's staining procedure for characterization of organism/s. The growth was identified as either Gram positive or Gram negative.

Antibiotic Sensitivity Test (ABST)

Sensitivity to antibiotics was done by Kirby-Bauer method or the disc diffusion method in Mueller-Hinton Agar (MHA). The antibiotics used were Oxytetracycline 30 µg (HiMedia), Levofloxacin 5 µg (HiMedia), Cephalexin 30 µg (HiMedia) and Cephapirin Benzathine 30 µg. Since antibiotic sensitivity disc for Cephapirin Benzathine was not commercially available, the antibiotic sensitivity disc for the same was prepared under laboratory conditions by infusion of the drug preparation into sterile antibiotic disc (HiMedia).

Preparation of antibiotic sensitivity disc for Cephapirin Benzathine

The trade preparation of Cephapirin Benzathine (METRICEF ®) is a 19g single use intrauterine infusion syringe containing 500 mg Cephapirin (as benzathine). Cephapirin Benzathine is a first generation cephalosporin group of antibiotic. Cephapirin was diluted with sterile distilled water so as to contain a concentration of 30 µg per 20 µL of the diluted drug. 20 µL of diluted drug was infused into sterile antibiotic disc for preparing the antibiotic sensitivity disc of Cephapirin Benzathine.

Procedure for Antibiotic Sensitivity Test

The bacterial colony/ colonies obtained on the BHIA agar plate were streaked using sterile rod and transferred into sterile nutrient broth. The nutrient broth was then streaked onto Mueller-Hinton Agar plate and antibiotic discs were placed. The culture plates were incubated for 24-48 h.

RESULTS AND DISCUSSION

Microbial culture for isolation of uterine pathogens

The endometrial samples collected using modified cytobrush were subjected to microbial culture on day one on Brain Heart Infusion Agar (BHIA) for isolation of bacteria. After an incubation period of 24-48 h at 37°C, there was bacterial growth in 75 per cent (18/24) of the samples. While no growth could be detected for six (6/24, 25 per cent) samples.Gram positive cocci were detected in nine (9/18, 50 per cent) samples. Gram negative bacilli were present in five (5/18, 27.78 per cent) samples and four (4/18, 22.22 per cent) samples showed the presence of both Gram positive and Gram negative bacilli.

Antibiotic sensitivity of uterine pathogens

The antibiotic sensitivity of uterine pathogens to antibiotics was measured as diameter in millimeter indicating Zone of Inhibition exhibited by the antibiotic discs on Mueller-Hinton Agar. Standard reference for the range indicating the sensitivity of the organism/s as sensitive, intermediate or resistant was taken (HiMedia). For cephalexin, bacterial growth showing equal to or less than 14 mm was taken as resistant, oxytetracycline (18 mm) and levofloxacin (15 mm). For cephapirin benzathine, the standard range was taken as 16 mm (Wiesner *et al.,* 1972).Out of 18 samples cultured, three samples were resistant to cephapirin benzathine, two samples were resistant to oxytetracycline and three samples were resistant to levofloxacin. While all the cultures were sensitive to cephalexin.The bacterial organism/s showed a sensitivity of 100 per cent to cephalexin, 88.89 per cent sensitivity to oxytetracycline and 83.33 per cent to cephapirin and levofloxacin.Statistical analysis revealed that there is no statistical difference between the different intrauterine antibiotics used in the study in the sensitivity parameter (p-value > 0.05).The findings of the present study with special reference to cephapirin benzathine was in agreement

with the findings of Malinowski *et al.* (2011) who reported that *A. pyogenes* showed 77.5 per cent sensitivity to cephapirin.

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Table 1. Microbial culture for isolation of uterine pathogens

No. of endometrial samples cultured	Positive for growth	Gram positive cocci	Gram negative bacilli	Gram positive and Gram negative bacilli	No growth
24	18/24 (75 %)	9/18 (50 %)	5/18 (27.78 %)	4/18 (22.22 %)	6/24 (25 %)

Table 2. Antibiotic Sensitivity of intrauterine pathogens

Animal no.	Cephapirin (30 µg)	Cephalexin (30 µg)	Oxytetracycline (30 µg)	Levofloxacin (5 µg)	P - value
C2	12 mm	19 mm	13 mm	14 mm	
C3	11 mm	15 mm	21 mm	27 mm	
C11	> 40 mm	38 mm	23 mm	31 mm	
C17	-	-	-	-	
C21	> 40 mm	34 mm	29 mm	27 mm	
C22	> 40 mm	34 mm	23 mm	22 mm	

C1	-	-	-	-	
C5	36 mm	37 mm	27 mm	24 mm	
C6	-	-	-	-	
C13	38 mm	31 mm	27 mm	29 mm	
C19	> 40 mm	31 mm	27 mm	29 mm	
C24	38 mm	36 mm	26 mm	35 mm	
C8	24 mm	21 mm	35 mm	13 mm	
C10	23 mm	23 mm	23 mm	21 mm	
C12	-	-	-	-	
C14	40 mm	38 mm	31 mm	37 mm	
C16	-	-	-	-	
C23	29 mm	23 mm	6 mm	15 mm	
C4	31 mm	29 mm	20 mm	27 mm	
C7	-	-	-	-	
C9	33 mm	30 mm	23 mm	21 mm	
C15	10 mm	15 mm	23 mm	33 mm	
C18	23 mm	28 mm	23 mm	29 mm	
C20	37 mm	30 mm	29 mm	28 mm	
No. of animals resistant	3	0	2	3	
% sensitivity	83.33	100	88.89	83.33	0.642^{ns}

P-value = 0.642^{ns}, ns - Non significant; (mm: Diameter in millimeter indicating Zone of Inhibition exhibited by the antibiotic discs on Mueller-Hinton Agar.

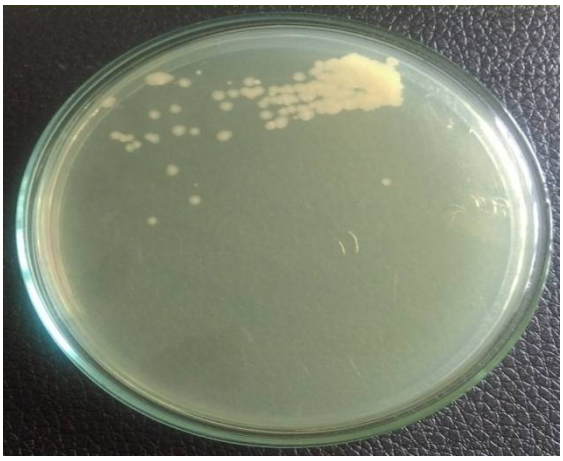

Fig. 1 Bacterial growth on Nutrient Agar after 24 h incubation at 37˚C

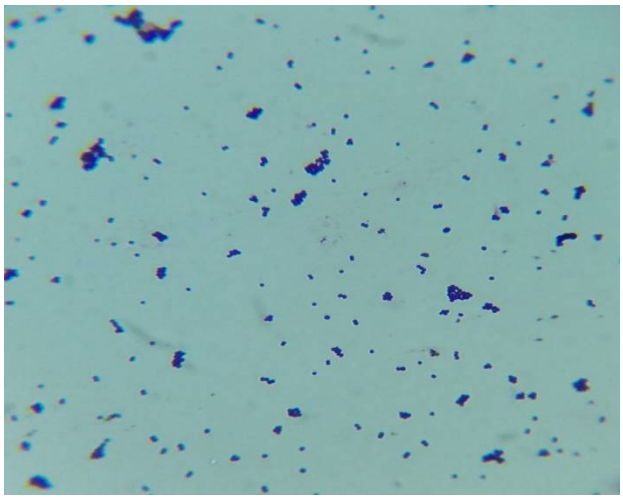

Fig. 2 Gram staining - Presence of Gram positive cocci

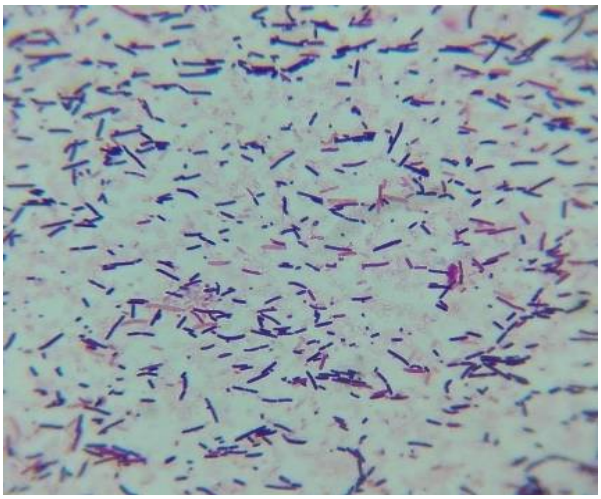

Fig. 3 Gram staining - Presence of Gram positive and Gram negative bacilli

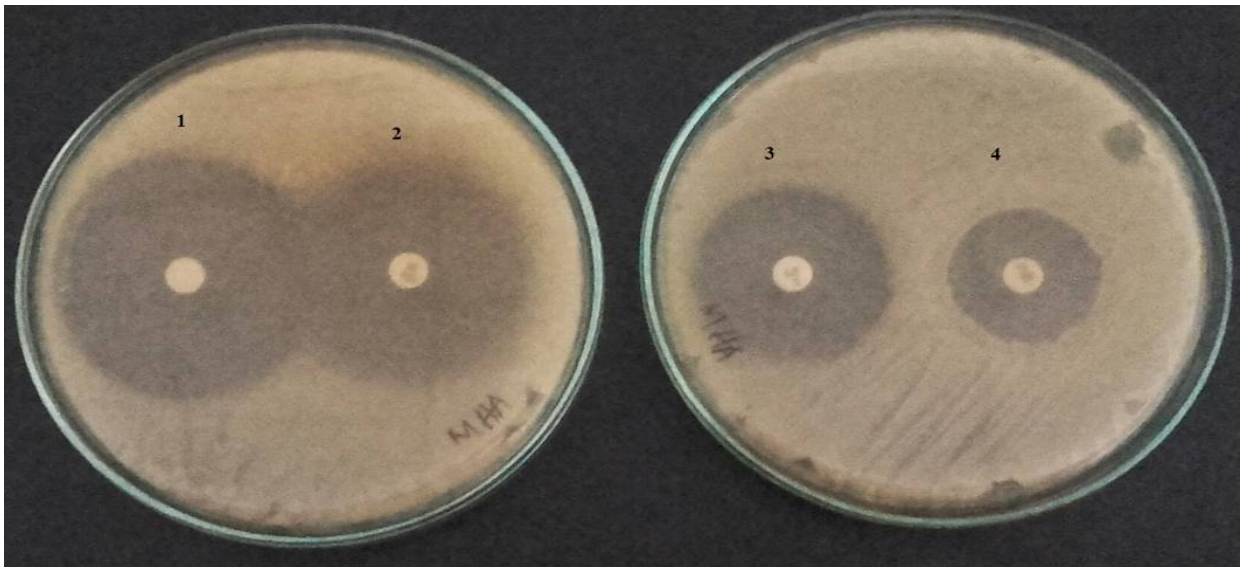

Fig. 4 Results of antibiotic sensitivity test (ABST) on Mueller-Hinton Agar

1. Cephapirin 30 µg
2. Cephalexin 30 µg
3. Levofloxacin 5 µg
4. Oxytetracycline 30 µg

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RESEARCH ARTICLE

Fresh Semen Characteristics of Vechur Bull Spermatozoa

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ABSTRACT

The Vechur breed of cattle is a native breed of Kerala. It is the only recogniseddwarf breed from the state. The study was carried out using a total of 18 ejaculates, collected from three adult Vechur bulls maintained at the Vechur farm, College of Veterinary and Animal Sciences, Mannuthy. The semen ejaculates were assessed for ejaculate volume, pH, concentration, sperm progressive forward motility, sperm viability, sperm abnormalities, sperm acrosome integrity and sperm functional membrane integrity in this study.

Keywords : Vechur bull, semen characteristics.

INTRODUCTION

Vechur is a recognised indigenous dwarf breed of cattle named after the Vechur village of Kottayam district, Kerala. The current estimated population of the Vechur breed is 500-600 animals (Anilkumar, 2016). Vechur cattle have gained popularity among farmers due to their ability to withstand the sub-tropic climate of the region, high disease resistance and ability to utilize locally available low quality fodder as roughage. Very little is known about the fresh semen characteristics of this breed. Hence, this study was planned to study the fresh semen characteristics of Vechur cattle which would provide valuable data required to plan further conservation efforts by germplasm cryopreservation.

MATERIALS AND METHODS

Three adult healthy Vechur bulls maintained at the Vechur farm, College of Veterinary and Animal Sciences, Mannuthy,aged between four to six years and with a history of optimum fertility were selected for the study. Intact adult Vechur bulls were used as teasers for semen collection.The bulls were stewed twice by allowing to mount on teaser bulls before semen collection.The semen ejaculates were collected using a Danish type Artificial Vagina (AV) maintained at a temperature of 42°C- 44°C with optimum pressure. Semen was collected at a frequency one collection per bull per day, twice a week, at an interval of two to three days between collections. Immediately after collection, the semen was transferred to a water bath maintained at 37°C. A total of 18 ejaculates from three Vechur bulls (six ejaculates from each bull) were used for the entire study.

The volume of the semen was assessed by direct visualization of the graduated collection vial. The pH of the semen was identified using a narrow range pH paper (Himedia®,India).The concentration of the semen was determined using a Bovine photometer (IMV® Technologies, France). The concentration was expressed in millions/ml. Initial progressive forward motility of the collected semen was assessed under 400 × magnification afterextending 10 µl of fresh semen with 0.9 ml of 2.96 per cent sodium citrate solution.Sperm viability and sperm abnormalities were assessed using eosin- nigrosin staining technique as described by Campbell*et al.* (1953). Sperm acrosome integrity and functional membrane integrity was assessed using the hyposmoticsperm swelling-Giemsa(HOS-G) test as described bySelvaraju*et al.* (2008).Statistical analysis was conducted using Statistical Package for Social Studies (IBM SPSS Statistics 21®, USA).

RESULTS AND DISCUSSION

In the present study, the volume of fresh semen obtained from Vechur bulls was 2.36±0.21 ml (Table 1). Venkatachalapathy*et al.* (2004) had reported Vechur bull semen volume of 2.9±0.3 ml. However, the semen volume recorded in Vechur bulls was much lower than that of other indigenous breeds such as Nellore bulls (4.8± 0.1 ml, Koivisto*et al.,* 2009) and in Kankrejbulls (4.84 ± 0.01 ml, Patel and Siddiquee, 2013). The size of the bull and testicular size influences the volume of ejaculate (Sane *et al.,* 1994). It was also reported that the volume of *Bosindicus*semen ejaculates is less than that of *Bostaurus*(Koivisto*et al.,* 2009). Another important factor which could have led to the lower semen ejaculate volume was the period of study, which was, from March to June in the Thrissur district of Kerala, the temperature and humidity are high. Ghasemi and Ghorbani, (2014), in their studies carried out at Iran,had found that season of collection had an impact on the sperm volume.The pH of Vechur bull semen ejaculates was found to be 6.87±0.02 (Table 1). Venkatachalapathy*et al.* (2004) had observed a pH of 6.72 ±0.05for the Vechur bullsemen. Kankrej bull semen was found to be having a pH of 6.88± 0.01 (Patel and Siddiquee, 2013).

The sperm concentration in Vechur bull semen ejaculates was found to be 653.78±70.09 million/ml (Table 1) which was found to be lower than the sperm concentration reported earlier (1390±85.2 million per ml) by Venkatachalapathy*et al.* (2004) in Vechur bulls. In addition, the sperm concentration obtained in this study was lower

than that reported for Kankrej bulls (Patel and Siddiquee, 2013) and zebu crossbred bulls (Rahman *et al.,* 2014). The normal range of sperm concentration in bull semen was reported to be between 600-2800 million/ml (Parkinson, 2001) and the observations of the present study lies within this normal range towards its lower limit.The sperm progressive motility was found to be 85.83±0.93, which was found to be higher than the reported fresh semen motility per cent in the same breed(73.42 ± 5.40, Venkatachalapathy*et al.,*2004). The viability of spermatozoa in the fresh semen ejaculate was found to be 90.75±0.35 per cent (Table 1) which was considerably higher than that reported in other breeds of bull semen. The observation of Venkatachalapathy*et al.* (2004) with respect to the viability of spermatozoa in fresh Vechur bull semen ejaculate was lower than that of the present study(85.15± 2.6 per cent). The fresh semen sperm viability per cent of Karan Fries bull (82.75±2.29, Wodajo *et al.,* 2003)was lower than that of Vechur sperm viability.

The total sperm abnormality in fresh Vechur bull semen was found to be 9.33±0.68per cent (Table 1). The observation was found to be lower than the sperm abnormality per cent reported (12.16 per cent) in crossbred bull semen (Mathur*et al.,* 1991). In the present study, the value obtained was within the normal range described for the other known breeds of bulls.The functional membrane integrity was assessed using the HOS-G and the average HOS positive spermatozoa per cent in fresh semen samples was found to be 85.97±1.11 per cent (Table 1) and this is comparable with the observations of Uysal *et al.* (2007) in Holstein bull fresh semen. However, the results of this study were found to be higher than that of the earlier works in Karan Fries bulls (81.086±0.823per cent, Wodajo *et al*., 2003and; 79.75±2.18per cent, Das *et al.* 2003). The acrosome integrity of the fresh semen ejaculates,as stained with Giemsa stain, was found to be 95.58±0.35 per cent (Table 1). The observations were found to be higher than those in Karan Fries bulls (69.17±0.328 per cent, Wodajo *et al.,* 2003) and Kankrej bulls (75.13±0.42 per cent, Patel and Siddiquee, 2013).

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Table 1: Fresh semen characteristics of Vechur bull spermatozoa (Range, Mean ± SE)

Semen characteristics	**Range (n=18)**	**Mean ± SE (n=18)**
Volume (ml)	1-5	2.36±0.21
pH	6.7-6.9	6.87±0.02
Motility (%)	80-90	85.83±0.93
Concentration (million/ml)	322-1320	653.78±70.09
Viability (%)	82.50 - 96.50	90.75±1.09
Abnormality (%)	4-14	9.33±0.68
Functional membrane integrity (%)	76.00-92.50	85.97±1.11
Acrosome integrity (%)	92.50-97.50	95.58±0.35

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RESEARCH ARTICLE

Effect of Complete Feed with Tween 80 on Weight Gain and Fibre Digestibility on Dairy Calves

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ABSTRACT

The experiment was conducted to assess the effect of addition of non-ionic surfactant Tween 80 on weight gain and fibre digestibility in dairy calves fed on complete diets. Twelve female dairy calves of six to nine months of age selected from Instructional Livestock Farm Complex, Pookode Theywere randomly divided into two groups G_1 and G_2 as uniformly as possible with respect to body weight. The animals in the group G_1 was fed on a complete ration with 40 percent Neutral Detergent Fibre (NDF) while those in group G_2 were fed on the same complete ration as in G_1 with 0.1 percent Tween 80. The duration of feeding trial was for 90 days. A digestibility trial of five days was conducted at the end of feeding trial. The treatment group of animals (G_2) showed better weight gain ($P<0.05$) than G_1 animals. The crude fibre (CF), NDF and Acid detergent fibre (ADF) digestibilities were higher in G_2 than G_1 animals. . Better fibre digestibility observed in G_2 may be due to the increased release of cell bound microbial enzymes.

Keywords : Non-ionic surfactant, complete ration, feeding trial, digestibility trial.

INTRODUCTION

Availability and affordability of feed are the two major factors affecting the profitability of any dairy enterprise. Lack of cultivable land, scarcity of fodder, unavailability of area for cultivation are major threats to dairy sector in the state.Densification of fodder helps to reduce the transportation and handling cost and thereby reduces the feeding cost. Non-ionic surfactants alone or in combination with digestion enhancing agents are added generally in ruminant feed and can have growth promoting effect (Shelford*et al.,* 2001).Surfactants are surface acting agents. They can be defined as molecules capable for associating to form micelles (Annual Report on Progresses in Chemistry. 2003). In ruminants that are fed with forage based diets, surfactants are used as promising feed additives (Singh and Kewalramani, 2009a).

Increased dry matter disappearance was observed at an application level of 1 % of Tween 80 compared to lower application levels (Lee *et al.* 2007). Various studies have shown that Tween 80 improves fibre digestion. The exact mechanism by which surfactants improve fibre digestibility is still unknown. Non-ionic surfactant Tween 80 is commonly used and incorporated in feed. The present study was conducted with aims to evaluate the effect of feeding complete ration with Tween 80 on weight gain and fibre digestibility of dairy calves.

MATERIALS AND METHODS

Twelve healthy female crossbred dairy calves of age six to nine months were selected from calf shed, Instructional Livestock Farm Complex (ILFC), Pookode. These animals were grouped into control and treatment groups based on body weight and age. The animals were given individual pens to facilitate individual feeding and watering.. Ten days of adjustment period was given prior to feed trial to the calves to get accustomed with individual feeding and watering.Complete feed which met all nutrient requirements of calves as per ICAR 2013 standards was formulated (Table 1). The feed was manufactured in Kerala Feeds Muthalamada, Palakkad. Tween 80 feed grade manufactured by SRL was purchased from Chemind chemicals, Calicut. Bottle containing liquid form of Tween 80 of 500 gram was purchased. It was added at regular intervals into feed mixer during feed mixing to ensure thorough mixing and blending. The Tween 80 was mixed at a level of 0.1 per cent with feed.

The animals were weighed on fortnightly basis. Feed and feed residue samples were collected on daily basis to analyse dry matter intake of calves. A digestibility trial of five days was conducted towards the end of the trial by total collection method. Each animal in separate cage was assigned a bucket for collection of dung voided by it. Dung voided by each animal was collected at the time of voiding and was collected in respective buckets. Amount of feed offered, amount of feed left and amount of dung voided by each animal was recorded. Feed sample, feed residue and dung was collected on 24 hour basis. Samples of complete feed, residue and faeces were analysed for proximate analysis as per the methods of Association of Official Analytical Chemist (AOAC, 2012).

RESULTS AND DISCUSSION

Fortnightly average bodyweight of calves are presented in Table 2. The average bodyweight of Group I and Group II at the beginning of the experiment were 70.37 and 70.22 kg respectively.Significant increase ($P<0.05$) in weight gain was observed in group II in comparison to Group I from the third to seventh fortnight of trial. The average bodyweight of Group I and Group II were 114.35 and 135.50 kg, respectively at the end of the experiment.A significant increase in bodyweight gain was observed from third fortnight of the trial shown in Figure 1. The results are in accordance with the study conducted by Singh and Kewalramani (2010) in fourteen crossbred male calves where the treatment group of animals fed with Tween 80 (0.1 %) had shown significantly higher total body weight gain and average daily gain on comparison with the control group. Similar effect in average daily gain and total

weight gain were observed by Shelford and Kamande (2001) when feed lot cattle were fed with a total mixed ration with Tween 80 added to it at a level of 0.1 percent. Results of this study has shown that supplementation of Tween 80 with total mixed ration at a level of 0.1 percent led to better weight gain in crossbred dairy calves.

Digestibility coefficient of fibre fractions in calves fed on the respective experimental rations (%, on dry matter basis) is shown in Table 3. The crude fibre digestibility was significantly (P<0.05) higher in Group II than Group I, the values in groups I and II being 55.36 and 61.27 per cent, respectively. The NDF digestibility was significantly (P<0.05) higher in Group II than Group I, the values being 54.41 per cent in Group I and 59.26 per cent in Group II. The acid detergent fibre (ADF) digestibility was also significantly (P<0.05) higher in Group II than Group I, the values being 45.48 percent in Group I and 49.72 percent in Group II.Singh and Kewalramani (2010) conducted a study in fourteen crossbred male calves where the treatment group of animals fed with Tween 80 (0.1 %) has shown significantly higher digestibility of crude fibre, neutral detergent fibre, acid detergent fibre and cellulose. Singh and Kewalramani (2009b) observed enhanced fibre digestion due to effective utilization of fibre fractions. Addition of Tween 80 resulted in increased activity of the fibre degrading enzymes carboxymethylcellulase, ß glucosidase, cellobiohydrolase and xylanase. Kim *et al.* (2004) observed that in Hanwoo steers, increased levels of supplementation of Tween 80 led to better digestibility of crude fibre fraction.

Thus it may be concluded from the above study that feeding a complete feed with 0.1 per cent Tween 80 added to it can result in better bodyweight gain and better digestibility of fibre fractions. Increased release of cell bound microbial enzymes can be a reason behind the better digestibility of fibre fractions.

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Table 1: Composition of complete feed

Ingredients	Inclusion level (kg)
Maize	6
De oiled rice bran	5
Coconut cake (de oiled)	14
Straw	40
Molasses	8
MinMix	0.5

Calcite	2
Salt	1
Rape seed meal	10
Vegetable oil	3.5
Cotton seed cake	10
Total	**100**

Table 2. Fortnightly bodyweight of calves (kg)

Fortnight	Body weight		SEM	P value
	G. I	G. II		
Day 1	70.37	70.22	10.37	0.989
1st	77.18	82.19	13.39	0.082
2nd	86.32	91.48	15.97	0.058
3rd *	90.97a	100.18b	16.39	0.009
4th *	99.76a	110.10b	18.64	0.006
5th *	106.86a	119.22b	19.81	0.006
6th *	110.26a	127.82b	19.98	0.008
7th *	114.35a	135.50b	22.23	0.002

*Means bearing different superscripts in a row differ significantly (P< 0.05)

Table 3. Digestibility coefficient of fibre fractionsin calves fed on the respective experimental rations (%, on dry matter basis)

Attribute	Group		SEM	P value
	I	II		
CF*	55.36a	61.27b	3.86	0.049
NDF*	54.41a	59.26b	1.46	0.029
ADF*	45.48a	49.72b	0.15	0.049

*Means bearing different superscripts in a row differ significantly (P< 0.05)

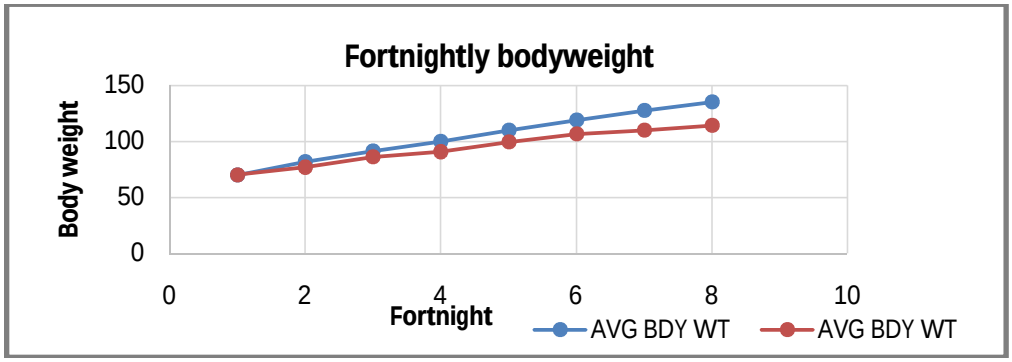

Figure 1. Fortnight weight gain in calves

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RESEARCH ARTICLE

Effect of Temperature and Salinity on Survival and Nauplii Production Rate of *Parastenhelia* sp. (Copepoda: Harpacticoida) – An Optimization Study

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ABSTRACT

In this study, the effect of salinity (24±1to 40±1PSU) and temperature (18±1 to 38±1^{o}C) on survival and nauplii production rate of a marine harpacticoid, *Parastenhelia* sp. in a laboratory condition was investigated. The species was first obtained from Point Calimere situated at the low headland on the Coromandal coast, South India along the Palk Strait where it meets the Bay of Bengal at the southeastern tip of Nagapattinam District, Tamil Nadu. The vegetation of the estuary comprises: tropical dry evergreen forest; mangrove forest (estuary, lagoon) and seashore vegetation. It has been cultured in the laboratory condition. The findings show that there is a significant ($p< 0.05$) detected between the salinity treatement and it shows that difference in salinities give different effects on the survival and nauplii production rate of the harpacticoids cultured in the different temperature. Harpacticoids reared in the 32 PSU show the highest survival (80%) and nauplii production rate (54.33±0.41 nauplii female^{-1} day^{-1}) followed by 36 PSU while the highest survival rate (80 %) and nauplii production rate (59.33±0.77 nauplii female^{-1} day^{-1}) were obtained at temperature 28^{o}C. From this experiment, it can be concluded that 32 PSU

and temperature 28°C is the optimum condition for the mass culture of a tropical copepod *Parastenhelia* sp. in the laboratory condition.

Keywords : *Parastenhelia* sp.; nauplii production rate; survival rate; salinity; temperature.

INTRODUCTION

Copepods are the most plentiful zooplankton in the oceans and constitute a trophodynamic link between primary and tertiary production, copepods act as fundamental step in the ecological recycling and energy in marine environment (Camus and Thomas, 2012). They serve as larval diets for fish, providing them with better survival, growth and improve the stress resistance of cultured larvae better than commonly use live prey (Camus and Zeng, 2012; Santhanam et al., 2013). Marine harpacticoids are usually resistant to different environment conditions but they got optimum temperature and salinity, and these will be species and strain dependent (Zaleha and Jamaludin, 2010). The study of the effects of environmental factors on the physiology of organisms is essential to determine the tolerance limits and to assert the most suitable conditions for the optimal energy balances of the individuals (Matias-Peralta et al., 2005).

Harpacticoid copepods can synthesize several nutritionally important essential fatty acids, making them desirable as food for rearing marine fish. Harpacticoids are generally tolerant of wide environmental conditions and they are better food for fish larvae than *Artemia* and rotifer because of their power to synthesize highly unsaturated fatty acids which are very essential for normal growth and survival. Moreover, the harpacticoid copepods are energetically poorer but appear to have an appetite stimulatory effect (Koedijk et al., 2010a). Harpacticoid copepods maintain tank hygiene (Cutts, 2003). Because of their small size and natural abundance they are a favorite prey for a vast assemblage of marine fish larvae (Stringer et al., 2012). Among tropical copepods with high potential for aquaculture hatcheries, *Parastenhelia* sp., is debatably the best suited candidate for tropic larval fish rearing (Camus and Thomas, 2012). More research is consequently required to optimize their culture techniques to fully understand their potential. Hence, through with a series of laboratory experiments the present study analyzed the optimum temperature and salinity for the intensive cultivation of *Parastenhelia* sp.

MATERIALS AND METHODS

Copepods collection and identification

Zooplankton samples were collected from the Point Calimere coastal waters (Lat. 10°15.21'N; Long. 78°48.50'E), Southeast coast of India, during early in the morning by using plankton net with 158µm mesh by horizontal towing according to Santhanam et al. (2015). The collected samples were immediately transported to the laboratory provided with sufficient aeration using a battery aerator. At laboratory, the samples were thoroughly rinsed to reduce the contamination from other organisms. Grading accomplished by using a set of superimposed sieves with varying mesh sizes from 500µm mesh to remove fish and prawn larvae to 190µm to remove rotifers and nauplii of copepods. After grading, individual copepod species were isolated using fine brush and needle under a stereo zoom microscope. After isolation, both sexes of copepod individuals will be identified using the morphological characters by referring the standard books and manuals (Davis, 1955, and Wells et al., 2012). For morphological identification the individuals was preserved with 5% formaldehyde. The stock culture of *Parastenhelia* sp. was maintained in the laboratory using the method of Santhanam et al. (2015). 25 individuals of female *Parastenhelia* sp. was isolated and stocked initially in 250 ml beaker filled with filtered seawater and *Isochrysis galbana* was given as feed twice per day at a wet concentration of 0.00018 mg/L.

Experimental design

For survival and fecundity bioassays Santhanam and Perumal (2012 a & b) method was adopted. In brief, for a 12-days survival experiment, 10 adults of *Parastenhelia* sp. were placed into new 50 ml beakers filled with filtered water with the corresponding water salinity (eg. 24±1, 28±1, 32±1, 36±1 and 40±1 PSU) for preparing the different salinity, the natural seawater diluted with filtered fresh water to reduce the salinity or heated the seawater to increase the salinity and incubated at five temperatures (eg. 18±1, 23±1, 28±1, 33±1 and 38±1°C) for maintaining the different temperature levels, the samples were kept in different air conditionered rooms and fed with *I. galbana* and provided with 12L: 12D light: dark cycle. The water in the beakers was exchanged every two days. During the experiment the copepods were fed with *I. galbana* once per day at 0.00018 mg/L. The dead organisms and fecal pellets were removed with a pipette. Survival of the animals was determined under a stereo microscope from the start of the experiment. The salinity was checked with a portable refractometer and the temperature was measured everyday using a mercury thermometer.

For nauplii production rate (NPR) experiment individual female *Parastenhelia* sp. carrying first egg sacs was placed in 20 ml glass vial filled with filtered seawater. All the experimental vials were placed in dark, but were exposed to light for 3 to 4 hours every day during which period they were examined. Every day the copepods were checked to ascertain the time of their nauplii release and the culture media were changed every second days. Once the female copepod released its nauplii it was transferred to a new 20 ml vial filled with a fresh culture medium. The nauplii released from the eggs were fixed by adding a drop of 5% formalin and counted under a dissecting microscope.

Statistical analysis

Data collected were all analyzed using one way analysis of variance (ANOVA). Turkey's comparison test was used to test for significant differences within the different salinity and temperature levels. The statistical analysis was performed using a Graph Pad Prism Version 6.

RESULTS AND DISCUSSION

Effects of salinity on survival and NPR of *Parastenhelia* sp.

The recorded mean survival was above 50% at all tested salinities, although maximum survival rate was found at 32 PSU salinity with final survival of 80% after 12 days (Fig. 1a). Though salinity had considerable effect on survival, maximum negative effects were observed at lower salinities. Lowest survival close to 20% was observed for copepod maintained at 24 PSU salinity.

Salinity significantly influences the nauplii production rate of *Parastenhelia* sp. The salinity tolerance of *Parastenhelia* sp. seemed to be 24-40 PSU (*P<0.05)* (Fig. 1b). NPR was found to be highest (54±10.01 nauplii female^{-1} day^{-1}) when the salinity was maintained at 32 PSU followed by 28 PSU (44±3.21 nauplii female^{-1} day^{-1}). The lowest nauplii production (16±2.08 nauplii female^{-1} day^{-1}) was observed at 40 PSU which was about half the NPR observed at 28 and 32 PSU. Higher salinity appeared to have adverse effect on nauplii production of *Parastenhelia* sp. as evidenced by the present results.

Effects of temperature on survival and NPR of *Parastenhelia* sp.

The recorded mean survival was above 50% at all the temperatures tested until 4th day. Total mortality was recorded at 38^{o}C by the end of 9th day (Fig. 2a). However survival was higher (80%) at 28^{o}C by the end of 12th day, followed by 45% survival at 23^{o}C with.Though the final survival of 40% was higher at 18^{o}C followed by 15% at 33^{o}C. Temperature significantly influences the naplius production rate of *Parastenhelia* sp. The copepod tolerate temperature variations between 18 and 38 ^{o}C (*P<0.05*) (Fig. 2b). NPR was found to be highest (59.33±7.57 nauplii female^{-1} day^{-1}) when the temperature maintained at 301.15 K followed by 23^{o}C with an NPR of 39.66 ± 3.79 nauplii

female^{-1} day^{-1}. The lowest nauplii production (12.67±3.06 nauplii female^{-1} day^{-1}) was noticed at 38^{o}C. In the present study the highest temperature employed had more adverse effect on the NPR of the test species.

Under natural conditions, copepods are frequently well adapted to address with seasonal variations in temperature and salinity (Marcus, 2005; Milione and Zeng, 2007; Santhanam and Perumal, 2012a). Estuarine copepods especially have wide tolerance ranges of salinities (Castro-Longoria, 2003; Chinnery and Williams, 2004; Bollmohr et al., 2009). This is a helpful trait for aquaculture, as copepods used as live feed for fish larvae will be alive and stay obtainable in the water column under a range of temperature and salinity conditions that are appropriate for the culture of fish larvae (Payne, 2000 and Rippingale; Santhanam and Perumal, 2012a). An abrupt diminishing in salinity and temperature does affect the survival. The result of this study shows that *Parastenhelia* sp. is more sensitive to a sudden change of temperature than to a change of salinity. Its physiological response and tolerance towards temperature and salinity might be the reason for the different sensitivity to the two parameters. The results of our study show that high survival of over 80% was achieved between 32 and 36 PSU. Sun and Fleeger (1995) stated that harpacticoid copepods grew best in the salinity regime of 25-35 PSU, while it can continue to survive in the salinity range of 10 to 60 PSU.

Harpacticoids in general are known to have a wide tolerance to salinity changes due to their different natural habitats with periodic exposure to salinity fluctuations. Rhodes (2003) reported that the salinity tolerance of *Nitocra lacustris* ranged from 10 to 40 PSU. Recently, Zaleha and Jamaludi (2010) observed the optimum condition for the maximum production of a tropical harpacticoid copepod *Pararobertsonia* sp. to be around 35PSU. Although *Parastenhelia* sp. was able to survive in a wide range of salinity levels, the overall reproductive capacity has been observed to decrease under high salinities as agreed by many workers (Chen et al., 2006, Hall and Burns, 2002, Calliari et al., 2008 and Santhanam and Perumal, 2012b). In this study, the nauplii production rate peaked at 32-36 PSU and decreased at both higher and lower salinities. Under these salinities, copepods matured and were able to form eggs in earlier. This may be explained by the fact that these are copepods well adapted to salinities of 32-36 PSU in our laboratory for more than a year. However, it is often hard to define a salinity threshold for estuarial species (Uye and Fleminger, 1976, Milione and Zeng, 2008, Santhanam and Perumal, 2012a), as different species may acclimatize to different salinities at a variety of stages of their life history due to their habitat variations.

According to Santhanam et al. (2013), only few laboratory studies have explored the relationship between temperature and fecundity of harpacticoid copepods. The various species might have various thermal limit in terms of nauplii productive response. Miliou and Apostolopoulou (1991), Zaleha and Jamaludin (2010) reported that a decrease in the number of egg sacs and the total number of nauplii produced by the Greek strain of *Tisbe holothuriae* was observed when the temperature was lower or higher than the optimal (19°C). The results of our study show that high survival and nauplii production was achieved at optimum temperature 28°C. Data on the period of the different stages of marine harpacticoid copepods and the influence of environmental factors (specifically temperature) and their potential relations on nauplii production are relatively limited. Nevertheless, Matias-Peralta et al. (2005), Zaleha and Jamaludin (2010) elucidated that nauplii production rates of harpacticoids are strongly temperature dependant and nauplii production is reduced at higher temperatures above 40±1^{o}C.

CONCLUSION

It is understood that the survival and nauplii production of *Parastenhelia* sp. were influenced by salinity and temperature. Increase in salinity give positive effects to the species as it can increase the survival and nauplii production rate in 32 PSU. The effect of higher temperature might be more crucial than salinity as shown in this study. Hence the study suggests that salinity range of around 32 PSU and a temperature of around 28^{o}C were suitable water quality conditions for effective mass culture of *Parastenhelia* sp. in laboratory condition. Further study

should be carried out to understand the growth rate and population of the species in response to the environmental stress from salinity and temperature change.

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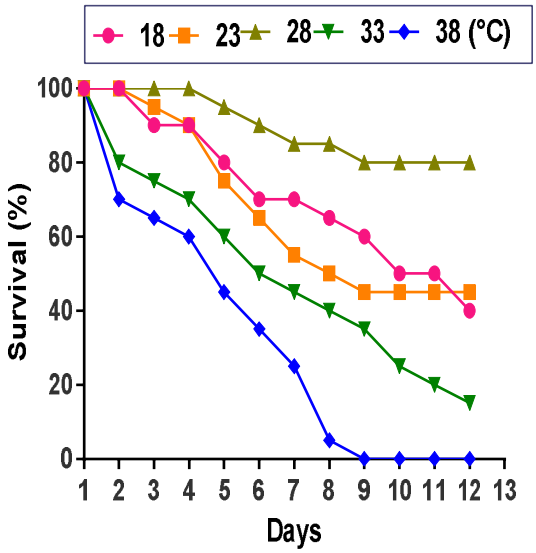

Fig. 1. (a)

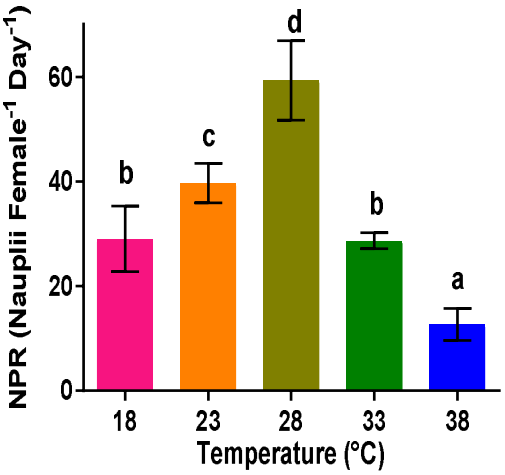

Fig. 1. (b)

Fig. 1. (a) Survival, (b) Nauplii production rate of copepod *Parastenhelia* sp. exposed to different salinities. Copepods were incubated under identical condition of 28°C.

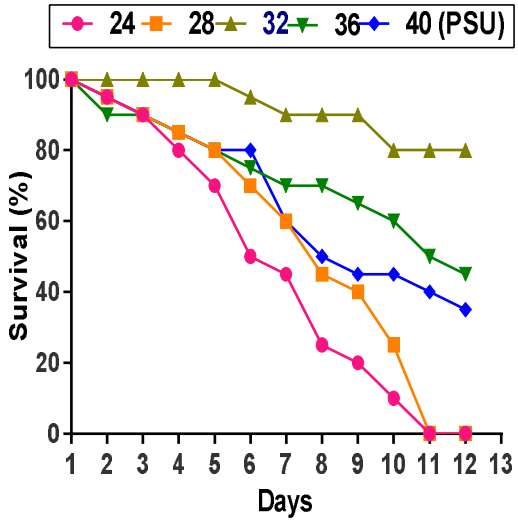

Fig. 2. (a)

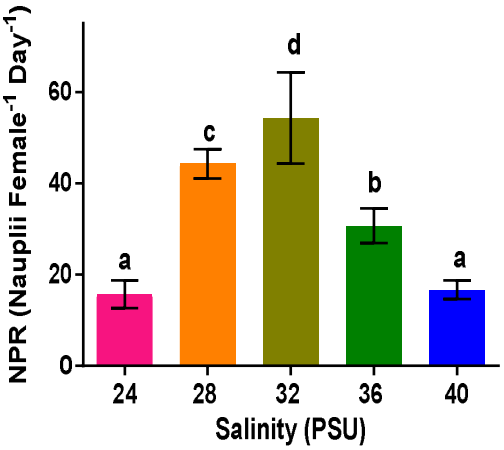

Fig. 2. (b)

Fig. 2. (a) Survival, (b) Nauplius production rate of copepod *Parastenhelia* sp. exposed to different temperatures. Copepods were incubated under identical condition of 32 ppt salinity.

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RESEARCH ARTICLE

Effect of Titanium Dioxide TiO_2 Doped Graphene as an Electron Transport Layer on P3HT:IC70BA Organic Solar Cell

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ABSTRACT

Graphitic thin films of titanium dioxide (TiO_2:G)(v:v) were used for the first time as a cathode buffer layer introduced in poly(3-hexylthiophene) (P3HT): Indene-C70 Bis-Adduct (ICBA) bulk heterojunction (BHJ) solar cell resulting in a remarkably improved cell efficiency due to its superior electron conductivity. Five volume ratios of mixing were used (TiO_2:G1, TiO_2:G2, TiO_2:G3, TiO_2:G4 and TiO_2:G5). Using these interfacial layers led to enhance devices performance especially the interfacial layer TiO_2:G2 which gave the best results. The open-circuit voltage, short circuit current and fill factor increase respectively to 0.55 V, 33 mA/cm^2 and 35%with low band gap energy of 1.88 eV, due to the enhanced electron extraction by inserting a TiO_2:G2 layer between the active layer and Al cathode. Thus, the power conversion efficiency increases to be6.38%.the XRD analysis revealed that the prepared thin films showed high crystallinity structure.

Keywords : Titanium dioxide, Bulk Hetero Junction solar cell, Electron conductivity.

INTRODUCTION

Bulk heterojunction (BHJ) polymer solar cells (PSCs) have attracted considerable attentions in both academia and industry due to their low cost, flexibility and large-area fabrication features [1–3] compared to traditional SCs [4–6]. Although the power conversion efficiency (PCE) of 9.2% has been reported for single BHJ PSCs [7], Graphene, the 2D carbon nanomaterial,has drawn much attentionnowadays. It is a zero band gapmaterial, [8] and electrons in it are

just likemassless relativistic particles, so it has an excellentelectrical conduction in twodimensions,[9-13] even at temperatures closeto absolute zero and the Dirac point.[8] Onthe basis of its astonishing advantages inelectrics, graphene has been used in conductive glasses [14], organic photovoltaiccells,[15, 16] field-effect transistor devices [17], andultrasensitive sensors [18, 19].

To date, diverse research efforts to improve the overall conversion efficiency have been intensively taken including the rational design of sensitizers for increasing the light harvesting ranging from visible to near IR [20-22] optimization of semiconducting titanium dioxide (TiO_2) nanostructures, which strongly depends on their dimensional (e.g., size and shape) and morphological features. Yang et al. [23] introduced graphene as two-dimensional (2D) bridges into the TiO_2 nanostructure photoanode, which led to faster electron transport and lower recombination indye-sensitized solar cells (DSSCs). However, despite their potential for enabling the improved efficiency, facile, and viable strategies of coupling carbon structures into OPVs along with plausible analysis on the operation mechanism have rarely been conducted. Therefore, big challenges still remain to clearly understand the influence on the charge collection and transport at the interface between active layer and electrodes. Here we introduce a definitely different and inspiredmodel to integrate graphitic TiO_2 thin layers into conventional P3HT:IC70BA based OBHJ for enhanced device performance.

MATERIALS AND METHODS

OPVs were fabricated using pre-patterned ITO-coated glass substrate. Prior to the use, the substrate was cleaned in ultrasonic using 20% Decon90, deionized water, isopropanol, and acetone in the clean room, and later dried with N_2 compressor. All cleaned substrates weretreated with O_2 plasma treatment for 25 min. The solution for hole transporter PEDOT:PSS solution was spin-coated at 5000 rpm for 40 s onto the cleaned substrates and then annealed at 140 °C for 10 min. The photoactive layer P3HT:ICBA (1:1) w dissolved in Chlorobenzene with a concentration of 15mg/ ml was spin-coated at 1500 rpm for 35 s in the glove box and annealed at 170 °C for 45 min.. Later, In order to get TiO_2:G solution, a volume mixture was made between the graphene solution and the prepared TiO_2 solution. One drop of (25 µl) from graphene solution was added to (5 ml) of TiO_2 solution using a laboratory pipiter to get TiO_2:G ratio which was mentioned as a TiO_2:G1, two drops to get TiO_2:G2, three drops to get TiO_2:G3, four drops for TiO_2:G4 and five drops for TiO_2:G5. TiO_2:G solutionswith all mixing values were spin-coated at 4000 rpm for 25 s onto the photoactive layer and annealed at 75 °C for 25 min. To complete the device, 120 nm thick Al was thermally evaporated at rate 1A/s through a shadow mask at a base pressure of 10^{-6} mbar. The active area of the complete devices is 0.12 cm^2. Devices were tested under AM 1.5 illumination with an intensity of 100 mW/cm^2 at room temperature. The idealized device configuration is illustrated in Fig. 1.

RESULTS AND DISCUSSION

XRD Analysis

Fig. 2 declare the effect of introduce TiO_2 doped with Graphene with different concentration as an electron transport layer of the x- ray pattern of P3HT:ICBA blends prepared on glass covered with ITO layer. The most interesting results is the appearance of the main diffraction peak for the P3HT located around $2\theta^o = 5.49^o$ which are correlated with the inter chain arrangement in P3HT linked with the interdigitated alkyl chains [11]. On the other hand, all the peaks are found to related with In_2O_3 i.e. to ITO base layer, also there is diffraction peak related to Graphene located at $2\theta=43.77^o$. The increment of Graphene concentration lead to significant variation in the hole pattern. The grain size D of the preferred plane for crystal growth of P3TH show nonsystematic variation with Graphene concentration, i.e. Increases and decreases. Indeed, D increases from18.4 to 28.4 nm but then decreases to15 nm followed by explicit increase to 44nm for high Graphene concentration. Also the peak related to Graphene and ITO show similar behavior

with Graphene concentration, i.e. with Graphene concentration. The most interesting result is the disappearance of the Graphene peak for high Graphene concentration. where the effect of doping of TiO_2 became more pronounce.

Surface Morphology

To obtain additional insight into the device performance, we examined the morphology of the metal oxide (MO)derived films. Atomic Force Microscopy (AFM) was used to examine the MO films on top of the active layer, before the electrode deposition, in order to obtain the topographies shown in Figure 3. Uniform films with all ETL materials composed of MO nanoparticles are observed. For pristine MO films cast from as-diluted solutions (as described in materials synthesis), the root mean square (RMS) roughness was 10.8 nm for TiO_2:G1, while for TiO_2:G2 these value increased to be 33 nm and then decreased to became 13.6 nm, 18.3 nm and 11.3 for TiO_2:G3, TiO_2:G4 and TiO_2:G5 respectively. High roughness obtained using TiO_2:G2 is ascribed to better crystallinity of the MO nanoparticle compared to the precursor, this contributes to improved charge extraction which is a known factor that affects the performance of devices [24].

Optical Characterization

The absorption spectra of different ETLs within devices of the same architecture are shown in Fig.4. The absorption spectra are very similar for all devices indicating there is minimum absorption from the ETLs; this suggests that it does not interfere with the ETL's secondary function as an optical spacer [25, 26]. On the other hand a red shift was observed with the TiO_2:G2 hybrid ETLs,alsothe enhanced efficiencies observed, can be ascribed primarily to their improved electrical characteristics. The optical energy gap values (E_g^{opt}) of TiO_2:Gr (v/v) buffer layers on P3HT:$IC_{70}B$ on ITO/PEDOT:PSS are shown in Fig. 5. This figure reveals that the values of direct optical energy gap, decreases drastically for TiO_2:G2, this reduction is accompanied with abrupt increase in the extinction coefficient value, as shown if Fig. 6.

Electrical Characterization

The corresponding results obtained from P3HT:IC70BA based solar cell with different configurations of TiO_2:G were summarized in Table 4. Fig.8 shows the J–V characteristics for the devices using different buffer layers. The short-circuit current density (Jsc) is significantly increased with the presence of thin Graphitic TiO_2(TiO_2:G2) layer and results in enhanced power conversion efficiency (P_{CE}) compared with the reference electrode, while the values of open-circuit voltage (V_{oc}) and fill factor (FF) are similar with all representative samples. The values of J_{sc} obtained from the devices using Graphitic TiO_2 thin layer introduced in the upper part of P3HT:IC70BA film are 30.5 mA/cm^2, which exhibited lower J_{sc} than a J_{sc} of 35.25 mA/cm^2 for pristine TiO_2 electrode. Furthermore, the highest performance, J_{sc} of 33 mA/cm^2 and P_{CE} of 6.38%, was achieved in solar cells using (TiO_2:G2) thin layer. This indicates that the energy levels aligned successfully promoting an efficient electron extraction from the device. This indicates that the high P_{CE} for the device with TiO_2:G2 buffer layer is directly influenced by the efficiency of charge extraction – collection mechanism. The relatively well matched energy levels of the TiO_2 conduction bands match well with the work function of graphene ~4.4eV, forming an efficient energy transfer layer for fast electron extraction. [24].

CONCLUSION

The effect of TiO_2:G interlayer as an electron transport layer on the P3HT:IC70BA BHJ organic solar cell had been investigated. Five volume ratios were applied, TiO2:G2 gave the best results.Inserting TiO_2:G2 layer between the active layer and Al cathode decreases the work function of cathode and reduces the series resistance. Therefore, VOC increases to be 0.55 V, FF increases to 35% and significantly increment in PCE to 6.38%and the short circuit current density JSC to 33%. Besides of the above, the insertion of TiO_2:G layer prevent the Al atoms from diffusion into the active layer and thus improve the lifetime of BHJ cells.

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Table 1. X-ray diffraction data for P3HT:IC70BA BHJ with different buffer layers

Sample	2θ (Deg.)	FWHM (Deg.)	d_{hkl} Exp.(Å)	G.S (nm)	d_{hkl} Std.(Å)	hkl	Phase	card No.
	5.2166	0.4333	16.9268	18.4	16.084454	(100)	P3HT	[Ref.]
	21.0864	0.3333	4.2098	24.3	4.1315	(211)	In2O3	96-101-0342
	30.0578	0.3949	2.9706	20.8	2.9214	(222)	In2O3	96-101-0342
TiO_2:G1	34.9679	0.3320	2.5639	25.1	2.5300	(400)	In2O3	96-101-0342
	43.8064	0.3500	2.0649	24.5	2.0600	(002)	Graph.	96-901-2471
	45.0707	0.3200	2.0099	26.9	1.9847	(341)	In2O3	96-101-0343
	50.3014	0.4667	1.8125	18.8	1.7890	(440)	In2O3	96-101-0344
	59.9376	0.3867	1.5421	23.7	1.5256	(622)	In2O3	96-101-0345
	5.3142	0.2750	16.6161	28.9	16.0845	(100)	P3HT	[Ref.]
	21.0848	0.3300	4.2101	24.5	4.1315	(211)	In2O3	96-101-0342
	30.0426	0.3436	2.9721	24.0	2.9214	(222)	In2O3	96-101-0342
TiO_2:G2	34.9612	0.3267	2.5644	25.5	2.5300	(400)	In2O3	96-101-0342
	43.7814	0.3600	2.0660	23.8	2.0600	(002)	Graph.	96-901-2471
	50.3014	0.4133	1.8125	21.2	1.7890	(440)	In2O3	96-101-0344
	60.0443	0.2400	1.5396	38.2	1.5256	(622)	In2O3	96-101-0345
	5.1620	0.4800	17.1057	16.6	16.084454	(100)	P3HT	[Ref.]
	21.0673	0.2650	4.2136	30.5	4.1315	(211)	In2O3	96-101-0342
	30.0480	0.3855	2.9716	21.3	2.9214	(222)	In2O3	96-101-0342
	34.9569	0.2980	2.5647	28.0	2.5300	(400)	In2O3	96-101-0342
TiO_2:G3	43.7714	0.3200	2.0665	26.8	2.0600	(002)	Graph.	96-901-2471
	45.1107	0.3200	2.0082	26.9	1.9847	(341)	In2O3	96-101-0343
	50.3047	0.3667	1.8124	23.9	1.7890	(440)	In2O3	96-101-0344
	59.9119	0.2750	1.5427	33.4	1.5256	(622)	In2O3	96-101-0345
	5.2634	0.5300	16.7764	15.0	16.084454	(100)	P3HT	[Ref.]
	21.0748	0.3300	4.2121	24.5	4.1315	(211)	In2O3	96-101-0342
	30.0598	0.3667	2.9704	22.4	2.9214	(222)	In2O3	96-101-0342
	34.9662	0.3200	2.5640	26.0	2.5300	(400)	In2O3	96-101-0342
TiO_2:G4	43.7847	0.3600	2.0659	23.8	2.0600	(002)	Graph.	96-901-2471
	45.0807	0.4200	2.0095	20.5	1.9847	(341)	In2O3	96-101-0343
	50.3281	0.4000	1.8116	22.0	1.7890	(440)	In2O3	96-101-0344
	59.9459	0.3767	1.5419	24.4	1.5256	(622)	In2O3	96-101-0345
	5.4232	0.1800	16.2824	44.2	16.084454	(100)	P3HT	[Ref.]
	21.0848	0.3300	4.2101	24.5	4.1315	(211)	In_2O_3	96-101-0342
	30.0433	0.3734	2.9720	22.0	2.9214	(222)	In_2O_3	96-101-0342
TiO_2:G5	34.9550	0.3257	2.5648	25.6	2.5300	(400)	In_2O_3	96-101-0342
	45.0257	0.3100	2.0118	27.8	1.9847	(341)	In_2O_3	96-101-0343
	50.3189	0.4317	1.8119	20.3	1.7890	(440)	In_2O_3	96-101-0344
	59.9194	0.4700	1.5425	19.5	1.5256	(622)	In_2O_3	96-101-0345

Table 2 The extracted and calculated photovoltaic parameters of the fabricated devices with different buffer layers.

Device	V_{oc} (V)	J_{sc}(mA/cm²)	FF (%)	P_{CE} (%)	R_s Ω	R_{sh} Ω	β
TiO_2:G1	0.54	30.5	34	5.62	6.06	41.6	2.41
TiO_2:G2	0.55	33	35	6.38	4.67	40	1.15
TiO_2:G3	0.50	25.5	32	4.13	5.61	39	1.00
TiO_2:G4	0.42	20.5	34	3	5.61	30	1.15
TiO_2:G5	0.40	18.83	33	2.5	5.60	40	1.92

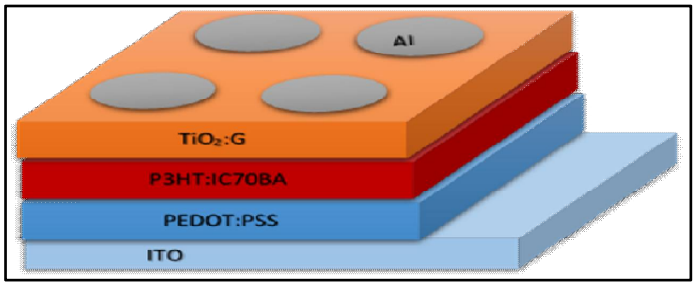

Fig. 1 Schematic diagram of P3HT:IC70BA based OBHJ solar cells with TiO_2:G (ETL) layer

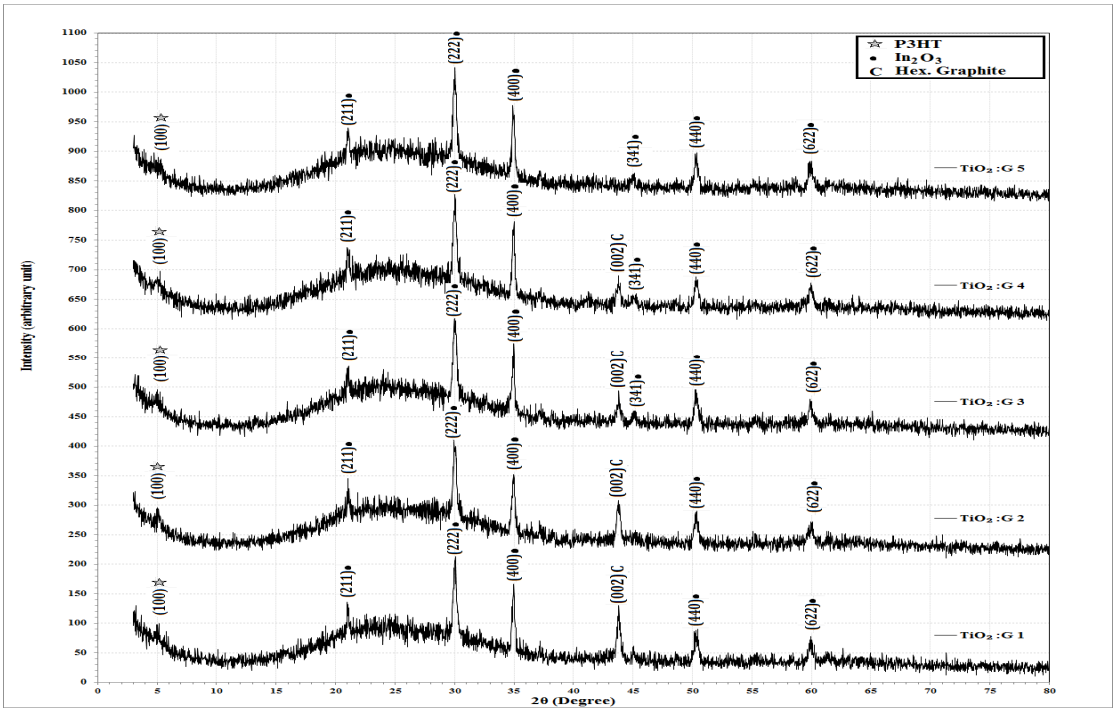

Fig. 2 X-ray diffraction pattern for P3HT:IC70BA BHJ with different buffer layers.

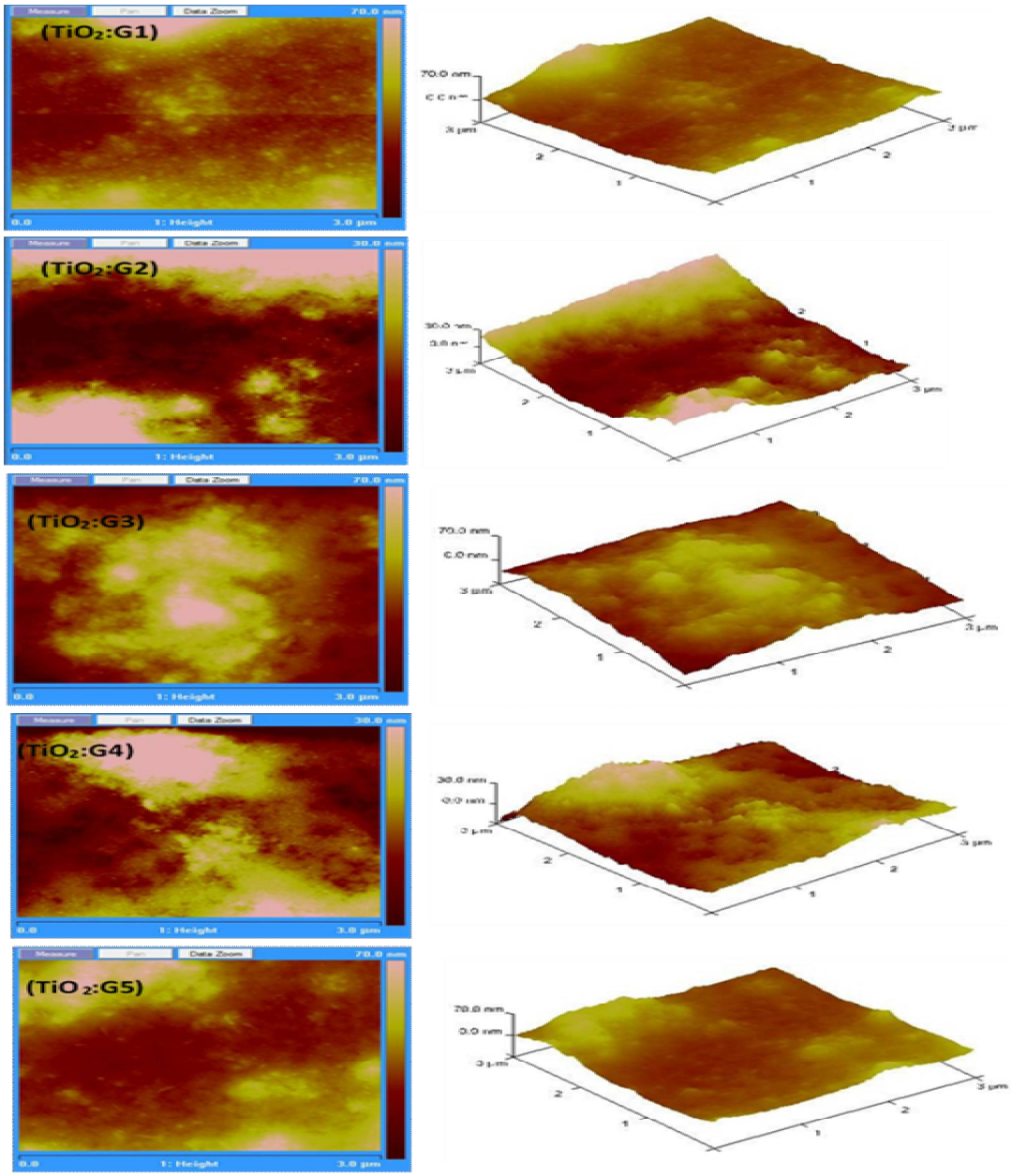

Fig. 3 AFM topography and phase images of different TiO_2:Gr (v/v) buffer layers on P3HT:$IC_{70}B$ on ITO/PEDOT:PSS. (TiO_2:G1, TiO_2:G2, TiO_2:G3,TiO_2:G4, TiO_2:G5)

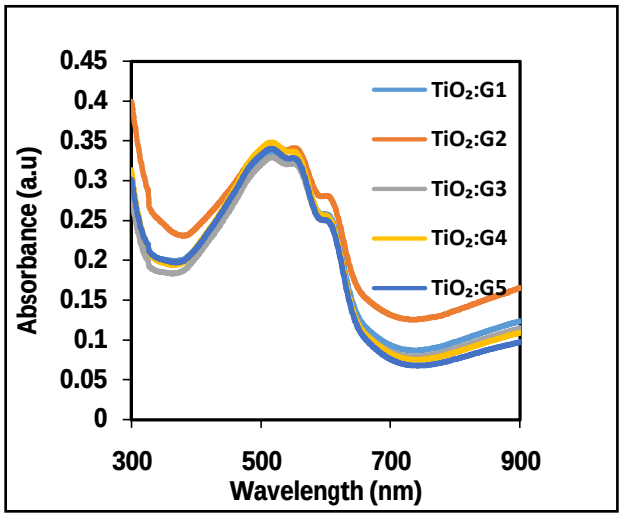

Fig. 4 UV-visible absorbance depending with different buffer layers.

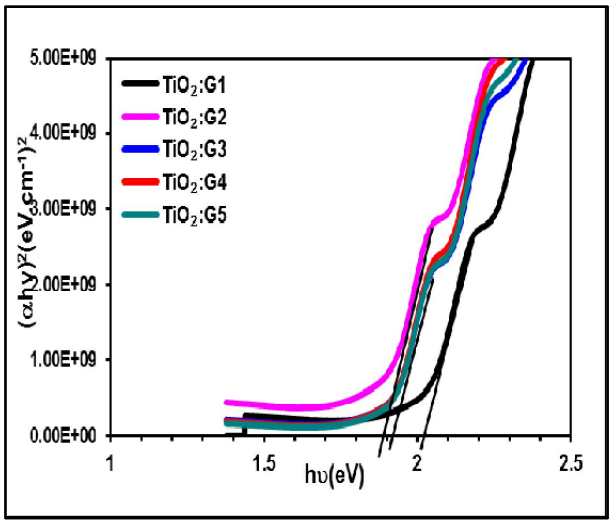

Fig. 5 Variation of$(\alpha h\upsilon)^2$ versus photon energy ($h\upsilon$) with different buffer layers.

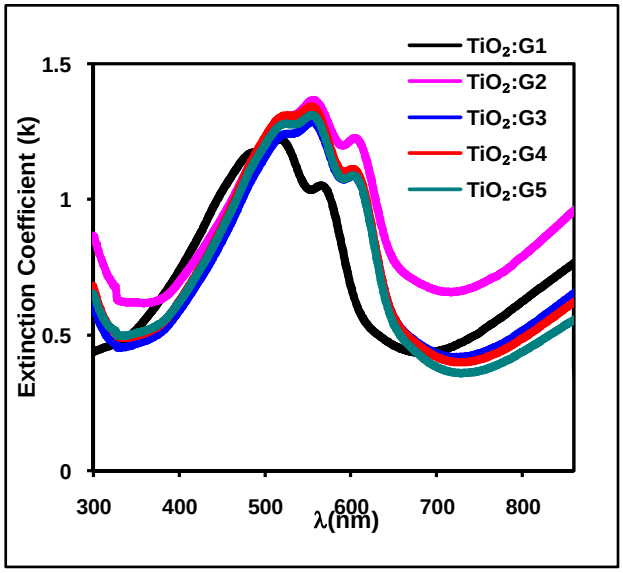

Fig. 6 Variation of extinction coefficient (k) with wavelength forOPV devices with different buffer layers.

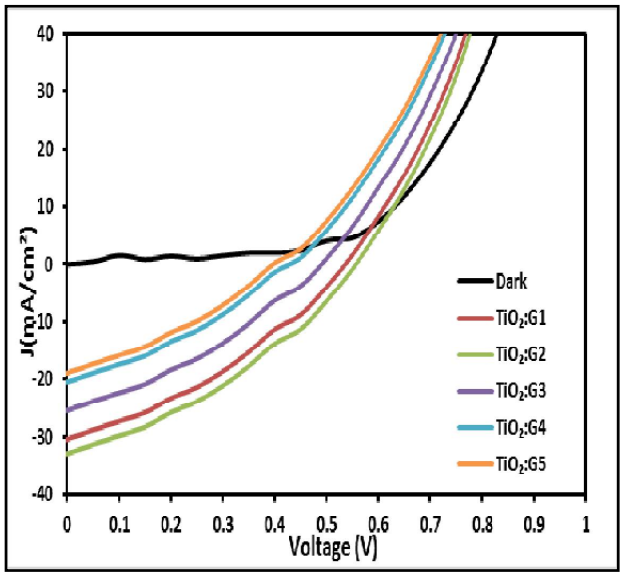

Fig. 7 shows J-V characteristics of the OBHJ solar cells under the illumination of 100 mW/cm² white light.

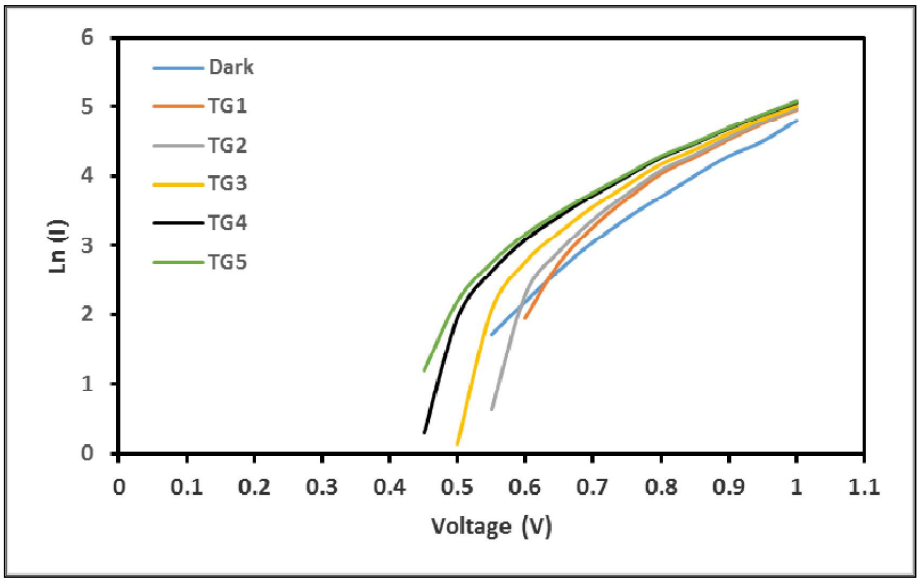

Fig. 8 Variation of Ln(I) versus the bias voltage for P3HT:IC70BA OPV devices with different buffer layers.

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RESEARCH ARTICLE

Role of GABA and ATPases Activities in the Process of Development and Ageing

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ABSTRACT

Neurotransmission involvingGamma amino butyric acid (GABA) along with energy consumption as a function of ATPase activity, has a specific role in development and aging process. In this study, we focused mainly on age dependent change of levels of both the GABA and with ATPase activities in brain and liver of rats of Long Evans species to shed some light on their possible involvement on the ageing process. In this study age related alteration in the levels of GABA and ATPase activities were determined in brain and liver of Long Evans rats as modulators of the development and aging process.
GABA levels and ATPase activities in brain and liver of Long Evans rats of different age groups from 4 days to 420 days were determined following standard methods. Results were analyzed in relation to development and aging process.Results were expressed as the mean of 5 determinations ± Standard deviation. GABA is an inhibitory neurotransmitter. At the age of 12 months GABA content per gram of wet tissue was recorded maximum. At the age of 4 days after birth the level was very low and is followed

by a rapid increase within 30 days. The same result was found both in brain and liver. GABA levels both in brain and liver was found to decrease slightly at higher at advanced age.Since the Na^+ K^+ ATPase activity has been implicated in the Na^+ pump and the regulation of membrane polarization. This suggests that in adult there is no deficit in learning and memory occurs. It may be concluded that for maintenance of life ATP synthesis and breakdown are essential through the metabolic pathways, even may be continued after death for quite a long time. All the phenomenon needs further investigation.From our experiment we drew the conclusions,GABA are working synergistically with ATPase to stimulate the ageing process in rats of Long Evans species.In the process of development and aging in Long Evans species GABA has a modulatory relation between inhibitory neurotransmission of GABA and ATP consumption through Mg^{+2} and Na^+K^+ ATPase activities with the progression of development and aging of rats of Long Evans Species.

Keywords : Aging, Development, Neurotransmitters, GABA, ATPase

INTRODUCTION

Growth is not merely a change of size or enlargement of the existing organs or tissues. Rather, it is the continuous dynamic process of physiological, conceptual and intellectual growth in an orderly manner in all species of animals and human. Ageing is the decay or discontinuity in such a developmental proces[1]. Neurotransmitters playning a vital regulatory role in the process of development, growth and ageing for the elderly[2].Alterations in neurochemical indices of synaptic functions has been considered to be indicators of age related impairment of central functions like locomotion, memory and sensory performance[3].Ageing of the brain is characterized by several neurochemical modifications involving structural proteins, neurotransmitters, neuro peptides and related receptors[4]. During the life span of an organism, each tissue, organ or part or its physiological activities have inherent sequential timing for cognitive development. Late-life depression and dementia is the ultimate result of any kind of impairment in such cognitive development. With such development the organism aquire skills; become able to adapt more readily to stress; to assume maximum responsibilities and to achieve freedom in creative expressions[3].

However, the process of development and ageing in relation to neurotransmitters like gamma amino butyric acid (GABA),energy utility through ATPaseand their synergistic functions are not understood yet. Age dependent modification in brain may be reflected in the alteration of the regional distribution levels of these neurotransmitter[5,6]. Since, protein phosphorylation using ATP is a key process in biological regulation, it may play avital role in modulation of neuronal signals and stimuli generated by neurotransmitters[7].Along with the inhibitory neurotransmission of GABA, there may be an age dependent change in sodium and potassium ATPases, may be suggestive of an interrelationship between these two.It would be justified to predict that there may be a change in level of ATPase in the brain during old age to maintain its enormous functions particularly that of the central nervous system. Liver may have some synergistic function to produce more ATP filling the gap of the energy requirement of the brain. In this study, age dependent change of levels of both the GABA along with activities of ATPase both in brain and liver are addressed to understand their possible involvement on the development and ageing process. Levels of GABA and ATPase activities are determined in brain and liver considering them as modulators of the development and ageing process of Long Evans rats.The present study addresses change in the level of GABA and ATPase activities with the progression of development and ageing.

MATERIALS AND METHODS

Duration and place of work

This work was performed in the Drug Testing Laboratory, the Institute of Public Health, Mohakhali, Dhaka 1212, Bangladesh. Five Batches of Rats of Long Evans species were bred in ICDDR,B animal house to fulfil the need of the experimental period covering the age of day 4 to day 420 and transferred to the Animal House of Drug Testing Laboratory. Only 50 male siblings were taken just after birth in phages and grouped into 8 groups with proper documentation of date of birth to maintain as per preset experimental period from 2000 and 2003.

Animal maintenance and tissue sampling

Rats of different age groups were maintained in the Animal House of Drug Testing Laboratory, IPH, Dhaka under proper 12/12 hours day and night synchronization having temperature between 26-30°C. All the tasks of animal handling were performed according to the NIH Guide for the Care and use of laboratory animals.Rats of were maintained on diet containing 14 % proteincomposed mainly of wheat and rice flour, milk and oil which has been fortified with vitamins and minerals. This work was approved by the Ethical Committee of the IPH, Mohakhali, Dhaka.Brains and livers of male rats of different age groups were used in the experiment. Three or five rats were taken for both control and experiment for each age group. The experimental animals were sacrificed under anesthesia of chloroform and the brains and livers were removed instantaneously and placed on ice immediately. The samples were washed by cold normal saline and stored in deep freeze of -40^{o}C until analysis.

Estimaton of protein

Protein was esteimated by the method of Lowry's[8,9].

Reagents and chemicals

Sucrose solution (0.32M) in water, Perchloric acid volume by volume solutions of 60%, 12% and 6 % ; 0.6% W/V ammonium molybdatein 6% percholoric acid; and 0.2 % W/V ascorbic. Reagents and chemicals used in this work are purchased from either Sigma Aldrich or BDH.

ATPase assay

ATPase activities were determined following the method of (1973) Atkinson, A., etal[10] and Hunt et al.[11]. Working solutions (A) Each 50 ml of total ATPase incubation media consists of sodium chloride (0.1M), potassium chloride (0.03M), Magnesium Chloride (0.003 M), ATP (0.003M), and Tris (0.025M). (B) Each 50 ml of Mg-ATPase incubation media consists of sucrose (0.32M), Magnesium Chloride (0.003 M), ATP (0.003M), and Tris (0.025M).

Estimation of ATPase activity

For total ATPase activity 0.4 ml of tissue homegenate incubated with 3.0 ml of incubation media (A). Incubation continues for 10 minutes at 37^{0}C. The reaction was stopped with 3.0 ml of 12 % perchloric acid. The tubes were centrifuged at 3000 rpm for 20 minutes and clear supernatant transferred to new tube. The liberated inorganic phophate in supernatant was determined as described in the section assay of inorganic phosphate below.

For Mg-ATPase activity 0.4 ml of tissue homegenate incubated with 3.0 ml of incubation media followed by all the procedures as indicated in the toal ATPase. Other procedures are same as total ATPase determination.The $Na^{+}K^{+}$ATPase activity was determined by subtracting MgATPase from total ATPase activity. The results were expressed as mg of liberated Pi per hour of incubation per mg of protein at 37^{0}C.Inrganic phosphate was determined follwing the method of King et al[12].

Estimation of GABA

Gamma amino butyric acid was determined following the method of Saad[13].Wet tissue wighing 0.5 gm of brain and 1.0 gm of liver were homogenized in 10 volumes of ethanol and centrifuged at 3000 rpm for 10 minutes.The pallet was defatted with 6 ml diethyl ether and by 6 ml of chloroform. Extracted with 2 ml normal saline. Saline extract (0.5 ml) representing 125 mg of original brain and 250 mg of liver tissue were dried in an oven and reconstituted with distilled water. Extracts (0.01 ml) representing 25 mg of wet brain and 50 mg of liver were applied by finpippet to a 4x60 cm band of Whatman No 1 chromatographic paper. One dimensional descending chromatograph was run by Phenol-Water solvent for about 12 hours. The chromatograph was dried in air and developed with 0.1 % ninhydrin and dried in an oven at 90^0C. Standard GABA sample of same concentration was applied along with the experimental sample.The GABA spots were identified from Rf value.Spots were extracted with waterand OD of the extract was taken at 540nm in Spectrophotometer. GABA concentration was calculated according to least square method[14].

Statistical analysis

All the results were expressed as the average of 5 experiments ± SD. Standard deviations were calculated.

RESULTS AND DISCUSSION

GABA was determined in both in brain and liver of rats of different age groups as shown in the Table 1. At the age of 4 days GABA level was quite low. A rapid increase in total GABA was observed at the age of two months both in brain and liver. Throghout the aging period the leve was found to be increased gradually. Maximum level was attained at the age 360 days. The same pattern of GABA was also observed in GABA per mg of protein.Specific Mg^{+2}- ATPase activity (µg of inorganic phosphate liberated per hour per mg of protein at 37^oC in whole wet tissue) was determined both in brain and liver of rats of different age groups as shown in the Table 2. The specific Mg^{+2}- ATPase ATPase activity wa sfound to be maximum at age 360 days having a very low level of increase throghout the experimantal period. But total Mg^{+2}- ATPase ATPase activity was observed to be increased at age of 30 days. The value showed to be increased throghout the experimentral period.Specific Na^+K^+- ATPase activity (µg of inorganic phosphate liberated per hour per mg of protein at 37^oC in whole wet tissue) was determined both in brain and liver of rats of different age groups as shown in the Table 3. The specific Na^+K^+- ATPase ATPase activity had a rapid increase upto age 30 days having a gradual increase throghout the experimantal period. Total Na^+K^+- ATPase ATPase activity was observed to be increased at age of 30 days. The value showed to be increased throghout the experimentral period.

Age dependent change in brain reflects the alteration of neurochemicals within different brain regions. Levels of neurochemicals in different regions and their change in region specific functions causes neuro-pathology [15]. The dysfunctions may be considered in various angles; anatomical, metabolic, synthetic, secretory, electrical or nervous. The different parts of nervous system performs and controls different behavioral functions. Occasional abnormal cerebral and hypothalamic malfunctions cause physical and neuro-humoral disorders resulting ageing of an individual. Levels of neurotransmitters are thus involved in central mechanism of development, growth and ultimate ageing. Ageing is accompanied by various cellular challenges which led to homeostatic alterations and ultimately result in the reduction of neuronal functions and cognitive performance[16].Therefore, in the present study we investigated the age dependent modifications in neurochemical profile in Long Evans rats. The present study addresses change in the level of GABA and ATPase activities with the progression of development and ageing. Present study results are in good agreement with theobservation that there no age dependent change in total protein content[17]. In our study no age dependent change in the protein content observed both in wet brain and liver.Increased GABA level in association with the release of accumulated GABA from brain has been reported[18,19] with age. At the age of 4 days after birth the level was very low and is followed by a rapid increase within 30 days.

At the age of 12 months GABA content per gram of wet tissue was recorded maximum. The same result was found both in brain and liver. GABA levels both in brain and liver was found to decrease slightly later at the age of day 420. Same pattern of age dependent GABA levels were reported[20,21].In dogs GABA level was higher at the age of 3 years than one year but at the age 15 years the level decreased to lower than that at the age of three[21,22]. Significant changes observed in functions related to GABA neurotransmission occur due to age dependent change within the GABA receptors in the aged rats including significant decreases in GABA content[23], GABA release, GABA neurons, glutamate decarboxylase enzymatic activity, and GABA receptor binding[24,25].

In adult the ATPase activity was maximum in synapses.Change at the level of which may affect the transmission process, inhibit cation transport and alter brain activity. These clinical changes occur in older humans and animals than in adults[23,26]. Our results are in good agreement with this hypothesis. Present study revealed that specific ATPase activity showed maximum value at the age of days 360 and 420 in brain and liver respectively, suggesting the smooth synaptic transmission during developmental process.At day 4 total ATPase activity in brain and liver 25.64 ± 1.18 and 11.69 ± 2.14 µg of Pi released respectively followed by a rapid increase at day 120 until attained the maximum value at day 180. It is evident from this finding that up to day 180 after birth is the growth and developmental period. During the remaining experimental period the total ATPase activity observed static though the weight of the tissue increased.Present study showed a minimum value of GABA at the age of day 4, GABA level is quite low that as indices of both per milligram of tissue or per gram of protein. A rapid increase in total GABA is observed at the age of day 30 in brain and liver. Up to the age of day 60, GABA level shows a rapid increase and reached to the plateau and throughout experimental period the level increased and attained maximum value at day 360. After day 360 a short fall of GABA is observed.

The level of GABA determined as per mg of protein is maximum in brain. At the age of day 4 it is low, followed by gradual increase up to the age of day 320 in both brain and liver tissues. Percentage of GABA varies between 0.006-0.011 % and 0.0032-0.0061 % of wet tissue of brain and liver respectively.Present study findings of GABA level is in good agreement with the levels of Na^+ K^+ATPase levels both in brain and liver may be suggestive of a programmed and synergistic relation of GABA neurotransmission and energy consumption during later stage of development and onset of ageing through Na+ K+ATPase mediated pathway[26]. It is known GABA is an inhibitory neurotransmitter reflected as the age-related response resulting decreased motor function under synergistic control of Na^+K^+ ATPase mediated electrochemical gradient[27].GABA synaptic signaling usually lead to a net influx of Na^+, $K^+$and Cl^-ions whichmay regulate intracellular water movement during development and ageing[28].Massive water influx may consequently lead to neuronal ageing[29].The specific ATPase activity wasfound to be maximu at the age of day 360 having a gradual increase throghout the experimantal period. Total ATPase activity was observed to be increased at age of 30 days. The value showed to be increased throghout the experimentral period.

The specific Na^+K^+ATPase ATPase activity had a rapid increase upto age 30 days having a gradual increase throghout the experimantal period. Total Na^+K^+ATPase activity was observed to be increased at the age of day 30 in wet brain and liver. Our result is in good agreement with the finding up to the age of 420 days.It is revealed from present study that specific Mg^{+2} ATPase activity (µg of inorganic phosphate liberated per hour per mg of protein at 37^{o}C in whole wet tissue) is maximum at age 360 days having a very low level of increase throghout the experimantal period. But total Mg^{+2}ATPase activity was observed to be increased at age of 30 days followed by a gradual increase throghout the experimentral period.In synaptosomal membrane with the progression of development and ageing, peroxidation of polyunsaturated fatty acids observed with a reduction of theNa^+K^+ATPase[23]. Since the Na^+K^+ ATPase activity has been implicated in the $Na^±$ pump and the regulation of membrane polarizationhas a differential effect on Na^+K^+ ATPase resulting loss of the learning and memory in animals. Moderate activity of ATPase was observed during present studies. This suggests that in adult there is no deficitoccurs in learning and memory. ATPaseactivity may be dependent on neurotransmitter concentration Na^+K^+in brain[26,28,29].

Since the most of the neurohumoral disorders are the results of aberrant chemical processes and changes within nervous system a correlation between development, ageing and neurochemical variables can be found. In the present study, change in the level of neurotransmitters and change in the $Na^{+}K^{+}$ATPase activities may be involved in the turnover of neurotransmitters and energy utilizationthrough different sodium potassium transporters.

CONCLUSION

Therefore, present study reveals a strong evidence in favor of modulatory relation between inhibitory neurotransmission of GABA and ATP consumption through Mg^{+2} and $Na^{+}K^{+}$ ATPase activities with the progression of development and ageing of rats of Long Evans Species. Genetic and epigenetic studies shall be designed to understand clearly the mechanism of onset of development and ageingto identify the GABA and ATPase activity traits in different functional areas of brain.

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Table 1.Effect of Aging on Gaba Content of Brain and Liver Tissues of Rats. Each Value is the Mean of 6 Determinations ± Standard Deviation

Age in days	Brain			Liver		
	GABA /mg of protein (µg)	GABA/g of Brain Tissue(µg)	Total GABA(µg)	GABA /mg of protein (µg)	GABA/g of Liver Tissue(µg)	Total GABA (µg)
4	0.692 ± 0.022	56.19 ± 0.289	30.09± 0.991	0.174 ± $5.69x10^{-3}$	31.99 ± 1.20	7.76 ± 1.14
30	0.822 ± 0.042	69.04 ± 0.23	88.13± 4.90	0.183 ± 0.018	34.52 ± 0.43	87.04 ± 4.79
60	0.952 ± $3.21x10^{-3}$	85.803 ± 0.289	112.66 ± 1.41	0.212 ± $2.72x10^{-3}$	41.76 ± 0.89	147.13 ± 5.72
120	1.112 ± 0.032	99.22 ± 0.277	139.54 ± 2.31	0.237 ± $4.88x10^{-3}$	45.22 ± 1.23	231.36± 5.10
180	1.75 ± $7.92x10^{-3}$	106.26 ± 0.18	168.16 ± 1.95	0.278 ± 0.020	52.13 ± 1.03	383.82 ± 7.32
360	1.193 ± 0.065	106.66 ± 1.00	166.17± 3.10	0.34 ± 0.026	61.04 ± 1.10	500.07 ± 4.56
420	1.132 ± 0.042	106.74 ± 1.80	164.58 ± 2.30	0.34 ± 0.012	60.08 ± 0.93	469.52 ± 3.79

Table 2. Effect of Aging on Total Atpase Activity in Brain and Liver of Rats. Each Value is the Mean of 6 Determinations + Standard Deviation

Age in days	Brain		Liver	
	Specific acitvity	Total ATPase in whole brain	Specific acitvity	Total ATPase in whole liver
4	589.97 ± 13.65	25.64 ± 1.18	258.88 ± 11.05	11.69 ± 2.14
30	634.23 ± 10.89	67.96 ± 3.78	269.58 ± 13.92	128.13 ± 9.91
60	658.23 ± 16.37	77.95 ± 5.54	287.10 ± 14.28	199.13 ± 13.90
120	690.79 ± 12.46	86.70 ± 4.37	288.84 ± 11.76	282.28 ± 7.89
180	704.27 ± 4.75	100.44 ± 1.08	303.94 ± 13.10	418.87 ± 11.61
360	731.27 ± 13.70	101.88 ± 1.72	314.24 ± 5.89	461.56 ± 9.94
420	727.80 ± 13.78	105.83 ± 3.68	340.16 ± 12.67	471.68 ± 17.70

Table 3.Effect of Aging on Mg^{+2}- Atpase Content of Brain And Liver of Rats. Each Value is the Mean of 6 Determinations ± Standard Deviation

Age in days	Brain		Liver	
	Specific acitvity	Total ATPase in whole brain	Specific acitvity	Total ATPase in whole liver
4	519.48 ± 10.22	22.59 ± 1.00	224.66 ± 8.22	10.065 ± 1.20
30	530.35 ± 12.68	56.87 ± 3.35	226.87 ± 9.37	108.57 ± 9.91
60	560.19 ± 16.61	66.30 ± 4.23	237.58 ± 10.10	164.92 ± 11.34
120	583.60 ± 12.21	73.17 ± 6.11	239.60 ± 11.76	234.06 ± 4.78
180	590.70 ± 9.88	84.23 ± 2.27	250.47 ± 10.59	345.18 ± 8.56
360	601.64 ± 13.70	83.83 ± 1.59	257.09 ± 5.82	377.69 ± 6.07
420	592.73 ± 13.53	82.88 ± 0.83	278.87 ± 11.44	386.53 ± 8.70

Table 4 .Effect of Aging on $Na^{+}K^{+}$- Atpase Content of Brain and Liver Tissues of Rats. Each Value is the Mean of 6 Determinations + Standard Deviation

Age in days	Brain		Liver	
	Specific acitvity	Total ATPase in whole brain	Specific acitvity	Total ATPase in whole liver
4	70.50 ± 10.22	3.50 ± 0.55	34.22 ± 3.40	1.62 ± 0.15
30	103.89 ± 8.73	11.08 ± 3.93	42.72 ± 5.36	20.37 ± 1.15
60	98.33 ± 7.34	11.64 ± 1.49	49.24 ± 4.18	34.35 ± 3.04
120	107.19 ± 7.58	13.44 ± 2.02	49.24 ± 0.57	48.22 ± 3.62
180	113.57 ± 9.88	16.21 ± 3.02	53.47 ± 3.09	73.69 ± 3.76
360	129.63 ± 9.16	18.04 ± 0.52	57.14 ± 1.31	83.87 ± 3.98
420	135.07 ± 4.19	22.94 ± 0.87	61.29 ± 5.41	85.14 ± 8.70

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RESEARCH ARTICLE

Comparative Efficacy of Vermiwash and Synthetic Nutrients on Plant Growth and Phytonutrients of *Solanum lycopersicum* and *Capsicum annuum*

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ABSTRACT

The present study was conducted to evaluate the effect of vermiwash on plant growth and phytonutrients of *S.lycopersicum* and *C.annuum* plants. Physico-chemical properties of the soil, vermiwash, synthetic nutrients were studied and interrupted with results. The results revealed that vermiwash and synthetic nutrients were equally enhanced the plant growth, but major phytonutrients were lacking in synthetic nutrients treated plants. Chlorophyll, Carotenoid, protein and carbohydrate were significantly increased in vermiwash treated plots. From the results it could be seen that vermiwash extracts offer a valuable resource which could be effectively exploited for increasing the plant growth and nutritional properties. Vermiwash can be economically and environmentally suitable for the soil environment. Therefore, it may conclude that significant increase in the plant growth as well as phytonutrients of vermiwash treated plants is due to high level of macro and micronutrients available in the vermiwash.

Keywords : Vermiwash, Synthetic nutrients, Phytochemical, *Capsicum annuum, Solanum lycopersicum*

INTRODUCTION

Due to increasing population and development of human Civilization Industrialization increased the problem of environmental degradation. The rapidly use of chemical fertilizer and pesticide destroyed the fertility of soil and also produces the harmful diseases for crops and human mankind [1]. Abundant use of chemical fertilizers and pesticides

leads to concentration of chemicals and metals, which ultimately affect the ecosystem. The agricultural production based on chemical fertilizers and pesticides are dangerous for soil fertility and conservation [2]. So, organic manures like vermicompost technology can be a good substituted for chemical fertilizers to overcome their adverse effects. The vermincomposting technology can also be utilized for generating a bioliquid termed as Vermiwash. Vermiwash is a liquid leachate collected by allowing excess water to saturate the actively vermicomposting substrate in such a way that the water washes the nutrients from the vermicast excreted by the earthworms feeding on the substrate as well as the earthworm's body surface. Vermiwash has excellent growth promoting effects besides serving as biopromotor. In recent days the vermiwash is used as liquid manure. Even though much work has been done on vermicomposting, very few reports are available related to vermiwash and its impact on the plant growth. The present study was carried out to find out the physico-chemical, nutritional quantities of vermiwash and microbiological approaches for sustainable development of plant growth promoting factor or as a soil conditioning agent.

MATERIALS AND METHODS

Soil and Seed Collection for Crop Study

Soil samples were collected from garden land, without stones and hard materials. This finely powdered soil was used for our study. Tomato (*Solanum lycopersicum*) and Chilli (*Capsicum annum*) seeds were collected from agri department, Erode. Seeds were washed with running water and planted into soil.

Extraction of Vermiwash

Vermiwash was extracted from vermiwash collecting device. The apparatus consists of a plastic or metal drum with a capacity of 5 liter and a tap at the bottom. The drum was filled with broken bricks to about 10cm which is followed by sand layer of 2-3cm thickness, lastly filled with vermicompost with heavy population of earthworms. Simultaneously fresh water was added into the drum and a container was kept below the tap of drum. The watery extract of vermicompost i.e. vermiwash was drained out off drum and collected, drop by drop into the container. The color of vermiwash ranges from yellowish to black. After 1 to 2 days the process of extraction was completed [3].

Preparation of Synthetic Nutrients

Macro and Micro nutrients were prepared according to Howard resh [4].

Experimental Design

The effect of Water, Vermiwash and synthetic nutrients on seed germination and growth development of *Solanum lycopersicum* and *Capsicum annum* were studied. Soil acts as a carrier for plant growth. In this experiment the selected vegetable seeds were planted in 10 numbers in each control pot I (Soil with water treated), experimental pot II (soil with 10% vermiwash treated) and experimental pot III (soil with synthetic nutrients). During the whole growing period, growth parameters at every 45 days interval was measured and recorded.

Growth Parameters

The effect of different carriers and nutrients on growth and Biomass of the edible crop *Solanum lycopersicum* and *Capsicum annum* were conducted. Plant growth and Biomass of the edible crop was recorded [5].

Shoot length

On the day of final count of the germination test, ten normal seedlings were selected from each treatment and in each replication. The shoot length was measured from the base of primary leaf to base of hypocotyle and mean shoot length was expressed in centimeters.

Root length

Ten normal seedlings used for shoot length measurement were also used for the measurement of root length. It was measured from the tip of primary root to base of hypocotyle and mean root length was expressed in centimeters.

Plant fresh and dry weight

Plant samples were weighed with the electrical weighing balance and recorded the fresh weight. Fresh Plant samples were placed in paper bags, these were air dried. After that, the dry weight was weighed and recorded.

Physico-Chemical Analysis

Physico-chemical analyses were carried out in soil, 10% vermiwash, synthetic nutrients, according to APHA, Standard Methods for the Examination of Water and Wastewater [6].

Phytonutrient Studies

Estimation of Protein [7], Estimation of Carbohydrates [8], Chlorophyll Analysis [9] and Qualitative Phytochemical Analysis [10] were carried out for both plants.

Extracellular Enzyme Activities of Vermiwash

Amylase Activity (Starch hydrolyis)

The sample was spot inoculated on starch agar medium plates and incubated at 30°C for 48 h. At the end of incubation period, the plates were flooded with iodine solution, kept for a minute and then poured off. Iodine reacts with starch to form blue color compound. Hence the colorless zone surrounding colonies indicates the production of amylase [11].

Protease Activity (Caseinase)

The qualitative assay of protease production was performed on sterile skim milk agar plates. Sample was spot inoculated and followed by incubation at 30°C and zone of clearance around the colony indicating the enzymatic degradation of protease [12].

Lipase Activity

The sample was inoculated on a tributytrin agar medium and incubated at 37°C for 72h. The observation for a clear zone was examined around each isolates throughout the incubation period. The clear zones around the colonies indicated a positive result [13].

RESULTS AND DISCUSSION

The pot study was conducted by using base material of soil with the nutrient supplements of water, 10% vermiwash and synthetic Nutrients. *Solanum lycopersicum* and *Capsicum annuum* plant were selected for this study. Neutral pH was maintained in the vermiwash (7.2), which was consistent with the previous report (pH 7.52) given by Amita Chattopadhyay [14] and also (pH 7.18) Maya jaybhaye *et al.* [15]. Nitrogen and TDS of vermiwash was found as 20.6mg/l and 5090 mg/l recpectively. Maximum nutrients level was observed in vermiwash, especially protein, iron, chloride, calcium and magnesium (Table 1). Abdullah Adil Ansari *et al.* [16] reported the nitrogen content was 0.02% and a total salt content was 9841.67 ppm.

Biometric analysis was taken on 45th day and 90th day (Table 2 and Plate 1, 2). Biometric study consists of shoot length (cm), root length (cm), total length (cm), dry weight (g), wet weight (g), total leaves (No's), flower buds (No's)

and branches (No's). In both plants, maximum shoot length, root length and total length was recorded in vermiwash and synthetic nutrients treated pots on 90th day. No major variation was observed in both vermiwash and synthetic nutrients. It was understandable that, water alone doesn't provide good growth in plants while growing in soil base. The maximum plant height and number of leaves observed in chemical nutrients it can be accounted for by the fact that chemical fertilizers are high in nitrogen, which is responsible for plant growth. The maximum circumference of stem in chemical fertilizers can be accounted for by the fact that chemical fertilizers have a greater percentage of available salts such as nitrate, phosphate and potassium, which significantly increases plant growth. Similar report was proved by Abdullah Adil Ansari *et al.* [16]. According to his study, the number of leaves observed after week six, was maximum for plants treated with chemical fertilizers, followed by vermiwash and vermicompost.

Ten seeds were sown identical day in all the 6 pots (3 pots for *S.lycopersicum* and 3 pots for *C.annuum*). Compared to water, the maximum germination was obtained by the influence of vermiwash and synthetic nutrients. The % of germination was 40%, 60%, 70% (*S.lycopersicum*) and 30%, 50%, 60% (*C.annuum*) for water, vermiwash and synthetic nutrients respectively (Figure 1). On fresh weight basis, the addition of vermiwash has significantly enhanced the content of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids in both plants (Figure 2). Chlorophyll a is recognized as the main pigment which converts light energy into chemical energy. Chlorophyll b as accessory pigment acts indirectly on photosynthesis by transferring the light it absorbs to chlorophyll a [17].

16 types of phytochemical tests (Qualitative) were conducted for *S.lycopersicum* and *C.annuum* fresh leaves on 90th day (Table 3). It is clearly understandable that the alkaloid, acidic compounds, protein, carbohydrate and carotenoids were present in all leaves. Out of 16, 10 phytonutrients were absent in synthetic nutrient treated plant. Homaira Afreen *et al.* [18] revealed the presence of different groups in the methanolic extract of the leaf of *Solanum lycopersicum* Lit showed the positive result with reducing sugar, tannins, flavonoids, steroid, saponins and alkaloid. Tannins, cardiac glycosides, anthraquinone glycosides, resins, oxalate, leucoanthocyanin, coumerins, quinine, starch, oil and fat, diterpenoids, emodins, cholesterol, anthocyanin were totally absent in all pots. Steroids, Indole alkaloids, phytosterols, phylobatannins and terpenoids were only present in vermiwash treated plant. The indole alkaloids itself appears to be an essential constituent of the perfumes of flowers also it is used in the antipsychotic drug. Terpenoids used as antimicrobial and antidiarrheal agent. Phlobatannins have wound healing properties. Plant steroids are known to be important for their cardiotonic activities. They possess insecticidal and antimicrobial properties.

Protein and carbohydrate estimation were carried out for *S.lycopersicum* and *C.annuu* (Figure 3). It was recognized that the level of protein and carbohydrate content of the plants were highly intensified by vermiwash. Among the diverse treatments, 5600, 4250 µg/ml of protein and 516.9, 467.0 mg/l of carbohydrate were found in *S.lycopersicu* and *C.annuum* respectively. The reason for increase in protein content in vermiwash might be due to the presence of proteolytic enzymes that were secreted by the gut of earthworms. In *Capsicum frutescens* the protein content was higher in the vermiwash treated pots when compared to control [19]. The extracellular enzyme activity was analysed in vermiwash, our study revealed the vermiwash produced zones around the plates which represented the enzymatic activity of protease (Skim milk agar), amylase (Starch agar plates) and lipase (Tributryin agar). Presence of proteases in soil helps in seed germination while amylases help for availability of simple carbon source for enhancement of plant growth and productivity as well. Similar results were reported by Zambare *et al.* [20].

CONCLUSION

In the present study, the effect of vermiwash and synthetic nutrients was observed on the plants of *Solanum lycopersicum* and *Capsicum annuum*. However, it can be concluded from the present research work that the vermiwash proves to be an effective biopromotor than synthetic nutrients. Synthetic nutrients only enhanced the growth of

plants, but vermiwash participate the nutrition development along with the growth. Vermiwash revealed the potential application in sustainable development of agriculture with respect to its origin, cost effectiveness, easy availability, time saving, reproducibility, reliability and eco-friendly. Thus vermiwash can be used as a substitute of commercial fertilizers available in market.

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Table 1: Physico Chemical Analysis of Soil and Nutrients

S.No	Parameters	Soil	Vermiwash	Synthetic Nutrients
1	pH	6.20	7.20	5.00
2	TDS (mg/l)	1820.00	5090.00	1210.00
3	EC (mhos/cm)	2800.00	7830.76	1861.53
4	Calcium (mg/l)	280.00	520.00	80.00
5	Magnesium (mg/l)	168.00	320.00	48.00
6	Acidity (mg/l)	30.00	Nil	70.00
7	Alkalinity (mg/l)	1000.00	750.00	400.00
8	Chloride (mg/l)	195.56	595.56	148.89
9	Iron (mg/l)	4.00	6.00	3.00
10	Phosphate (mg/l)	3.00	4.00	6.00
11	Nitrogen (mg/l)	12.00	20.60	21.20
12	Protein (µg/l)	233.30	6333.30	666.60

Table 2: Biometric Analysis on 90th day

S.No	Parameters	Water		Vermiwash		Synthetic Nutrients	
		S.l	***C.a***	***S.l***	***C.a***	***S.l***	***C.a***
1	Shoot length (cm)	15.6	20.3	19.8	26.7	21.1	28.6
2	Root length (cm)	10.2	09.9	13.1	15.4	16.0	17.9
3	Total length (cm)	25.8	30.2	32.9	42.1	37.1	46.5
4	Dry weight (g)	0.55	0.49	0.61	0.58	0.62	0.60
5	Wet weight (g)	2.60	2.26	3.26	2.85	3.42	3.13
6	Total leaves (No's)	31.0	27.0	61.0	46.0	67.0	50.0
7	Flower buds (No's)	2.00	1.00	5.00	4.00	6.00	4.00
8	Branches (No's)	10.0	9.00	13.0	11.0	14.0	10.0

S.l – Indicates *Solanum lycopersicum; C.a* – Indicates *Capsicum annuum;*

Table 3: Qualitative Phytochemical Analysis

S.No	Parameters	Water		Vermiwash		Synthetic nutrients	
		S.l	***C.a***	***S.l***	***C.a***	***S.l***	***C.a***
1	Alkaloids	+	+	+	+	+	+
2	Flavonoids	+	-	+	+	-	-
3	Saponins	-	-	-	+	-	-
4	Glycosides	+	+	+	+	-	-
5	Acidic compounds	+	+	+	+	+	+
6	Protein	+	+	+	+	+	+
7	Amino acids	+	+	+	+	+	+
8	Steroids	-	+	+	-	-	-
9	Indole alkaloids	-	+	+	-	-	-
10	Carbohydrates	+	+	+	+	+	+
11	Phytosterols	-	+	+	-	-	-
12	Phylobatannins	-	+	+	-	-	-
13	Gum and Mucillage	-	+	+	+	-	-
14	Carotenoids	+	+	+	+	+	+
15	Phenols	-	-	+	+	-	-
16	Terpenoids	-	-	+	+	-	-

S.l – Indicates *Solanum lycopersicum; C.a* – Indicates *Capsicum annuum;* + indicates present; - indicates absent;

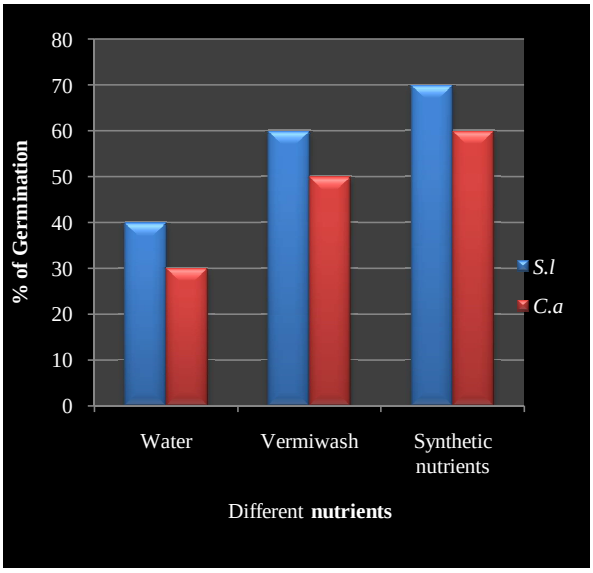

Figure 1: Percentage of Germination in *Solanum lycopersicum* and *Capsicum annuum* on 10th day

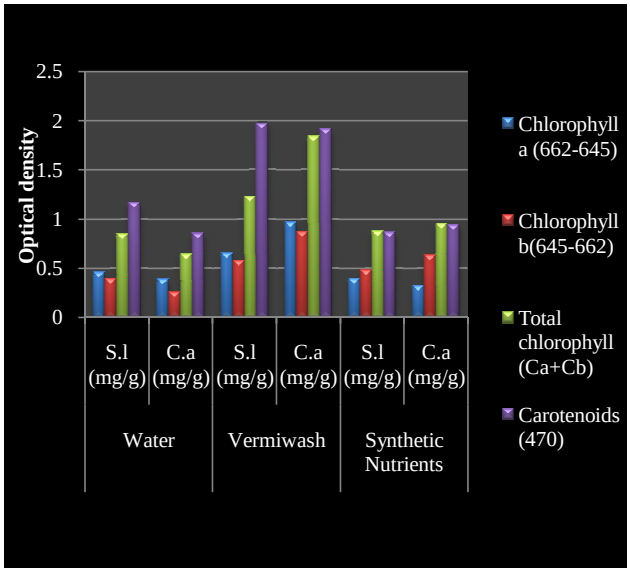

Figure 2: Chlorophyll, Carotenoid Estimation (80% Acetone) in *Solanum lycopersicum* and *Capsicum annuum*

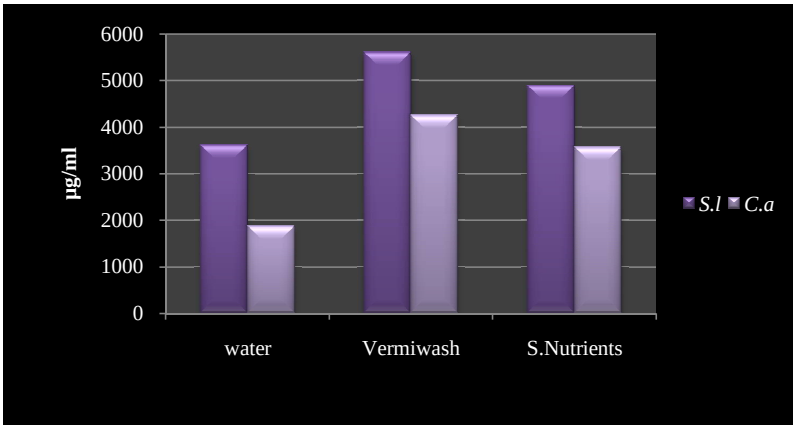

Figure 3: Analysis of Protein and Carbohydrate in *Solanum lycopersicum* and *Capsicum annuum*

Plate 1: *Solanum lycopersicum* treated with water, 10% vermiwash and synthetic nutrients

Plate 2: *Capsicum annuum* treated with water, 10% vermiwash and synthetic nutrients

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RESEARCH ARTICLE

Batch and Flow Injection Spectrophotometric Methods for Determination of Folic acid in Pharmaceutical Preparations using Cerium(IV) Ammonium Sulfate(CAS) as an Oxidant

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ABSTRACT

A simple, rapid and sensitive batch and flow injection spectrophotometric methods have been developed for the determination of folic acid (FA) in pure form and in its pharmaceutical preparations. The proposed methods involve the addition of a measured excess of cerium (IV) ammonium sulfate (CAS) in acid medium followed by determination of unreacted CAS by reacting with a fixed amount of methylene blue (MB) and measuring the absorbance at 665 nm. The optimum reaction conditions and other analytical parameters have been evaluated. Linearity was observed from 1.0-10.0 and 4.0-20.0 μg/ml folic acid by batch and flow injection procedures, respectively. Statistical analysis of the results and comparison with results by the British Pharmacopoeia method are also reported.

Keywords : Folic acid, Spectrophotometric, Flow Injection,Methylen blue,Cerium(IV)ammoniumsulfate.

INTRODUCTION

Folic acid (FA) is a member of the vitamin-B group(vitamin B9) in some term called folate.[1] chemically named as N-[4-[[(2-amino-1,4-dihydro-4-oxo-6-pteridinyl)methyl]amino]benzoyl]-L-glutamic acid (Fig.1).[2] The molecular formula is C19H19N7O6 and molecular weight 441.40 gm/mole. It is reduced in the body to tetrahydrofolate, which

is a coenzyme for various metabolic processes including the synthesis of purine and pyrimidine nucleotides, and hence in the synthesis of DNA. FA plays a major role in the synthesis of red blood cells, in the formation of RNA and DNA, in the development of tissues and the brain of the fetus and the growth of a baby[3]. There are various methods described for the determination of folic acid are based on high-performance liquid chromatography using different detectors.[4-6]Other methods use derivative spectrophotometry[7]spectrophotometry,[8-11], FTIR Spectroscopy[12] and adsorptive stripping voltammetry.[13].Flow injection spectrophotometry[14].

However, some of these procedures suffer from one or another disadvantage such as narrow range of determination; require heating or extraction, long time for the reaction to complete, and also instability of the colored product produced. The use of CAS as an analytical reagent offered adequate sensitivity and accuracy, also the simplicity and low cost of the analytical method. The use of CAS for the determination of FA has not been reported yet. Therefore, the present study was undertaken to evaluate CAS as an analytical reagent for the spectrophotometric determination of FA. Flow injection (FI) system is adequate procedure to use in routine analysis in pharmaceutical laboratories control due to their simplicity, high analytical frequency and capacity to reduce reagent consumption when compared with batch procedure.

The purpose of the present investigation was to develop, two simple, rapid and sensitive batch and FI methods using spectrophotometric detection were described for the determination of folic acid was presented. The method was based on the oxidation of folic acid by a known excess of cerium(IV)ammonium sulfate (CAS) in acidic medium followed by a reaction of the excess oxidant with methylene blue (MB) to bleach its blue color.The proposed methods have been successfully applied to the determination of FA in different brands of tablets.

MATERIALS AND METHODS

Materials

All chemicals were of analytical reagents grade.

Folic acid stock standard solution

A solution of 1000 μg.ml-1 was prepared by dissolving 0.1000 g of FA(provided by Company for Drug Industries and Medical Applications SDI, Samarra, Iraq) in 10 ml of 0.1 M sodium hydroxide and then the volume is made up to 100 ml in a volumetric flask with the same solvent and kept in the dark at 5C° in plastic container, this solution is stable for one week. Working standard solutions were prepared by suitable dilution of the stock standard solution.

Cerium (IV) ammonium sulfate (100μg/ml)

I prepared by dissolving 0.1g of CAS (BDH) (Ce (NH4)4 (SO4)4.2H2O) in a least amount of 0.5M H2SO4 and diluted to 1000 ml with distilled water. Working standard solutions were prepared by dilution with distilled water.

Methylene blue (100μg/ml)

I prepared by dissolving 0.1g of MB (E. Merck) (3,7bis(Dimethylamino)-phenothiazin-5-ium chloride) (C16H18ClN3S) in water and diluted to 1000 ml with distilled water. Working standard solutions were prepared by dilution with distilled water.

Sulphuric acid (1M)

A 1.0 M of H2SO4 (SDFCL) was prepared by diluting 5.4 ml of concentrated acid (Sp.gr. 1.8, 98%) to 100 ml with distilled water. More dilute solutions were prepared by dilution with distilled water.

Solutions of interferences

A stock standard solution of each interfering species (sodium chloride, glucose, sucrose, fructose, lactose, galactose, and starch) was prepared by dissolving 0.1 g of the compound in distilled water then the volume is completed to 100 ml in calibrated flask. Other solutions were prepared by serial dilutions of the stock solution.

Preparation of tablets solutions

Weigh and finally powdered an enough number of tablets followed by extraction of an accurately weighed portion of the powder equivalent to about 0.1 g of FA dissolving in 0.1 mole/l sodium hydroxide. Shake and filter the solution in to a 100 ml volumetric flask. Wash the residue on the filter with 10 ml portions of 0.1 M sodium hydroxide solution and dilute the combined filtrate and washings to the mark with the same reagent, to obtain (1000 μg/ml) of FA. Working solution was prepared by appropriate dilution of 10 ml of this solution to 100 ml by distilled water, to obtain (100 μg/ml) of FA. Other solutions were prepared by serial dilutions of the stock solution.

Instrumentation

All spectral and absorbance measurements were performed on a (Spectral and absorbance measurements were carried out by a Bio-Tek Instrument UV-Vis spectrophotometer (model J643002, Vermont, USA), using 1.0 cm quartz cell. The FI system comprised(Absorbance measurements of both reagents were carried out on a Bio-Tek Instrument UV-Vis spectrophotometer (model J643002, Vermont, USA). Spectral and absorbance measurements were carried out by an A&ELAB Instrument UV-Vis spectrophotometer (model AE-S60-2U, P.R.C.), using 1.0 cm quartz cell. Flow cell with 30μl and 10mm path length quartz was used.Multi-channel peristaltic pump (Watson-Marlow 5012, USA) used for propelling merged streams. A 6-way injection valve with various sample loops was used.

Procedures

Recommended batch procedure

In to a series of 25 ml volumetric flasks an increasing volume of FA solution (100 μg/ml) was transferred to cover the range of the calibration graph (1.0 – 10.0 μg/ ml). Then 0.4 ml of H_2SO_4 (1 M) and 2.5 ml of CAS (20 μg/ml) were added. The solutions were lifted for 10 min at room temperature (25°C), finally adding 1.5 ml of MB (10 μg/ml) then dilution to the mark with distilled water. The absorbance was measured after 5 min at 665 nm versus the reagent blank, prepared in the same manner but containing no drug.

Recommended FI procedure

A volume of 100 μl of prepared sample solution (FA) was loaded into the sample loop by means of a syringe. Sample was injected into a 0.02 M H_2SO_4 carrier stream pumped at a rate of 0.80 ml/min. The CAS solution (30 μg/ml) was added to the carrier stream at a rate of 0.80 ml/min in a confluence manner downstream to ensure rapid and adequate mixing. After that, the MB solution (15μg/ml) was added to a stream containing the unreacted CAS at a rate of 0.80 ml/min. After injection, the valve was returned to the load position when the maximum change in absorbance value had been reached. The absorbance was monitored at 665 nm in a quartz flow cell, at which the maximum absorption occurred and connected to a recorder, at 0.5 Mv and with a chart speed of 25 cm/h. FI system is shown in Figure (3.3).

RESULTS AND DISCUSSION

CAS has been used as an analytical reagent for many organic compounds .In the present work, it was found that CAS can oxidize FA in an acidic medium. In addition, it reacts immediately with MB in an acidic medium to bleach outits blue color. Therefore, after the oxidation of the drug under investigation by CAS, the excess CAS was reacted with the MB. The absorption spectrum of the MB which has maximum absorption at 665 nm shown in Fig.2.

Therefore, the different parameters affecting the oxidation reaction, and hence the subsequent determination of these drugs were optimized.

FA+ CAS (excess) → FA (oxidation product) +CAS (unreacted)
CAS (unreacted) + MB → Oxidation product of MB + Unreacted MB (indicator bleaching)
↓
(Measured spectrophotometrically at λmax=665 nm)

Optimization of variables

For spectrophotometric method

Effect of methylene blue amount

The effect of amount of MB dye concentration on the intensity of color was carried out in the range of 0.5 - 2 mL and it was found that the optimum concentration to maximum absorbance was obtained with 1.5 mL 10 μg.ml-1 of methylene blue dye in final volume of 25 ml.

Effect of oxidant amount

Cerium ammonium sulfate(CAS) was found to be a useful oxidizing agent, also the effect of different volumes (0.5 to 4.5) ml of 20 μg.ml-1 of CAS on the color of the dye was studied the results indicated that 2.5 ml of CAS solution was enough to obtain a maximum bleaching of the color of methylene blue dye therefor it was recommended in the subsequent experiments is shown in Fig.3.

Nature and amount of acid

The reactions were tested in HCl, H2SO4, HNO3, and CH3COOH solutions. The results indicated that the reaction is suitable in hydrochloric acid medium. A1 M H2SO4 as found to be adequate for the oxidation of the drugs. The variation in H2SO4 amount from 0.1 to 1.0 ml has been studied. The maximum absorbance was achieved when the H2SO4 was 0.4 ml of 1 M, which was used in all subsequent experiments.

Effect of temperature

The effect of temperature on the color intensity of methylene blue color studied. In practice a maximum absorbance was obtained when the color was developed at room temperature (25°C), decrease in color intensity and stability was observed in low or high temperature, therefor room temperature is recommended for subsequent experiments.

Order of addition

To obtain optimum results the order of addition of components should be studied. The results indicated that the order of addition of reagents should be followed as given by the procedure, otherwise, a loss in color intensity and stability are observed.

Effect of time on oxidation

It was observed that if methylene blue was added immediately to the solution containing FA and CAS in acidic medium the resulted solution is bleached rapidly and the absorbance is very low. This can be explained by the fact that the drug oxidation by CAS is a time developing reaction and thus the influence of the reaction time was studied. In this respect, solutions containing 3.0 ml of 100μg/ml FA, 0.4 ml of 1M H2SO4 and 2.5 ml of (20μg/ml) CAS have been let to react at darkness in different times before adding the indicator and measuring the absorbance at 665nm. The results indicated that 10 minute was the optimum time to give complete oxidation of FA and 5 minutes was

chosen as standing time to bleaching of the color of the dye MB, the color was remains constant for another 55 minutes.

Optimization of FI method

Optimization of chemical parameters

Batch method for the determination of FA was adopted as a basis to develop FI procedure. The manifold used for the determination of folic acid is shown in Fig.4. The parameters of flow in the determination of FA were optimized. According to the results of preliminary spectrophotometric studies concerning the effect of acidic medium on the absorbance of the product, hydrochloric acid was used for the FI method.

Effect of H2SO4 concentration

The effect of the concentration of sulphuric acid was studied in the range 0.01-0.1 M with fixed FA concentration of (10µg/ml). A gradual increase in the analytical signal was observed with increasing the H2SO4 concentration (Fig.5). However, the stability of the baseline and consequently the reproducibility of the results were significantly reduced at higher H2SO4 concentrations. Therefore, 0.02 M H2SO4was used as a carrier as it gives a reasonable sensitivity and baseline stability.

The effect of CAS concentration

The influence of the CAS concentration on the absorbance was studied at constant concentrations of FA. Fixed volumes (100 µl) of FA was used and injected into the H2SO4 stream. The effect of changing the concentration of CAS in the range (10-45) µg/ml on the absorption peak height was shown in Fig.6.The figure shows that, a maximum analytical signal was achieved when the CAS concentration reached to 30 µg/ml , and was chosen for further use.

Effect of MB concentration

The effect of a different concentration of MB in the range of (5-30) µg/ml at constant concentrations of FA (10 µg/ ml) on the absorption peak height is shown in Fig.7. The figure shows that maximum analytical signals were achieved when the MB concentration reached to 15µg/ml, and was chosen for further use.

Optimization of physical parameters

Effect of flow rate

Various flow rates (0.5, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2, 3.4 and 3.6) ml/min for propelled solutions for the system were studied. As it is shown in Fig.8, the maximum absorbance value was achieved when the final solution passed through the detector with a flow rate of 2.4 ml/min for the system.

Effect of coil length

In order to investigate the effect of this factor, three coils with different lengths (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100) cm were examined for the system. As shown in Fig.9, the optimum values of coils lengths for the mixing coil and for both first and second reaction coils were found to be (20, 60 and 20 cm) respectively.

Effect of sample volume

After injection the sample zone moves downstream through the manifold toward the detector, so the influence of different sample volumes (25, 50, 75, 100, 125 and 150) µl. The absorbance increases with increasing the volume of the sample introduced in to the flow system up to 75 µl for CAS system after which the absorbance slightly decreases as a result of sample dilution is shown in Fig.10.

Analytical characteristics

Analytical characteristics such as regression equation, linear range, relative standard deviation, relative error, molar absorptivity and Sandell's sensitivity values of each method were determined under the optimized conditions as shown in Table1.The limits of detection (LOD) and quantitation (LOQ) were calculated according to the International Union of Pure and Applied Chemistry (IUPAC) definition 18 using the formula:

LOD = 3S/b and LOQ = 10S/b

Where: S is the standard deviation of blank absorbance values and b is the slope of the calibration plot, are also presented in Table 1.The high values of molar absorptivity and low values of Sandell's sensitivity and LOD indicate the high sensitivity of the proposed methods.

Interference studies

In order to assess the possible analytical applications of the proposed analytical method described to the assay of commercial FA formulations, the effect of some excipients frequently present in the pharmaceutical preparationswere investigated by carrying out the determination of FA in the presence of different excipients. Experimental results showed that sodium chloride, glucose, sucrose, fructose, lactose, galactose, and starch had no effect on the determination of FA with concentration (10 μg/ ml) which having the recovery% by batch and FI-spectrophotometric methods that ranged from 98.5 to 100.0% and 98.0 to 100.0%, respectively for three replicates.

Application to analysis of tablets

The proposed batch and FIA spectrophotometric methods were successfully applied to the determination of FA in different brands of tablets. The results were summarized in Table 2. In comparison of the batch and the FIA procedure with standard method (HPLC), wider linear range of calibration graph, and good recovery were obtained. Also the results indicated that there was no significant difference between the proposed method and the reference method with respect to accuracy and precision at 99% confidence level.

CONCLUSION

In the current research study the followings are concluded, two simple methods have been developed to determine Folic Acid in pharmaceutical preparations. The developed procedures based on addition of a measured excess of CAS in acidic medium followed by determination of unreacted CAS by reacting with a fixed amount of methylene blue and measuring the absorbance at 665 nm. The proposed methods need neither temperature nor pH control and nor long time for the reaction to complete. The methods were successfully applied in different brands of tablets.

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Table(1): Analytical characteristics of the proposed method for folic acid

Parameter	Batch method	FI- method
Beer's law range (μg / ml)	1.0-10.0	4.0-20.0
Detection limits (μg / ml)	0.03	0.1
Quantitation limit (μg/ml)	0.1	0.4
Molar absorptivity (l /mole.cm)	4.753×104	0.8960×104
Sandell's sensitivity (μg /cm2)	0.009	0.04
Regression equation (Y = a + bC)*		
Intercept (a)	0.0073	0.0169
Slope (b)	0.1077	0.0203
Determination coefficient (R2)	0.9961	0.9982

Table (2):Application of the proposed method for the determination of folic acid in different brands of tablets

Method	Pharmaceutical products	Composition	Content (mg) declared	Found (mg)				Error%*
				standared method	Batch method	Error %*	FI method	
Using (CAS) oxidant	Folic awa	Folic acid	5	5.69	5.49	-3.51	5.44	-4.38
	Actavis Folic acid-	Folic acid	5	4.80	4.77	-0.62	4.60	-4.01
	Julphar Folicum-	Folic acid	5	4.38	4.59	4.79	4.25	-2.96
	Femitol	Folic acid	5	5.01	4.83	-3.53	4.83	-3.53

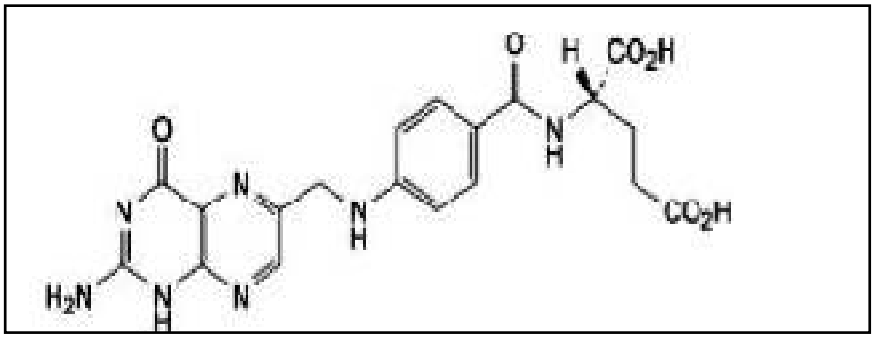


Fig. 1: structure of Folic acid

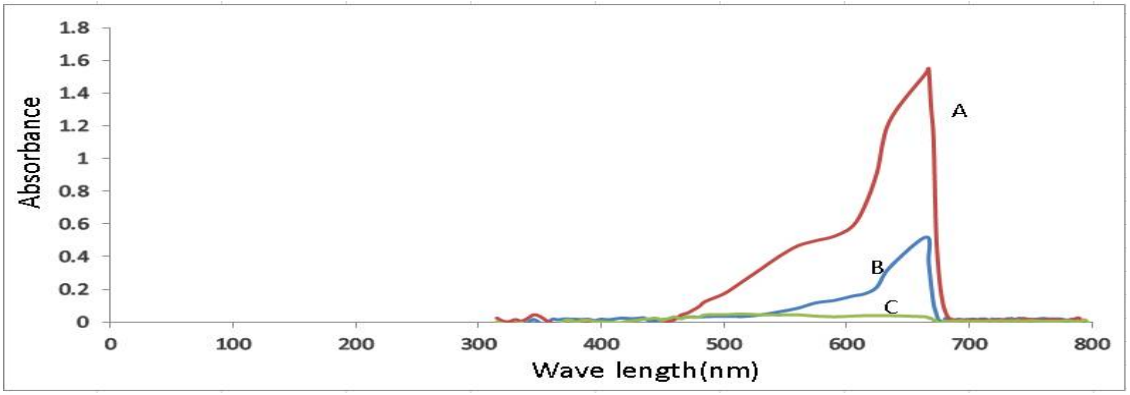


Fig. 2 Reactions of indirect determination of FA by oxidation with CAS:

(A) Absorption spectra of 1.5 ml of (10.0 µg mL-1) methylene blue, (B) Absorption spectra of 5.0 µg/ml folic acid against reagent blank, (C) reagent blank against distilled water.

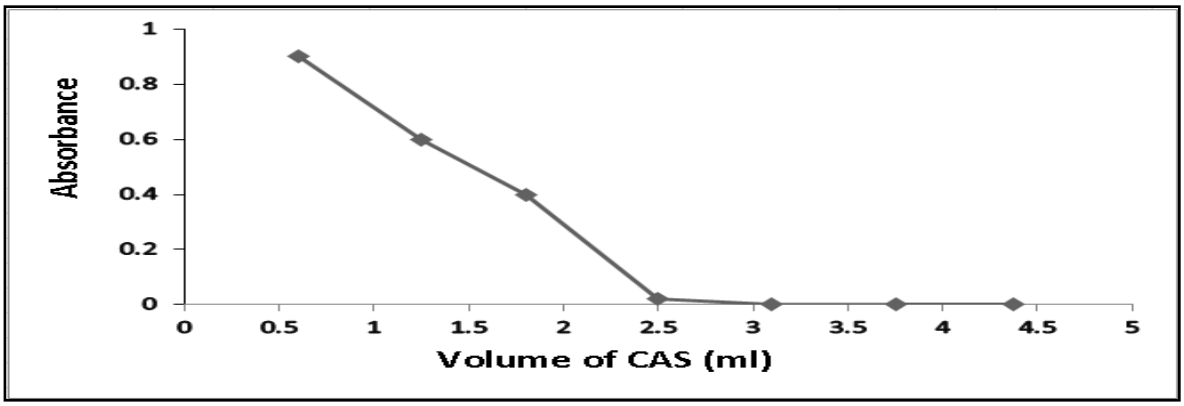


Fig.3: Effect of CAS amount on bleaching the color of MB dye

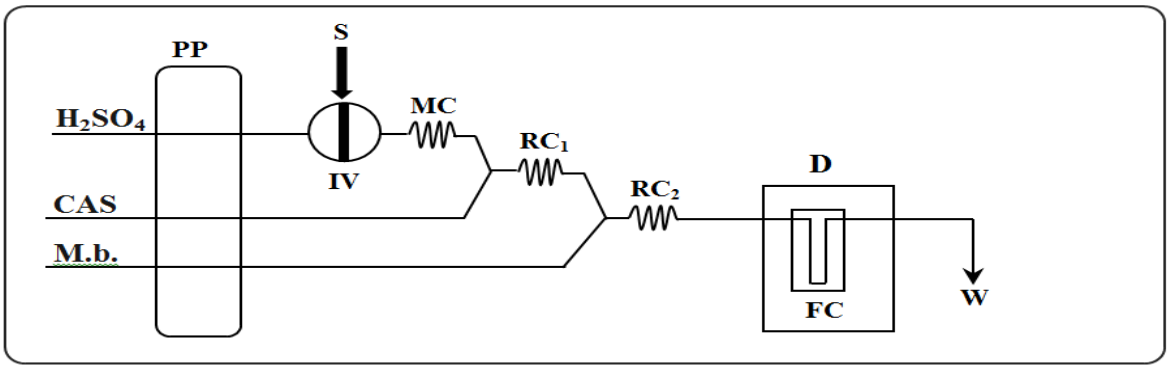
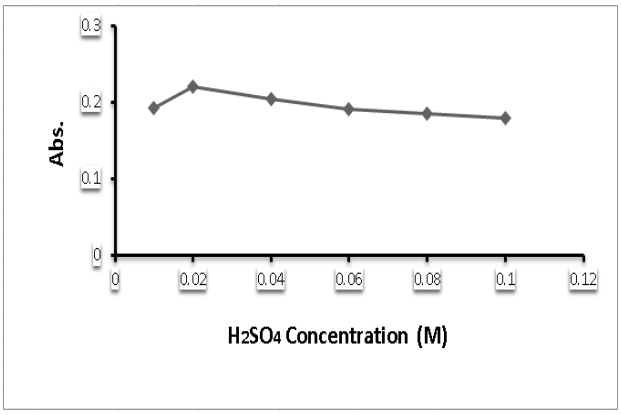


Fig.4: FIA manifold used for determination of folic acid using CAS as oxidizing reagent:
(PP) peristaltic pump, (S) sample injected, (MC) mixing coil, (RC1) 1st reaction coil, (RC2) 2nd reaction coil, (IV) injection valve, (D) detector, (FC) flow cell, (W) waste.



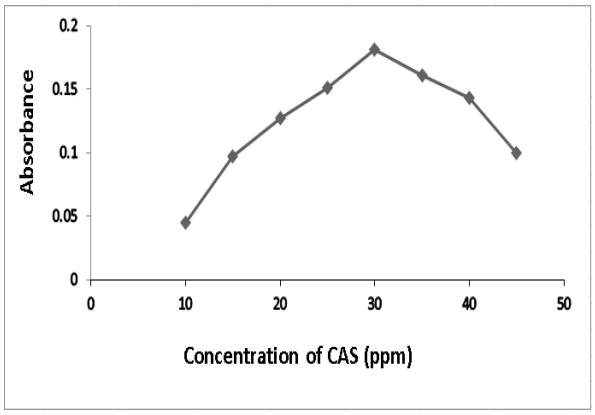


Fig.5: Effect of H2SO4 concentration on absorbance, using CAS as oxidizing reagent

Fig.6: Effect of CAS concentration on absorbance, using CAS as oxidizing reagent

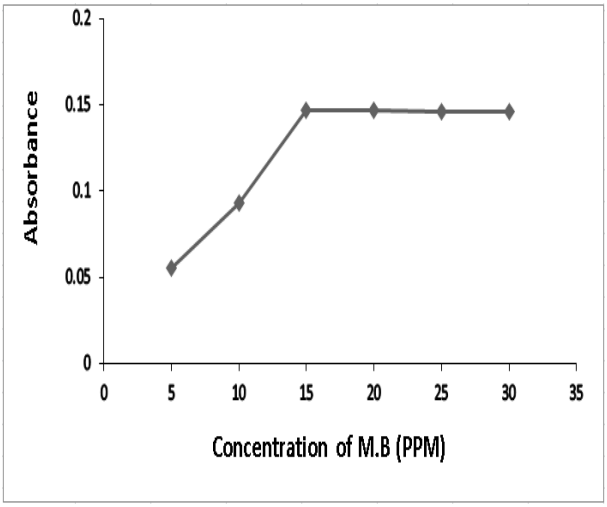


Fig.7: Effect of M.B concentration on absorbance, using CAS as oxidizing reagent

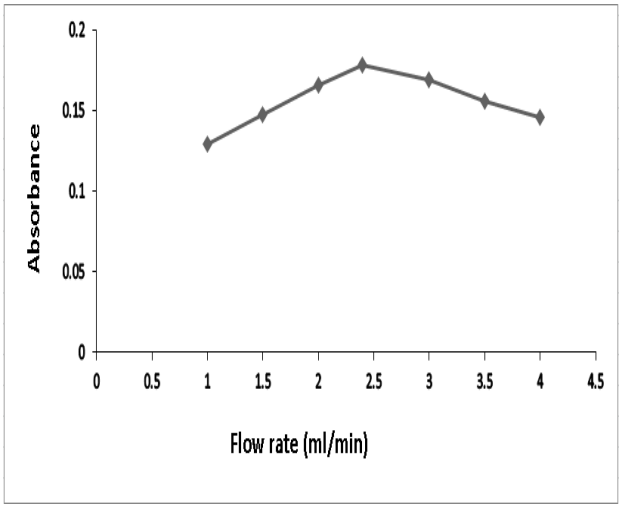


Fig.8: Effect of flow rate on absorbance, using CAS as oxidizing reagent

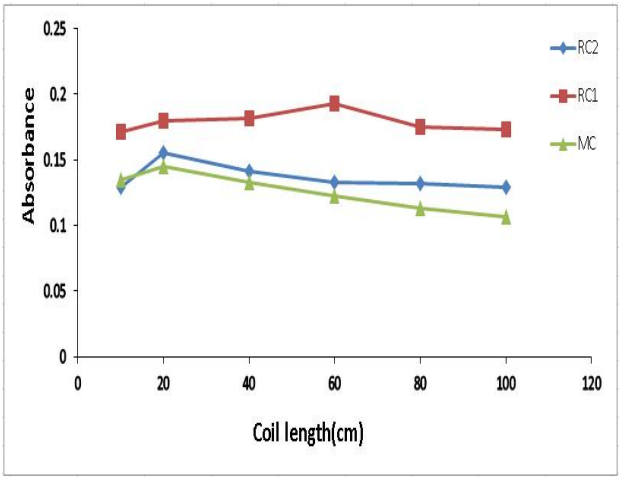


Fig.9:Effect of coil length on absorbance, using CAS as oxidizing reagent

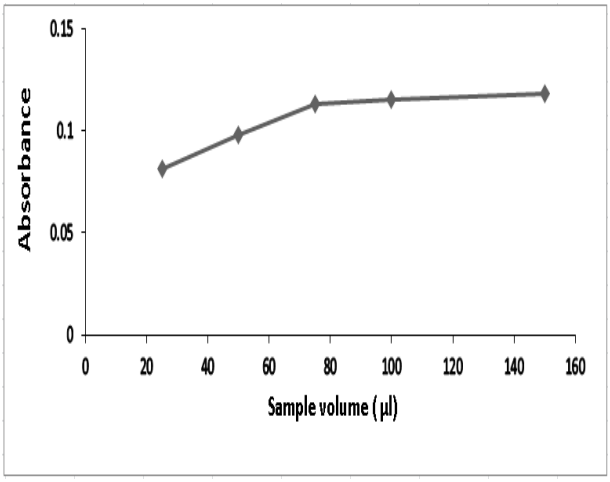


Fig.10: Effect of sample volume on absorbance, using CAS as oxidizing reagent

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RESEARCH ARTICLE

Design, Fabrication and Sensitivity Analysis of Different Types of Co-Axial Thermocouples using Oil Bath Based Calibration Technique

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ABSTRACT

Coaxial thermocouple is use for measuring highly transient surface heat flux because of small short response time (0.1 m/s) in the range of millisecond or less. Thermocouple gives permits to mounting directly through heat wall surface of any Geometry which is important for accurate measurement of rapidly changing surface temperature which is use in wall temperature directly interest. Coaxial thermocouple are generally fabricated by inserting one thermocouple element over the second element with an insulating material in between with a typical thickness of about 1 to 5µm and it is possible to fabricate at home. The purpose of this work is to fabricate different type of co-axial thermocouples (K-Type, E-Type and T-Type) using different type of materials (Alumel, Chromel, constantan, copper and Teflon) having different thermal property. After fabricated all these co-axial thermocouples are statically calibrated by using oil bath based technique and typical value of sensitivity for each co-axial thermocouples are calculated and then results was compared between them. In this calibration technique is used to check linearity between changes in voltage and temperature. The typical value of temperature coefficient of resistance also calculated from static calibration and it's found that these thermocouples are very sensitivity.

Keywords : Co-axial Thermocouples, oil bath calibration technique, sensitivity analysis.

INTRODUCTION

The measurement of transient heat transfer is very important in the engine areas, aerodynamics vehicle of high speed flow environment, cooling devices [1]. Due to rapidly changing of flow condition measurement procedure for accurate prophecy of heat fluxes must be transient and having fast response time. Coaxial thermocouple can measure suitably high transient surface temperature because of small response time. The co-axial are used to measure transient surface temperature by mounting the co-axial thermocouples embedded inside heating material surface. The surface heat fluxes are then estimated from the transient temperature data. The thermocouples are suitable for measuring temperature data in very short duration tests has been well established [2]. The coaxial thermocouple design has originally been proposed by [3] which are made of small wire coated with thin layer of aluminium oxide insulation.

Coaxial thermocouple have been used in the measurement of transient surface temperature and surface heat fluxes on internal combustion engine on model of aerodynamic facilities like shock tunnels [4] and as a tool study difference in heat fluxes of the frozen and equilibrium flows experiment carried out in high enthalpy One-dimension of co-axial sensor as per the definition of dimensionless thermal piercing extent is given in [5] and material properties thermal diffusivity, thermal conductivity, of Alumel, chromel, constantan copper and Teflon having temperature range [6]. K-type thermocouple (Alumel & Chromel) is most common general propose thermocouple having sensitivity of 41µV/^{0}C (Approximately) and with chromel positive relative to alumel. It is inexpensive and a wide diversity of probes is available range of -200^{0}C to 1350^{0}C, E-type thermocouple (chromel/constantan) has high output 68µV/^{0}C which make it well suited cryogenic use but itself non-magnetic and T-type (constantan/copper) thermocouple are suited for measurement is temperature range -200^{0}C to 350 ^{0}C often used as differential measurement since only copper wire touches the probes. Both conductors are non-magnetic; there is no abrupt change in characteristics, T-types thermocouples having sensitivity of about 43µV/^{0}C. Copper has much higher thermal conductivity than the alloy used in thermocouple construction, and so it is necessary to exercise extra care with thermally anchoring type T [7].

The fabrication of these thermocouples in the laboratory is always an art rather than the manufacturing process. The calculation of temperature data and convective rate is very important for devising and effective cooling system in internal combustion engine and typical high speed aerodynamics vehicle. The thin film gauge and thermocouple have highly sensitive and having very small time response (millisecond) and application reason of their prediction measurement capability as well as easy to fabricate in house.[8] and it's have been low coast and because of their simplicity and comparatively rapid response, fine wire thermocouple generally used, due to lack of strength and difficulties in the positioning the junction at the point interest fine wire thermocouple is not applicable reason of very small size of junction effected the response rate [9] which is limited to the wire diameter .

Heat flux calibration for demonstrated surface is applicable for thin film, resistive temperature gauge and thermocouple, an alternative calibration method has been proposed for estimating the surface heat fluxes for transient surface temperature data associated with thin film resistive temperature gauge and co-axial thermocouple [10], Determination of transient surface heat fluxes from the temperature history is one of the useful technique in engineering for short-time period,(millisecond), one dimensional heat conduction simulation are generally used for inter surface heating rate body, closed from temperature history measurement is very important for an analytical modelling. Due to environmental and industrial issue during transient heat and mass transfer there is no direct system to measure the heating rate, during the measurement in this process hating rate are predicted by temperature analysis[11] may types of measurement such as thermos couple liquid crystal gauge, infrared thermographs etc. have been discussed.

The propose of this work is experimentally analysis of sensitivity for three different type of coaxial thermocouple K-type, E-type and T-type which is contains three different type of material (chromel & alumel), (chromel & constantan) and (copper & constantan) respectively and isolated material Teflon with 1 micrometre thickness using static calibration also known as oil bath based calibration technique [13] measured voltage in temperature range 45^{0}C to 85^{0}C for each co-axial thermocouple, typical value of sensitivity and thermal co-efficient resistance (TCR) is calculated. The main purpose of this paper is to design, fabrication and static calibration of thermocouples (K-type, E-type and T-type) used for transient measurement.

MATERIALS AND METHODS

Design and Fabrication of Co-axial Thermocouples

The coaxial thermocouple are fabricated use of two different type of thermocouple materials having different diameters, inserting one wire on second and placed coaxially (Fig.1). The K-type coaxial thermocouple is prepared with alumel and chromel wire having diameter 3.25mm and 1 mm respectively, in E-type chromel and constantan wire having diameter 3.25mm and 1 mm respectively and T-type fabricated use of constantan and copper wire having diameter 3.25mm and 1 mm respectively.Due to the diameter of these wire is very small it's needs carefulness work during the fabrication process. The wire having small diameter inner element while the drilled wire from outer tube element, here chromel is inner element and alumel is outer tube element for K-type, constantan is inner element while chromel is outer tube element E-type and copper is inner element & constantan is outer tube in T-type coaxial thermocouple. Before assembling two wires to form the junction appropriate length of each wire is prepared as so keep overall size of coaxial thermocouples as smaller as possible.

The inner wire which is kept longer then outer tube is doused in insulating materials (Teflon) and after that leave it to cool in air for few hours until the insulating material coated has hardened then it is place co-axially in 1.01 mm hole. The insulating material (Teflon) is used to fix tightly over the chromel for K-type, on constantan wire for E-type and on copper for T-type so as to avoid electric contact between these two thermocouple materials. A hole of 1.01 mm diameter drilled on alumel, chromel and constantan wire while insulated the chromel, constantan and copper wire and inserted respectively and tightly fitted. Teflon is used as an insulating material thickness of one micrometre for these coaxial thermocouples. The junction is formed at flush end by means of grinding process with average impact load of about 2N/mm followed by creating scratches through grinder. It does create plastic deformation of one metal over the other by removing the bridge of Teflon between two thermocouple materials. In this process a microscopic junction on the surface (10-15µm) is formed with very low thermal mass and inertia [8, 14]. The connecting lead wire is then soldered to each of the thermocouple wire without affecting the thermocouples characteristics. Different types of co-axial thermocouple shown in the flowing picture(Fig.2).

Working principle of thermocouple

In 1821, an Estonian physicist named Thomas Johann Seebeck discovered that when any conductor (such as a metal) is subjected to a thermal gradient then it will generate a voltage. Thermocouples make use of this principle called as, Peltier-Seebeck effect.Thermocouples produce an output voltage which depends on the temperature difference between the junctions of two dissimilar metal wires. It is important to note that it measures the temperature difference between two points, not absolute temperature. For practical measurement of temperatures, the junctions of specific alloys are used which have a predictable and repeatable relationship between temperature and voltage. Different alloys are used for different temperature ranges. Properties such as resistance to corrosion may also be important when choosing a type of thermocouple because any erosion of the metal films alters the sensitivity of the thermocouples.

Where the measurement point is far from the measuring instrument, the intermediate connection can be made by extension wires which are less costly than the materials used to make the sensor. Thermocouples are usually standardized against a reference temperature of 0°C. Any attempt to measure this voltage necessarily involves connecting another conductor to the 'hot' end. This additional conductor will then also experience the temperature gradient, and develop a voltage of its own which will oppose the original. Fortunately, the magnitude of the effect depends on the metal in use. Using a dissimilar metal a complete circuit can be created in which the two legs generate different voltages, leaving a small difference in voltage available for measurement [7].

Static Calibration (Oil bath based calibration technique)

Static calibrations refer to the Act of evaluating and regulate the precision and accuracy of the measurement equipment. During the calibration it is also important that the time period of measurement is also conclusive. First the coaxial thermocouples must be calibrated to ensure that they are performing as designed. The static calibration or oil bath arrangement provides gradual set up variation in the temperature fed that co-axial thermocouple, there for an oil bath method is used to produced hot air in a beaker where K-type, E-type and T-type co-axial thermocouple is placed simultaneously. Heat is supplied by heat source (heater) that produced hot air in the beaker as shown in Fig.2. A thermometer is also mounted in the beaker along with thermocouples to record the temperature of hot air. It is also ensured that the temperature is uniform in the beaker. A multi-meter is used to monitor the change in voltage across these coaxial thermocouples corresponding change in temperature. For better result data acquisition system can use in place of multimeter. The calibration results of coaxial thermocouple recorded in two case first when temperature is increases from 45^0C to 85^0C and secondly taken when temperature decreases from 85^0C to 45^0C. During this calibration process the temperature and voltage monitored in multimeter were recorded in the step of 10^0C. The schematic diagram of static calibration is shown in Fig.3.

Determination of TCR and Sensitivity

The co-axial thermocouples made from two different materials that produced voltage proportional to temperature difference between ends of the pair conductors. The resistance of these thermocouples are very sensitivity to temperature and increasing with increasing of temperature when hot air is blow over it. This results in change in voltage of circuitry. For a change in temperature ΔT and if there is change in voltage across the thermocouple ΔV then TCR can be express as [1]. The mathematical expression for variation of resistance with temperature change is expressed as follows:

$$R(T) = R_0 \left[1 + \alpha (T - T_0)\right] \quad (1)$$

Resistance is converted to change in voltage using Ohm's low and α is calculated as

$$\alpha = \left(\frac{1}{V_0}\right)\left(\frac{\Delta V}{\Delta T}\right) \quad (2)$$

Where α TCR of thermocouple measured by static calibration and V_0 is the initial voltage.

Sensitivity analysis is the observation of variation in output of static model and its can be attributed to the different variation in input of the model or it may say that measurement technique for systematically changing in variable in model to determine the effect such changes. The sensitivity of materials is denoted by S which is depends on temperature and crystal structure. The material having half-field bands electrons (negative charge) and holes (positive charge) they have small sensitivity because both contribute to induced thermoelectric voltage as result so it cancel each other to contribution to voltage as result effect its sensitivity. In contrast semiconductors can be doped with an excess amount of electrons or holes and thus can have large positive or negative values of thermo power depending on change in the excess carriers. The sign of the sensitivity can determine which change carriers dominate

the electric transport in both metals and semiconductor. If ΔT is temperature difference and ΔT is thermometric voltage between two ends of a material, then sensitivity of that material is defined as [1].

$$S = \left(\frac{\Delta V}{\Delta T} \right) \quad (3)$$

RESULTS AND DISCUSSION

In this calibration three types co-axial thermocouples(K-type, E-type and T-type) has been taken. A multimeter is used to monitor change in voltage crossponding change in temperature range from 45^{0}C to 85^{0}C during heating and process is repeated druing cooling from 85^{0}C to 45^{0}C with a step change in temperature of 10^{0}C. The linear variation of voltage with temperature is recoreded which is shwon if Fig.4 in which Fig.4(a)-4(c) as shown variation for K-type, and E-type and T-type coaxial thermocouples respectively. A linear variation has been occurred in voltage corresponding variation in temperature during heating process and process is repeated during cooling (Fig.3) There are some voltage lose during cooling process due to loses of heat. and typical values α and S is calculated from equation (2) and (3) which are obtain as for K-type 0.067/^{0}C and 36.5µV/^{0}C. similarly for E-type and T-type 0.04/ ^{0}C, 45.25µV/^{0}C and 0.096/^{0}C, 34.5 µV/^{0}C respectivily.

The same value has been used for the further analysis to compute temepratures from measured change in voltage. Here E-type thermocouple is more sensible as compare to K-type and T-type thermocouple that can see in results. K-type thermocouple is most of common type of thermocouple having low cost, accurate reliable and has wide temperature range while E-type thermocouple has more strong signal and accuracy as compare to k-type and T-type co-axial thermocouples.

CONCLUSION

Three types of co-axial thermocouples (K-type, E-type and T-type) has been fabricate at home for static calibration. This technique is used to get a good linear response between temperature and voltage signals. These results are compared with each other and found that E-type co-axial thermocouples are more sensible than K-type and T-type and T- type having more TCR than K-type and E-type co-axial thermocouple. These co-axial thermocouples sensor can be used for routine measurement of transient temperature for short duration because of having high sensitivity.

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Table 1: Thermal properties of co- axial thermocouples materials [1, 4]

Metal	Thermal Conductivity (K)(w/mK)	Specific Heat (C)(J/kgK)	Density (ϱ)(kg/m³)
Chromel	19	300	8730
Constantan	19.5	390	8900
Alumel	30	110	8600
Copper	80.4	450	7870
Teflon	0.25	1010	2160

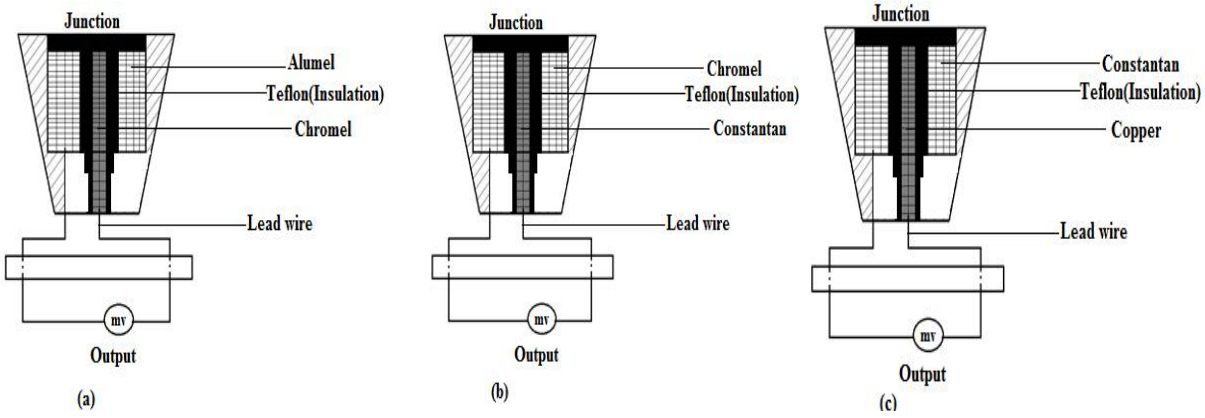

Fig. 1: The schematic diagram coaxial thermocouples, Fig.1 (a), Fig.1 (b) and Fig.1 (c) represent K-type, E-type and T-type coaxial thermocouples respectively.

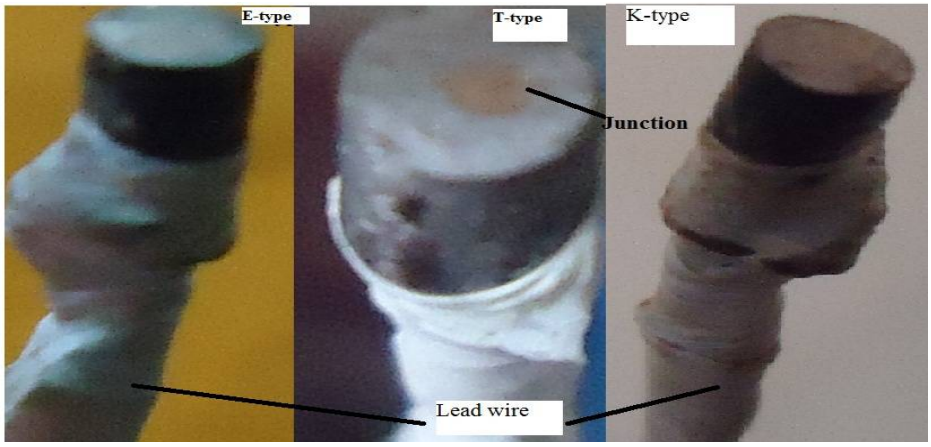

Fig. 2: Photograph of K-type, E-type and T-type coaxial thermocouples

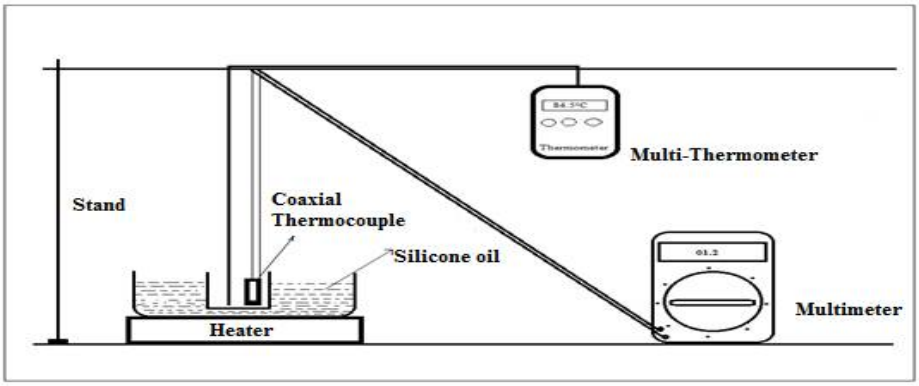

Fig. 3. The schematic diagram of static calibration set-up

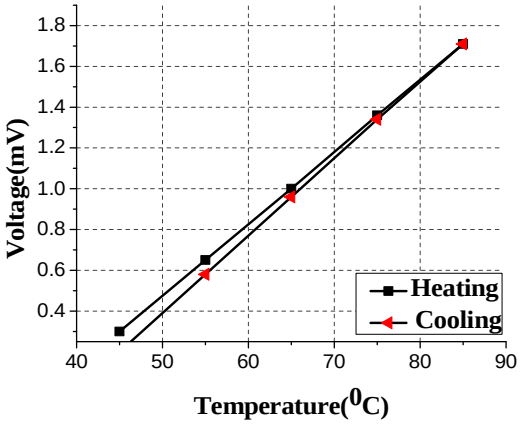

Fig.4(a) Variation of voltage with temperature for K-type

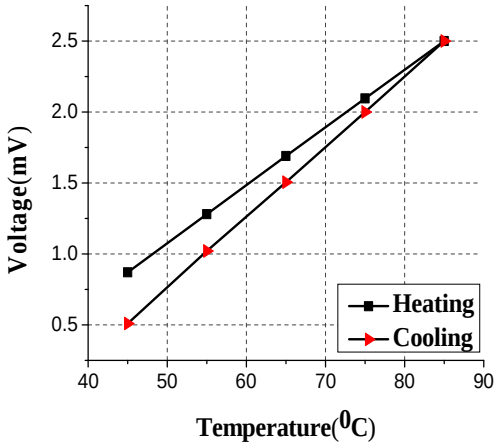

Fig.4(b) Variation of voltage with temperature for E-type

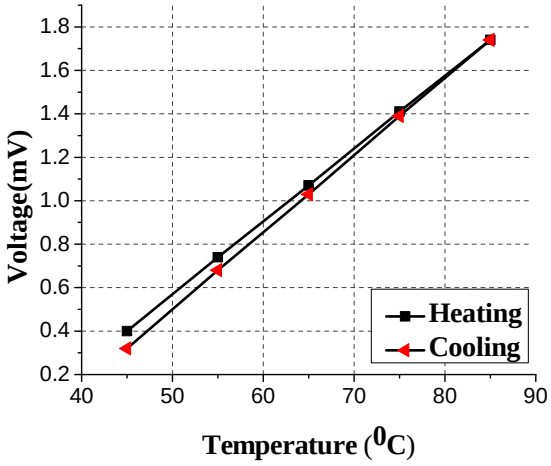

Fig.4(c) Variation of voltage with temperature for T-type

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REVIEW ARTICLE

Impact and Management of Extreme Weather Events in Groundnut Production in India

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ABSTRACT

The present paper describes the review of research work done on extreme weather events and its impact on groundnut production in India. Amongst the various extreme weather events, only those extreme weather events are considered which have direct relevance to groundnut production. These are low temperature extremes, high temperature extremes and rainfall associated extreme events. Impact of extreme weather events on growth, development and yield of groundnut are described. Since the 80% of groundnut production is from *kharif* season, more emphasis is given on impact of rainfall distribution, soil moisture, moisture stress and drought on groundnut.

Keywords : Extreme weather, heat wave, chilling, groundnut, moisture stress, drought.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an important food and oilseed crop grownin diverse agro-climatic environments in India. Among oilseeds crops in India, groundnut accounts for about 50 per cent of planted area and 45 per cent of oil production. In India, about 75 per cent of the groundnut area lies in a low to moderate rainfall zone (parts of the peninsular region and western and central regions) with a short period of duration (90–120 days). Most of the groundnut production is concentrated in five states – Gujarat, Andhra Pradesh, Tamil Nadu, Karnataka and Maharashtra which altogether account for about 86 per cent of the total area under groundnut cultivation. The remaining peanut-producing area is scattered among the states of Madhya Pradesh, Uttar Pradesh, Rajasthan, Punjab and Orissa. Although the crop can be grown in all seasons, it is grown mainly in the rainy season (*kharif)*, running

from June to September. The *Kharif* season accounts for about 80 per cent of the total groundnut production. In the southern and south-eastern regions, groundnut is grown in rice fallows during the post-rainy season (*Rabi)*, from October to March. If irrigation facilities are available, groundnut can be grown from January to May as a spring or summer crop.

Groundnut is essentially a tropical plant and requires a long and warm growing season. The favorable climate for groundnut is well-distributed rainfall of at least 500 mm during the crop-growing season, accompanied by an abundance of sunshine and relatively warm temperature. Temperature in the range of 25°C to 30°C is optimum for plant development (Weiss, 2000).Once established, groundnut is drought-tolerant, and to some extent it also tolerates flooding. Rainfall of 500 to 1000 mm will allow commercial production, although crop can be produced on as little as 300 to 400 mm of rainfall. Rainfall variations cause major fluctuations in groundnut production in India. Because groundnut is grown mainly as a rainfed crop, there is a high level of fluctuation in production depending on the rainfall.

The impacts of the extreme weather events such as floods, droughts, cyclones, hail storm, thunderstorm, heat and cold waves on the socio-economic state of India is increasing due to large growth of population and its migration towards urban areas which has led to greater vulnerability.De *et al.*(2005) have reported that in India the extreme weather events have increased considerably over the period of 100 years. Lunagaria, *et al*. (2015) analysed the trend of extremes of temperature and rainfall in Gujarat and concluded that warm nights are increasingand cold days are decreasing. Annual rainfall and wet days are reported to increase at most of the stations in Gujarat. In view of the increasing population and anticipated climate change and extreme weather events, production must continue to increase to meet the current and future demand for edible oil and vegetable protein in the country. This may be possible through improved agronomic management and genetic improvement of the crop to suite the target environments considering both the current and future climates.

Extreme weather and groundnut production

Basically, the climate of India is dominated by the summer monsoon (June to September). From meteorological point of view the entire year is divided into four seasons viz. (i) Winter (December, January and February), (ii) Pre-monsoon or Hot Weather season (March to May), (iii) Southwest or Summer Monsoon season (June to September) and (iv) Post monsoon season (October to November). However, from agriculture point of view there are mainly three crop seasons viz. (i) *Kharif* (rainy season), (ii) *Rabi* (winter season) and (iii) summer season crops. There are several weather aberrations and extreme weather events that occur in differentcrop growing seasons individually or in combinations with other events, however in the present study we will be confined to mainly those events which occur in groundnut growing regions of India and affect its production. These extreme weather events affecting agricultural production can be grouped into; (i).Low temperature extreme events (ii).High temperature extreme events (iii).Rainfall associated extreme events.

Impact of low temperature extreme events on groundnut

The temperature extremes i.e. lowest minimum and highest maximum temperatures recorded at Anand during 1958-2014 period along with monthly normal are depicted in Figure 1. It may be seen there is large difference between the two extremes in every month. During winter season (Dec-March) the differences are more than 35^0C while monsoon season these are less.The occurrences of extreme low temperature in association with incursion of dry cold winds from north into the Indian sub continent are known as cold waves. The cold waves mainly affect the areas to the north of 20^0 N but in association with large amplitude troughs, cold wave conditions are sometimes reported from States like Maharashtra and Karnataka as well.During winter season (December to March) when minimum temperature falls more that 5 ^{0}C from its normal, cold wave persists. In India the cold wave conditions during

December and January months did not have any effect on groundnut as during these periods groundnut are generally not grown during winter season. But the extreme cold in February and March, the middle of the planting season of the summer crop, proved to be harmful for the most of the summer crops including groundnut. Germination and growth are adversely affected if the temperature is less than 10 ^{0}C (Mohamad, 1984).

Groundnut and othercrops that are of subtropical origin are highly susceptibleto low temperature damage (Benedict and Ketring 1972; Christiansen 1971; Lyons1973;Mayland and Cary1970). There are two types of injury that can occur, chilling and freezing.Low temperature damage in the absence of freezing hasbeen defined as chilling injury (Levitt, 1972).Even though chillingand freezing injury are not the same, structural changesoccur in the cell membranes that are similar.Disruptionof cellular functions leads to the accumulation oftoxic substances and components responsible for off lavorsin fruit and vegetable crops (Singleton and Pattee, 1989).Figure 1.Shows that the minimum temperature sometimes fall below 5^{0}C during December, January andFebruary that may cause frost injury to new seedling of groundnut. Even during March when minimum temperature falls below 10 ^{0}C may also result in plant injury. In most of the months the minimum temperature departure from the normal are more than 10 ^{0}C.Foggy weather also affect the crop growth and development adversely in most of the groundnut as it reduces the receipt of PAR energy required for photosynthesis by the crop.

Impact of high temperature extreme events on groundnut

As winter season transforms into spring the temperature rises initially in the southern parts of India, giving rise to thunderstorms and squally weather which are hazardous in nature. While the southernmost part of the country is free from dust storm and hailstorm, such hazardous weather affect the central, northeastern, north and northwestern parts of the country. Extreme positive departures from the normal maximum temperature result in heat wave during the summer season. These extreme weather events are observed during March to June. However, heat wave like situation arises some times in February also when temperature crosses 35^{0}C. (Figure 1) shows that at Anand the maximum temperature may exceed by more than 5^{0}C from its normal in almost every month and sometimes departure may exceed 8^{0}C resulting in heat wave like situation. Such extreme weather events adversely affect the summer crops.

Hailstorm and thunderstorms causes physical damage to plant resulting in poor growth and yield. The high temperature during flowering stage of the groundnut affects the pollination (Mohamad, 1984).Vara Prasad *et al.*(2003) reported from the growth chamber study that the seed yield of groundnut decreased progressively by 14%, 59% and 90% as temperature increased from 32/22 to 36/26, 40/30 and 44/34 ^{0}C, respectively. Decreased seed yields at high temperature were a result of lower seed-set due to poor pollen viability, and smaller seed size due to decreased seed growth rates and decreased shelling percentages. Seed harvest index decreased from 0.41 to 0.05 as temperature increased from 32/22 to 44/34 ^{0}C (Figure 2). Correlations are based on data during the period 1949–50 to 1997–98. The values shown inFigure 3(b) are backward-differenced crop production and monsoon rainfall indices expressed as percentage change from their respective previous year's values (After Krishna Kumar *et al*. 2004).

Impact of rainfall associated extreme events

Rainfall is the most significant climatic factor affecting groundnut production, as 70 per cent of the crop area is found in semi-arid tropical regions characterized by low and erratic rainfall. Low rainfall and prolonged dry spells during the crop growth period are reported to be main reasons for low average yields in most of the regions of Asia and Africa, including India (Reddy *et al*. 2003), China (Zeyong, 1992) and several parts of Africa (Camberlin and Diop, 1999). In India groundnut yields were reported to be vulnerable from year to year because of large inter-annual variation in rainfall (Sindagi and Reddy, 1972). Bhargava,*et al*. (1974) reported that 89 per cent of yield variation over four regions of India could be attributed to rainfall variability in the August to December growing period. Challinor,

et al. (2003), analysing 25 years of historical groundnut yields in India in relation to seasonal rainfall, concluded that rainfall accounts for over 50 per cent of variance in yield. Gadgil, (2000) observed that the variation in groundnut yield in the Anantapur district arises to a large extent from the variation in the total rainfall during the growing season.Sivakumar and Sharma (1986) compiled a summary water use of groundnut and reported that the water use varies from 250 mm in the rainfed conditions to 830 mm under irrigated conditions. It was observed that seasonal rainfall up to 50 cm is required to sustain a successful groundnut crop in this region. Krishna Kumar *et al*.(2004) correlated all-India total groundnut production with rainfall over India. They found that July, August and September rainfall index had significant correlations with groundnut production index (Figure 3). However, it may be seen from the (Figure 4).That at local levels the experimental yield at Anand, Gujarat was not in linear relationship with yield of groundnut. Seasonal rainfall at Anand Gujarat exceeding 800 mm had adverse effect on the production resulting in decrease in yield. Also in Anantapur of Andhra Pradesh, the pod yield of groundnut showed a highly significant curvilinear relationship with the use of moisture, namely, adding rainfall and soil moisture (AICRPAM, 2003). A total moisture use of 350–380 mm was found to be optimum for obtaining a maximum yield; a moisture use of either less than this amount or more reduced pod yield. This is because the excess rainfall may have caused more vegetative growth and less reproduction.

Even monthly rainfall at district level had differential effect on pod yield of groundnut in Rajkot and Junagadh districts of Gujarat. At same amount of monthly rainfall there islarge difference in the pod yield of groundnut in both the districts (Figure 5 and 6). The vast differences in yield from year to year may be attributed to uneven distribution in the rainfall during a month. The polynomial relationship was found to explain better in comparison to linear relationship particularly in the months of June and July. The higher rainfall during early stages of crop growth may not be useful to groundnut as it may result in more vegetative growth causing disease infestation and less reproductive growth and development. Ong (1986) also showed a poor relationship between groundnut yield and seasonal rainfall, thus highlighting that rainfall distribution is more important to groundnut yield than the amount of rainfall. The yield variability may also be the result of extreme weather events experienced during the crop season. The extreme weather events causing yield losses in groundnut are primarily (i) delay in onset of monsoon rainfall, intermittent dry spells resulting in moisture stress and drought like situation during different stages of groundnut, heavy wet spells and post harvest losses due to unseasonal or prolonged monsoon rainfall.

Effect of moisture stress and drought

Rainfall in the semi-arid regions is erratic in duration and distribution, which leads to droughts of varying intensities and durations during the crop season. Hence, the total water use could vary with the stage of crop growth during which moisture stress or droughts occur, and the water-use requirements of the crop at these stages. Results from a series of experiments at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT, 1984) showed that early stress or lack of rainfall/soil moisture between 29 and 57 days after sowing did not influence pod yield significantly, whereas pod yields increased by 150 kg/ha/cm of water applied during the seed-filling stage (93–113 days after sowing).

Sivakumar and Sarma (1986) studied the effect of drought stress imposed at different growth phases on the total water use by groundnut and showed that the total water use during the three seasons of experimentation was different for any given growth phase because of the differences in the rainfall during the preceding rainy season (and hence the initial-profile water content) during the three years and because of the differences in the amount of water applied. Williams *et al*., (1986) have shown that the drought-stress effects on groundnuts depend primarily on the stress pattern. The differential responses of groundnut cultivars to drought were assessed relative to the mean response of all genotypes to three major aspects of drought, (i.e., duration, intensity, and timing relative to crop phenophases) varying independently. The timing of drought was found to have large impact on the variation about the mean response. In general, the sensitivity of a genotype to drought increases with yield potential, increasing the

closer the drought ends to final harvest. The large variations in the response of genotypes to midseason droughts were due to recovery differences after the drought is relieved.The soil water deficits occurring during flowering and up to the start of the pod growth phase significantly reduced pod yields (in a range of 17–25 per cent) relative to the well-watered control plots for two Spanish and two Virginia cultivars (Wright *et al*., 1999). The reduction in yield was greatest when severe stress occurred during the pod-filling phase. Several other reports also observed the pod development stage to be most sensitive to moisture deficit (Rao *et al*., 1985; Stirling, *et al.*, 1989; Patel and Gangavani 1990).

Analysis of the relationship between simulated groundnut yield and climate showed that yield was predominantly influenced by rainfall from flowering to maturity (Christensen *et al*., 2004). Naveen *et al*., (1992) found that water stress imposed during the flowering and pegging stages produced the greatest reductions in pod yield, followed by water stress at early and late pod stages. Nageswara Rao *et al*., (1985) confirmed that irrigations could be withheld during much of the vegetative period without any apparent effect on pod yield, implying that water stress during the vegetative stage has no effect on yield. Nautiyal*et al*., (1999) proved that soil moisture deficit for 25 days during the vegetative phase was beneficial for growth and pod yield of groundnut, while Stirling*et al.,* (1989) observed the insensitivity of pod yield to early moisture deficit. Sivakumar and Sharma (1986) imposed drought stress or a soil moisture deficit at all the growth phases of groundnut during three growing seasons and observed that stress from emergence to pegging gave increased yields relative to the control group in the three years of the study, while stress at other stages decreased the yield. Not just yields, but other yield attributes, growth and development are affected by soil moisture deficit or water stress.

The start of flowering and pod elongation are delayed by drought stress (Boote and Ketring, 1990). The rate of flower production is reduced by drought stress during flowering but the total number of flowers per plant is not affected due to an increase in the duration of flowering (Gowda and Hegde, 1986). Boote and Hammond (1981) reported a delay of 11 days in flowering when drought was imposed between 40 and 80 days after sowing. Stansell and Pallas (1979) found that the percentage of mature kernels was reduced to 34 per cent of the control when drought was imposed 36–105 days after sowing. Vorasoot, *et al.,* (2003) observed a drastic reduction in yield and also in yield-attributing characteristics such as total dry weight and shelling percentage when plants were grown at 25 per cent of the field capacity of the soil.

Management options

The management options to mitigate the adverse effect of extreme weather event includes farm management that includes time of sowing, selection of appropriate variety, maintaining plant population, scheduling and methods of irrigation, etc. In addition microclimatic modification such as mulching, shelterbelts etc can be attempted. And the most importantly the forecasting of extreme weather events well in advance.

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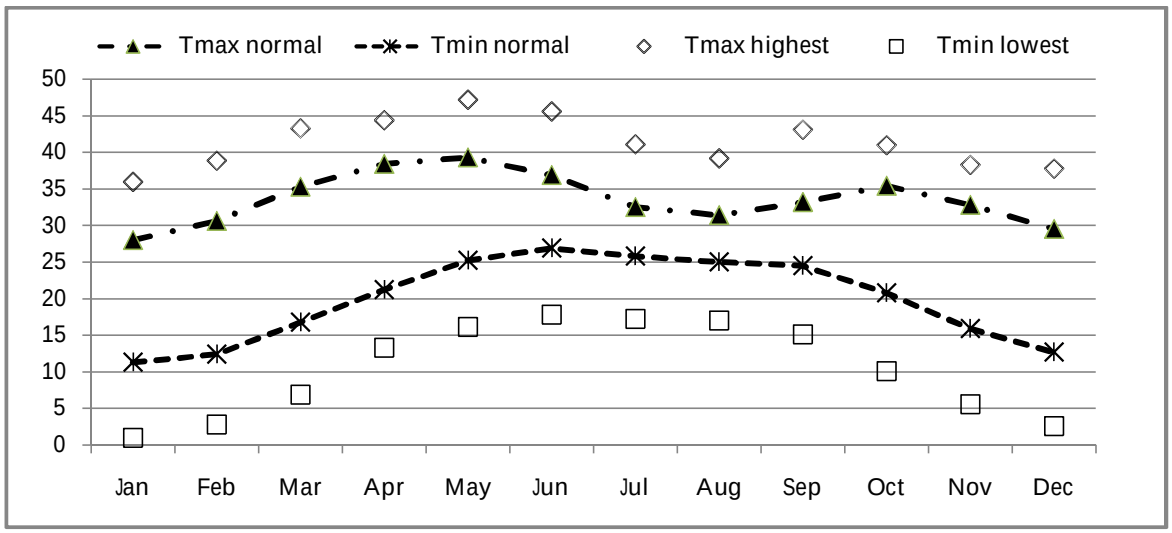

Figure 1.Mean monthly maximum temperature (Tmax) and minimum temperature (Tmin) with their extremes recorded at Anand during 1958 to 2014.

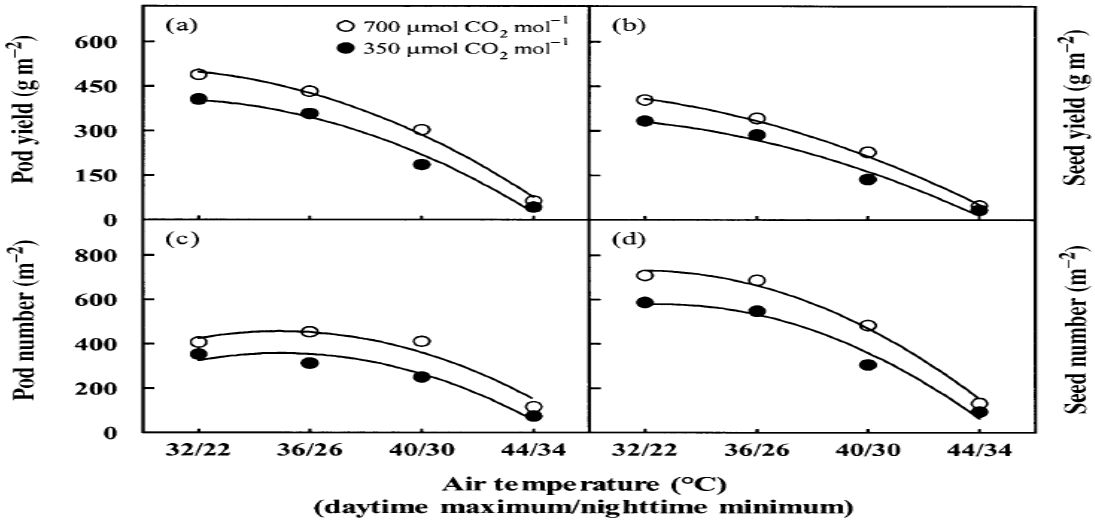

Figure 2. Relations between daytime maximum/nighttime minimum temperature and (a) pod yield; (b) seed yield; (c) pod number; and (d) seed number of groundnut at ambient and elevated CO_2.

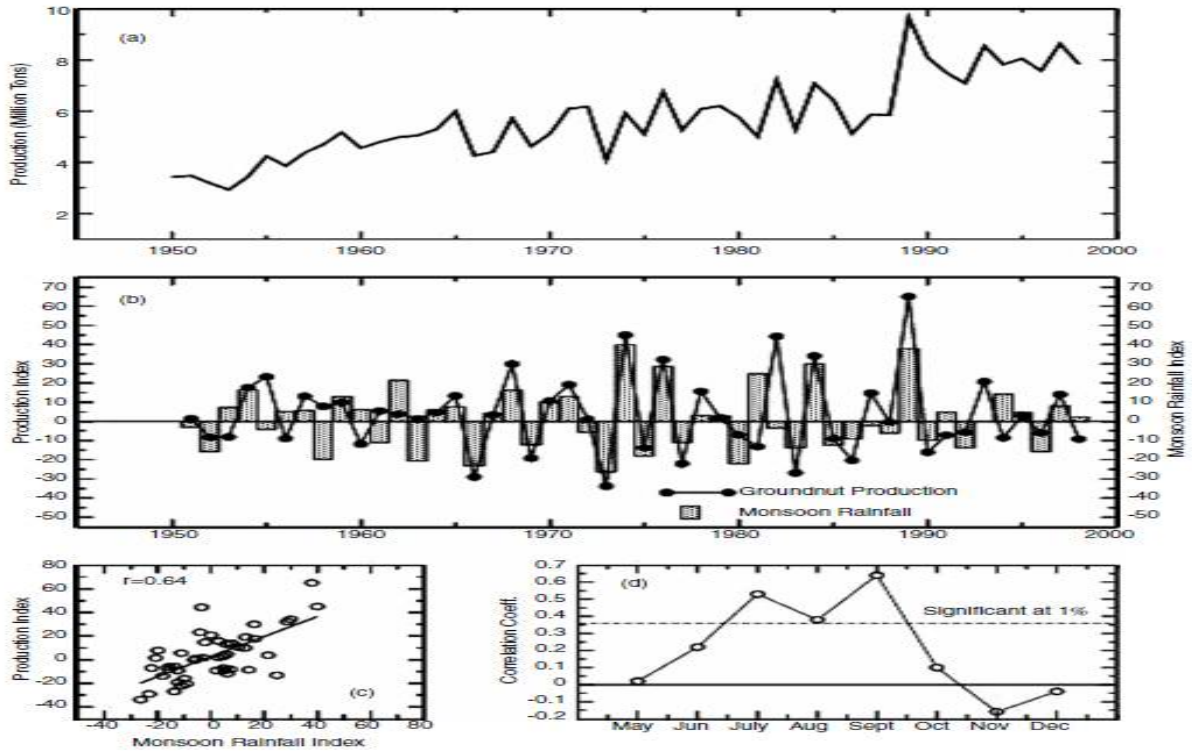

Figure 3. All-India total groundnut production and its association with rainfall over India. (a) Growth in food-grain production. (b) Year-to-year variations in food-grain production and monsoon rainfall. Correlation between groundnut production and (c) monsoon seasonal rainfall and (d) individual monthly rainfall during May to December.

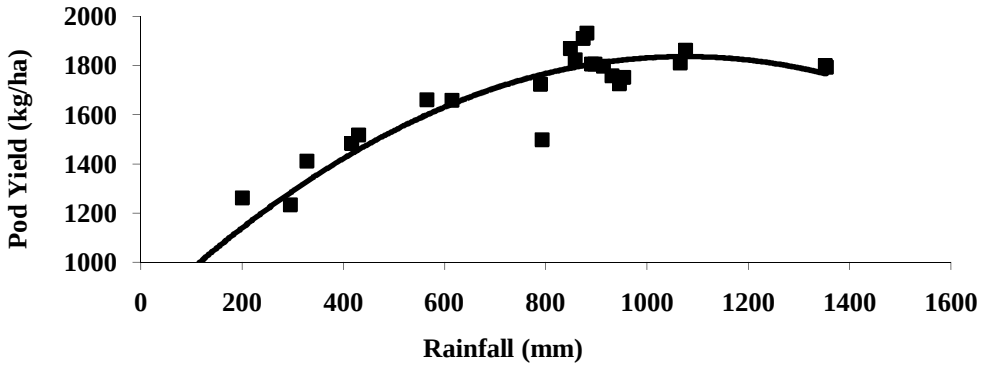

Figure 4. Relationship between pod yield of groundnut and seasonal rainfall at Anand

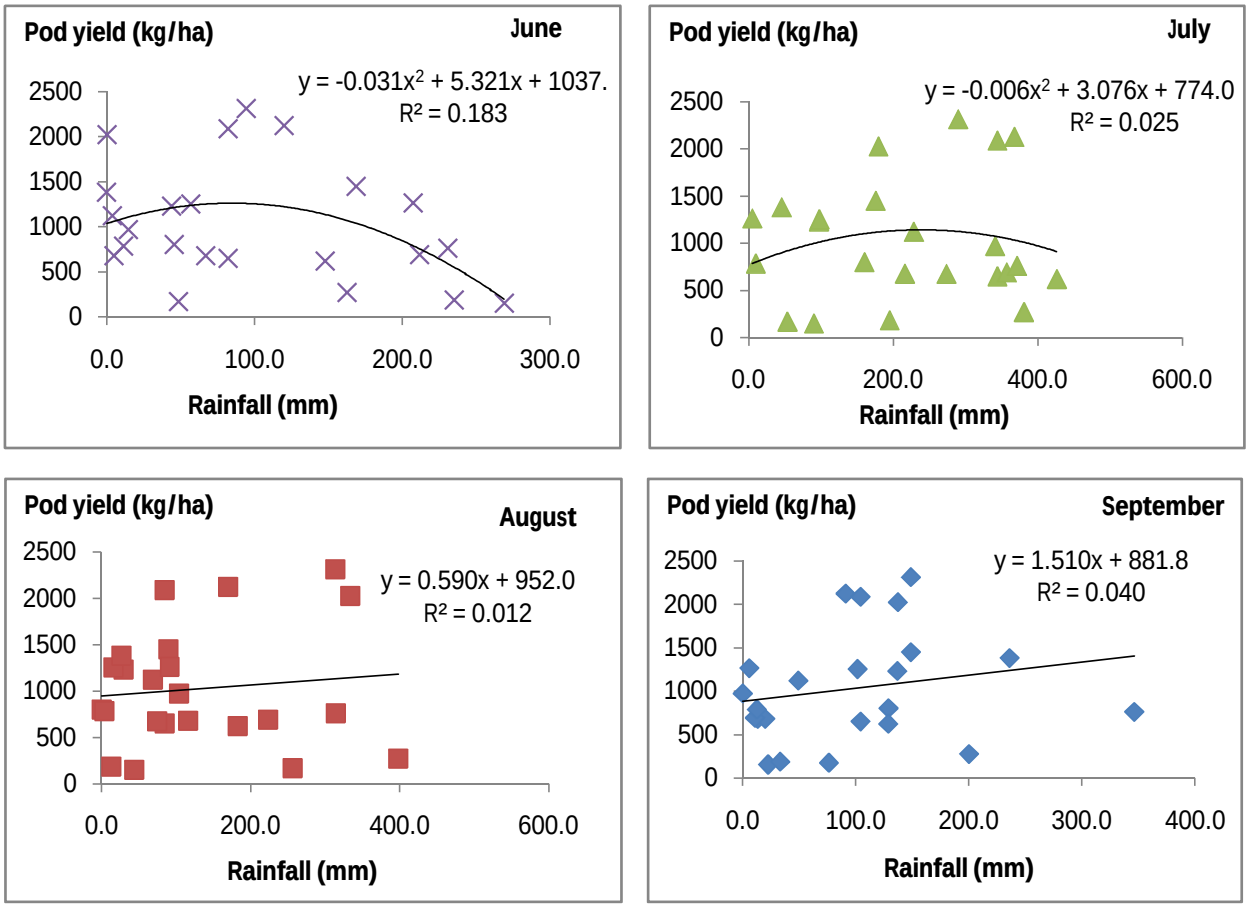

Figure 5: Monthly rainfall and groundnut production in Rajkot district of Gujarat

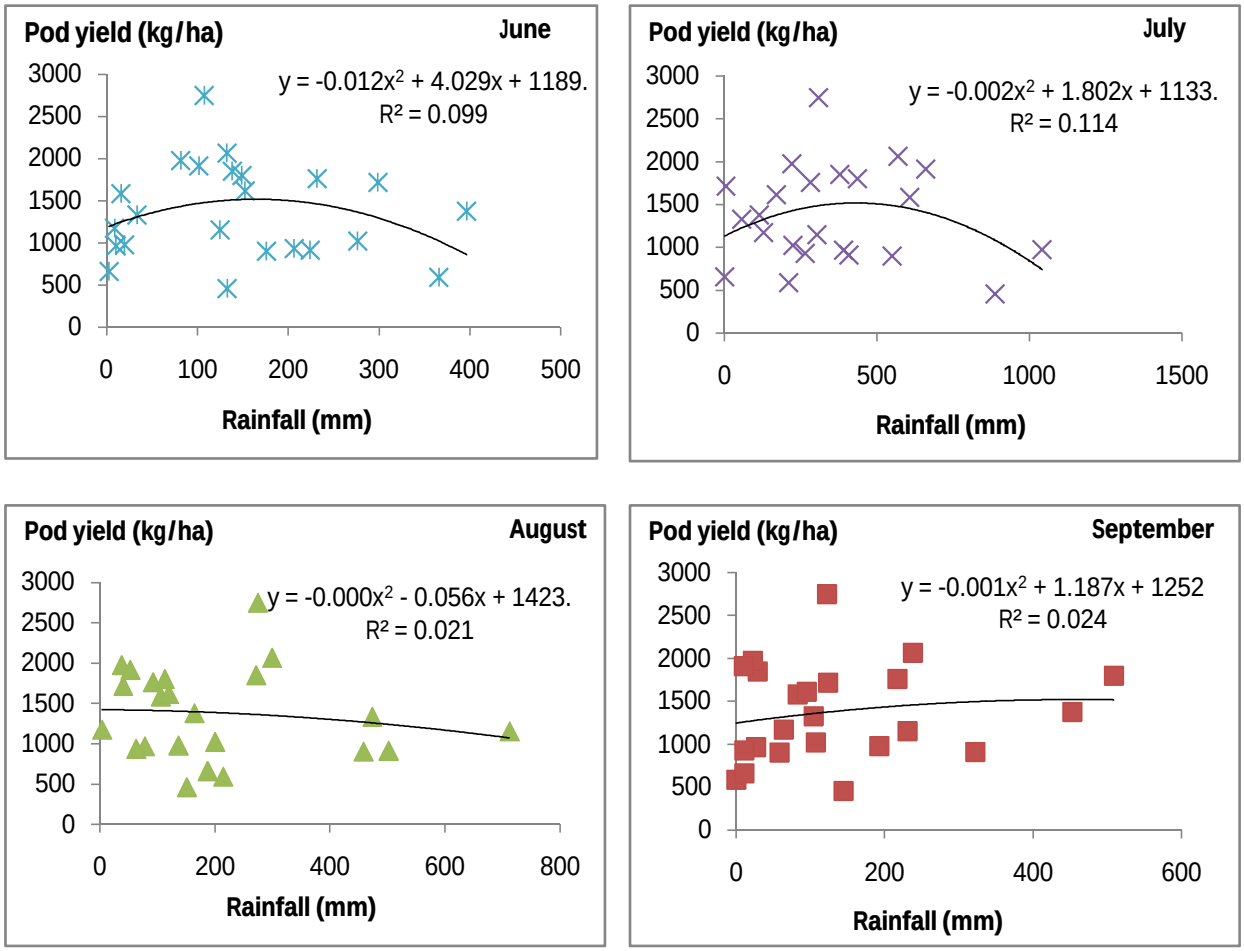

Figure 6: Monthly rainfall and groundnut production in Junagadh district of Gujarat

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RESEARCH ARTICLE

Quantitative Determination of Biochemical Constituents and Evaluation of Antioxidant Potential of Different Cultivars of Mulberry Species

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ABSTRACT

A study was undertaken to compute the metabolites and antioxidants present in the leaves of different varieties of *Morus* species,*viz.*,*M.alba* (S1635, TR4),*M.indica* (Suvarna, Vishwa, G4, C2038, V1, CNR, M5, S36, S54, DD),*M.latifolia* (BC259), *M.laeviegata*, *M.multicaulis*, *M.macroura*, *M.australis*, *M.cathayana* and *M.sinensis* (MR2).The level of metabolites as well as antioxidants significantly varied among different cultivars and species of mulberry.Average contents of carbohydrate and starch per 100g dry weight were 19.191mg and 15.482mg respectively.*M.latifolia* exhibited highest reducing sugar (3.10%), total polyphenol (13.160%) and Flavonoid (3.650%) content. Cultivar Vishwa (*M.indica*) showed higher protein content(6.44%)and amino acid content (3.41%).*M.sinensis* had the highest antioxidant (724.25 millimole/mg of sample) and DPPH scavenging activity(58.53%).*M.latifolia*was found to have the highest catalase activity (0.38µmol/min/mgprotein),peroxidase(178.47µmol/min/mgprotein**)** and ascorbate peroxidase activity **(**29.90 µmol/min/mg protein)among the cultivar studied. Cultivar C2038 (*M.indica*) exhibited higher Chlorophyll content (3.10 mg chl/gm tissue) in comparison to other cultivars of mulberry. Five polyphenolic constituents,*viz*., chlorogenic acid, caffeic acid, coumaric acid, rutin, and quercetin were identified and quantified by High Performance Liquid Chromatography. Relative distribution revealed that chlorogenic acid was the highest in concentration followed by rutin and quercetin. Present study generated the data for characterization of the *Morus* germplasm according to

their biochemical constituents in order to identify the plant as a therapeutically significant plant and a potent source of natural antioxidants apart from being used as a feed for silkworm.

Keywords : Antioxidants, DPPH, HPLC, morus,polyphenols.

INTRODUCTION

Traditional and folk medicines in healthcare trace their origins to the plethora of knowledge and benefits mentioned in ancient literature with regard to herbs.The World Health Organization (WHO) reports that 80% of the population in developing countries depend on traditional medicine for their primary healthcare.Exploration of new bioactive molecules from plants and screening their properties would provide a foundation for developing new lead molecules in strategic favour of natural product drug discovery.In recent times, natural products have beengrowing in popularity in their use as dietary supplements[1].These natural supplements present in the diet, being antioxidants, increase the body's resistance towards oxidative damages and bring about a substantial improvement in health[2]. Antioxidants are gaining widespread attention due to the effective role they play against aging.It is believed that phytonutrients can inhibit the propagation of free radical reactions that may ultimately lead to the development of diseases[3].

Traditional medicines make extensive use of phytochemical-rich plant extracts to cure various ailments. Mulberry, a plant of tremendous medicinal and economic significance, exhibits fascinating taxonomic groups due to its extant genetic variability and is of commercial importance in the sericulture industry. Medicinal properties ascribed to mulberry are wide-ranging and mulberry fruit, leaves, roots and bark have been used in Chinese folk medicine to treat numerous diseases.Pharmacological functions for mulberry have been partially attributed to the properties of the flavonoids and soluble sugar contained in mulberry leaf, fruit, and root bark[4]. The pharmaceutical value is also the result of its antioxidant and free radical scavenging capacity.

Studies have shown that the plant contains the phytoconstituents*viz*., tannins, phytosterols, sitosterols, saponins, triterpenes, flavanoids, morusimic acid, anthocyanins, anthroquinones, glycosides and oleanolic acid as active principles[5].Mulberry leaves have been generating widespread interest due to reports on their hypoglycemic, hypotensive, diuretic, bacteriostatic and antivirotic properties[6]. However, it is only in the recent past that the mechanism of their action has been related to their antioxidant properties. Srivastava*et al*. reported the nutritional composition as protein, fat, crude fiber, neutral dietary fiber and ash contents[7]. Reports indicate that mulberry leaves contain ascorbic acid, β carotenes, folic acid and elemental nutrients like iron, zinc, calcium, phosphorous and magnesium in substantial amounts[8].

While Sericultural Institutes in India under the aegis of Central Silk Board (CSB) are engaged in the collection of mulberry germplasm and their morphological identification, biochemical characterization and study of their antioxidant potential have been virtually non-existent. Furthermore, reports on identification and quantification of major phenolic compounds and the enzymatic antioxidants present in various accessions of mulberry leaves have also not emerged. It was hence thought expedient to carry out an in-depth study on metabolic profiling of different indigenous varieties of mulberry in order to identify biomolecules of economic and therapeutic interest. This would thereby enhance the significance of the mulberry beyond its traditional eminence as exclusive forage of silkworms.

MATERIALS AND METHODS

Collection of Plant materials

Fresh leaves of 19 accessions of mulberry were collected from various plantations in Tamil Nadu under Department of Sericulture and Tamil Nadu Agricultural University, Coimbatore.

Metabolic profiling of *Morus* Germplasm

Metabolic constituents such as carbohydrates, starch and reducing sugars[9], aminoacids[10], chlorophylls[11], protein[12], total polyphenols[13] and flavonoids[14] were quantified using the outlined protocols from the immature crop shoots of mulberry varieties.

Characterization of polyphenoliccompounds by HPLC

The phenolic constituents were determined using the HPLC method described by Rodriguez-Delgado *et al.* [15].The mulberry leaves were dried overnight at 90° C and extracted with 70% ethanol using soxhlet apparatus. The extract obtained was further separated with chloroform and ethyl acetate using a separating funnel. The polyphenolic ethyl acetate layer finally acquired was dried and used for quantification of polyphenolic constituents profiling[16]. The chromatographic conditions include separation in Waters HPLC (Model: 515) fitted with Photodiode Array detector (Model: 2998) and ODS column of size 250 mm × 4.6mm, 4µm (Hi Chrom, USA),solvents methanol- acetic acid-water in the proportion of 10:2:88 and 90:2:8 were used as mobile phase A and B respectively for binary gradient elution. Measurements were made at 254 and 280nm.Identification of phenolic acids was based on retention time and UV spectrum with those of standards. The solutions of standards at various concentrations from1to40µg/mL were injected into the HPLC-DAD system and the calibration curves were established for each standard compound. The concentrations of the compounds were calculated from peak area according to the respective calibration curves.

Antioxidant properties of *Morus*Germplasm

Estimation of Total Antioxidant Capacity and DPPH Free Radical Scavenging Activity

Total antioxidant activity was estimated according to the method suggested by Prieto *et al.*[17]. One ml of the extract was reacted with 1.0 ml of reagent solution (0.6 M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The solution was incubated in a thermal block at 95^0C for 90 minutes. Absorbance was read for the green colour at 695 nm. The antioxidant activity was calculated using the standard calibration curve derived from regularized concentrations (10 to 50 ppm) of Butylated hydroxy anisole (BHA), and was indicated as equivalents of BHA.The Free Radical Scavenging Activity was measured as the decrease in absorbance of methanol solution of DPPH (1, 1 diphenyl 2 picrylhydrazyl) according to Akachuchu and Fawus [18]. A stock solution of 0.1mM DPPH was prepared in methanol. Following this, 5 ml of the stock solution was added to 1 ml of methanolic extract of sample (10mg/ml), incubated for 30 minutes and the absorbance was measured at 517 nm. Scavenging activity wasexpressed as the percentage inhibition calculated by standard formula.

Antioxidant enzyme activity

Enzyme Extraction

Enzyme extraction was done by homogenizing the freshly plucked leaves (1 gm) in chilled mortar using 1mM EDTA and 50mM Potassium phosphate buffer at pH7.0. The homogenate was filtered and centrifuged to obtain the supernatant as crude enzyme source.

Assay of Ascorbate peroxidase (APX)

APX activity was determined by following the decrease in Absorbance at 290nm for 3 minutes. One ml reaction volume contained 100Mm potassium phosphate buffer (pH 7.5), 0.5mM ascorbate and 0.2mM hydrogen peroxide and enzyme extract [19]**.** APX activity was expressed as ascorbic acid oxidized µM/min/mg of protein.

Peroxidase (POD)

Peroxidase activity was determined using pyrogallol as the substrate with the amount of purpurogaline produced[20]. The assay mixture consisted of 0.05M pyrogallol in 0.1M sodium phosphate buffer (pH 6.0), enzyme extract and 1% hydrogen peroxide. The peroxidase activity was monitored at 420nm and expressed as unit mg/ protein.

Assay of Catalase (CAT)

Catalase activity was determined by involving the decomposition of hydrogen peroxide according to Mishra *et al*.[21]. The assay mixture consisted of 0.1M sodium phosphate buffer (pH 6.4), enzyme extract and hydrogen peroxide (1%). The catalase activity was estimated at 230nm and expressed as µM/min/mg of protein.

RESULTS AND DISCUSSION

Metabolic Profiling of *Morus*germplasm

Biochemical constituents present in leaves of mulberrygermplasm exhibited very large variations (Table 1). A significant variation was noticed in carbohydrate content that ranged from13.72%(*M.macroura*) to 23.93% (*M.cathayana*). Similarly, substantial variation in terms of starch content and reducing sugar content was observed among the mulberry accessions. Higher starch content of 19.33 % was recorded in *M.cathaya*naand the lowest was detected in *M.macroura* (10.62%) while reducing sugar was noticed to be high in *M.latifolia* (3.10%) and low in *M.macroura*(1.00%).A similar trend was observed in terms of aminoacids and protein contents (Table 1). The chemical composition and biomass yield of mulberry leaves differ according to their variety, degree of maturity, position of leaf and the type of soil in which the plants are grown.The starch content of the leaves wasreported to be higher than their soluble sugars[22]. As the leaf matures, the protein content decreases and carbohydrate content increases[23].

Total chlorophyll content of the *morus*germplasm aligned with the visual observations (Table 1). Colour of the leaves of certain cultivars and varieties isnot always correlated to the level of chlorophyll concentration. The level of chlorophyll content and other leaf biochemical constituents can be used as pointers to crop stress under conditions of nutritional deficiencies [24]. The content of chlorophyll is of vital importance in quantifying the photosynthetic efficiency of a plant and functions as an essential component in assessing the quality of foliage.A significant variation was noticed in total polyphenol content that varied from 2.28% (*M.cathayana*) to 13.16% (*M.latifolia)*ondry wt. of leaf. The quantity of total Flavonoids (catechin equivalents) expressed as percentage by weight of dried extract range from to 0.525% (*M.macroura*) to 3.65% (BC-259) in 80% ethanolic leaf extract. Phenolic compounds are the most active antioxidant derivatives in plants [25] and have generated much interest as a result of their free radical scavenging abilitiesthat can bring about substantial improvement in human health [26,27]. They are also believed to be an important part of general defense mechanism in many plants against infections.It has been reported that leaf polyphenols vary according to genotype and conditions such as temperature, drought, pollution, UV light and pathogen attack [28, 29]. The difference in total polyphenol among the mulberry germplasm in the present study is understood to be due to variations in the plant genotype.

Characterization of polyphenoliccompounds by HPLC

Chromatographic separation identified the major polyphenolic compounds to be chlorogenic acid, caffeic acid, coumaric acid, rutin and quercetin (Table. 3). Among the identified polyphenolic compounds, chlorogenic acid is found to be relatively prominent followed by rutin and quercetin in all the species investigated. Among the species, *M.latifolia*registered higher values (103.00mg/100gmdry wt.) for chlorogenic acid followed by *M.sinensis*(MR2) 91.04 and *M.indica* (CNR) 85.00. Rutin ranged from 53.00 in *M.latifolia*to 9.18 in *M.cathayana*. Quercetin ranged from 46.19 (*M.latifolia*) and 40.98*M.sinensis* (MR2). Caffeic acid was found to be in the range of 4.30 to 0.74mg/100gm. The presence of chlorogenic and caffeic acids were reportedto be constituents of *M.alba* leaves by Katsube *et al*. [30]. Our results are in accordance with Ayaz*et al*. [31], who reported the presence of chlorogenic acid and coumaric acid in *M.rubra, M.alba* and *M.laevigata* species of mulberry.Chlorogenic acid stimulate glucose uptake in skeletal muscle through the activation of Adenosine Mono Phospho kinase (AMPK), thereby acting as an anti-hyperglycemic agent. It has been reported to have anti-hyperlipidemic [32] and antiobesity effects [33]. Hence, proving from the *Morus*species investigated mainly *M.latifolia* to be an interesting natural phytochemicals source of chlorogenic acid for therapeutic applications.

Results indicate that mulberry leaves hold great potential as a dietary source of caffeic acidwhich is known as a selective inhibitor of leukotriene biosynthesis, significantly involved in a variety of diseases like asthma,inflammation and various allergic situations[34]. Caffeic acid is an effective antidiabetic agent due to its ability to enhance insulin secretion and decrease hepatic glucose output along with the increased level of adipocyte glucose disposal in the type 2 diabetic animals. It is beneficial against oxidative stress, thereby being helpful in preventing or delaying the development of diabetics and its complications. Coumaric acid has been reported to have anticancer properties. Rutin and quercetin are the major flavonoid compounds present in mulberry leaves that play a positive role in carbohydrate metabolism and antioxidant status in diabetic rats[35].Quercetin is reported to have antidiabetic effect in STZ induced diabetic wistar rats [36].

Antioxidant properties of *Morus*Germplasm

Total Antioxidant activity and DPPH scavenging Activity

The results for total antioxidant activity is depicted in Table 2. In the phosphomolybdenum assay, which is a quantitative method to evaluate water soluble and fat soluble antioxidant capacities (total antioxidant capacity), the varieties of mulberry plantsdisplayed wide variations in their activity. The total Antioxidant activity (ButylatedHydroxy Toluene) expressed asmmolof BHA/mg of fresh weight showed variations in reading ranging from 257.25 (*M.indica* var. vishala) to 724.25 (*M.sinensis*) . Report of Arabshahi-Delouee and Urooj (2007) also showed similar results [37]. As the extract demonstrated electron donating capacity, it is hoped that they can act as radical chain terminators, transforming reactive free radical species into more stable non-reactive products [38].

The DPPH scavenging effect of ethanolic extract among the mulberry species alternates from 30.08% (cathayana) to 58.53% (*M.sinensis*)(Table 2). The DPPH radical has been employed on a large scale to evaluate the free radicals scavenging ability of various natural products and has also been assessed as a model compound for free radicals that originate in lipids [39]. Results have demonstrated that the extracts by hydrogen and/or electron donation could aid in preventing reactive radical species from reaching biomolecules such as lipoproteins, polyunsaturated fatty acids (PUFA), DNA, aminoacids, proteins and sugars in susceptible biological and food systems [40].

Antioxidant Enzyme Activity

Reactiveoxygen species(ROS)have received special attention dueto their presence and accumulation in plant cells [41]during drought, cold,heat, the use of herbicidesandpresence of heavy metals.Scientific research has conclusively shown thatROSareharmful becausethey canraise theoxidative level in the cellthroughlossofcellularstructureand

function[42].ROSdetoxification agentsin cellsincludeantioxidative enzymessuchasascorbate oxidase,peroxidase, catalaseandascorbateperoxidase[43].The level of Peroxidase (POD) activity varied from 30.58-178.47 µmol/min/mg/protein with higher activity found in*M.latifolia, M.sinensis M.alba*(var.CNR,) and*M.australis*when compared to other cultivars studied. Low Peroxidase activity was witnessed in *M.indica* (var.DD, M5, *M.alba*(TR4) in contrast to the other plants selected (Table 2).

The catalase activity observed was in the range of 0.02-0.67 µmol/min/mg protein with its highest level evidenced in *M.latifolia*(0.66±0.04 µmol/min/mg/protein). Most of the species showed a lower enzymatic activity range varying from 0.02-0.17 µmol/min/mg/protein of which the lowest level was evidenced in *M.cathayana*. Other cultivars exhibited activity in the range of 0.34±0.02 to 0.38±0.052 µmol/min/mg/protein. The catalase activity noticed in the present researchis on par with the findings of Umesh Kumar *et al*. [44], who presented a report on the catalase activity of a few Indian medicinal plants.Ascorbate peroxidase activity ranged between 1.65-29.90 µmol/min/mg/protein.In contrast to other varieties studied,APX activity was significantly higher in *M.latifolia, M.sinensis, M.alba* (CNR) and *M.indica* (V1)with values ranging between 19.83 to 29.90 µmol/min/mg/protein (Table 2).Lower Range of activity between 5.31-8.33µmol/min/mg/protein was noted in *M.australis, M.multicaulis, M.macroura, M.cathayana*and*M.indica* (var.*C2038)* whereas other cultivars exhibited activity between 16.29 to 11.2 µmol/min/mg/protein.Reports indicate that enzyme activity is higher in *Morus sp.*when compared to other medicinal plants [44].

From the study we conclude that leaves of *Morus* speciesare a promising source of dietary antioxidants such as polyphenols which can be further used in nutraceutical industry.Further purification of the identified polyphenolic compounds in mulberry germplasm will serve as a major breakthrough in developing new natural drugs for human ailments. Present study confirms the metabolome that could be undertaken to utilize mulberry leaf for value addition apart from being used as fodder in sericulture.

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Table 1: Variation in the Metabolites among Mulberry Germplasm

Morus sp.	Carbohydrates (%)	Starch (%)	Reducing Sugar (%)	Chlorophyll (mg/g)	Total Protein (mg/g)	Total Aminoacids (%)
M. indica (CNR)	19.04^{x} f^{y}	15.48 l	1.78 f	1.386 e	3.16 f	1.228 e-g
M.latifolia(BC-259)	21.29 j	18.726 k	3.10 k	0.626 a	3.383 g	1.2049 e-g
M. indica (V1)	18.86e	14.816 e	2.407 i	1.173 d	3.166 f	1.43 h
M. sinensis(MR2)	15.61b	11.986 b	2.568 j	0.976 b	3.033 e	1.375 g h
M. indica(M5)	22.34 k	19.33 l	2.659 j	1.423 e	3.953 j	1.151 d e f
M.australis	19.55 g	15.84 l h	1.931 g	1.52 f	1.68 b	1.122 de
M.laevigata	18.60 e	14.496 d	1.994 g h	1.77 g	1.776 b	0.985 cd

M.multicaulis	20.29 h	16.216 i	1.163 b c	2.68 l	2.753 d	0.540 a
M.macroura	13.72 a	10.66 a	1.002 a	1.422 e	3.7 f h i	0.820 b c
M.cathayana	22.938 k	19.33 l	1.226 cd	1.56 f	3.13 e	1.347 f-h
M. indica (DD)	20.69 i	16.74 j	2.054 h	2.003 h	1.476 a	1.219 e-g
***M. indica* (C2038)**	19.68 g	15.736 g	1.263 c-e	3.1 m	3.603 h	1.679 i
***M. indica* (S54)**	16.22 c	16.03 hi	1.717 f	2.55 k	2.1879 c	1.1437 d- f
***M. indica* (S36)**	18.04 d	14.44 d	1.343 de	2.32 j	3.778 i	2.488 k
***M. indica* (Vishava)**	18.04 g	15.23 f	2.114 h	2.246 i	6.443 m	3.4120 k
***M. indica* (Suvarna)**	18.90 f	14.52 d	1.36 e	2.023 h	4.0027 j	2.626 k
***M. indica* G4**	16.38 c	12.723 c	1.079 ab	1.973 h	2.721 d	2.610 l
***M. alba* (S1635)**	22.67 k	19.143 l	1.377 e	2.213 i	4.874 k	1.909 j
M. alba (TR-4)	20.226 h	12.89 c	2.630 j	1.04 c	3.312 g	0.783 b
SE	0.193	0.426	0.056	0.028	0.052	0.092
CV(%)	1.232	3.339	3.712	1.917	1.959	7.343

[x]Values are mean of three replicates(n=3); [y]Different lower- case letters within the column are statistically significant by DMRT at $p < 0.05$

Table 2: Metabolic Profiling of Polyphenolic Constituents among Different Cultivars of Mulberry

Morus sp.	Chlorogenic acid (mg/g)	Caffeic acid (mg/g)	Coumaric acid (mg/g)	Rutin (mg/g)	Quercetin (mg/g)
M. indica (CNR)	85.46±0.44n	4.04±0.01l	9.21±0.09l	43.14±0.16k	39.66±0.33k
M. latifolia(BC-259)	103.04±0.02p	4.31±0.01m	11.72±0.07n	53.02±0.01m	46.29±0.09m
M. indica (V1)	33.61±0.20g	1.57±0.02e	3.56±0.10ef	16.97±0.09e	16.32±0.65fg
M. sinensis(MR2)	91.27 ±0.32o	3.60±0.03k	11.01±0.06m	48.30±0.04l	41.96±0.97l
M. indica(M5)	36.51±0.54i	1.60±0.04ef	3.91±0.04g	17.85±0.03f	16.02±0.51f
M.australis	45.21±0.34k	1.87±0.07h	5.41±0.10i	24.17±0.03h	22.04±0.30i
M.laevigata	33.02±0.24f	1.70±0.04g	3.85±0.05fg	16.88±0.05e	16.54±0.20g
M.multicaulis	30.47±0.65e	1.40± 0.01d	3.39±0.08de	15.68±0.33d	12.01±0.12cd
M.macroura	27.48±0.74c	1.18±0.04b	2.91±0.07bc	13.94±0.22c	11.78±0.55c
M.cathayana	18.96± 0.29a	0.83± 0.01a	2.99±0.51a	10.61±0.30a	8.29±0.59a
M. indica (DD)	28.19±0.31d	1.31±0.05c	3.84±0.09fg	13.88±0.9b	12.94±0.39d

M. indica (C2038)	61.06±0.03m	2.71±0.03j	7.50±0.25k	32.57±0.58j	28.09±0.32j
M. indica (S54)	21.29±0.04b	0.91±0.07a	2.56±0.11ab	11.05±0.50a	9.64±0.37b
M. indica (S36)	27.07±0.06c	1.18±0.05b	3.03±0.02cd	13.61±0.29bc	11.47±0.10c
M. indica (Vishava)	42.68±0.22j	1.81±0.06gh	4.88±0.07h	22.40±0.66g	19.57±0.24h
M. indica (Suvarna)	47.62±0.30l	1.99±0.10i	6.01±0.34j	24.27±0.11h	21.77±0.14i
M. indica G4	46.91±0.08l	1.98±0.13hi	5.24±0.16hi	26.18±0.09i	22.01±0.45i
M. alba (S1635)	35.64±0.18h	1.58±0.07e	4.10±0.35fg	17.44±0.22ef	14.64±0.72e
M. alba (TR-4)	21.81±0.38b	0.90±0.06a	2.90±0.40ab	11.10±0.55a	9.41±0.04b
SE	0.672	0.04	0.162	0.429	0.541
CV	1.879	3.226	3.964	2.334	3.375

Values are presented as mean ± S.D. (n=3); Different lower-case letters within the column are statistically significant by DMRT at $p < 0.05$

Table 3: Antioxidant Status of Different Varieties of Mulberry

Morus sp.	**Total Antioxidants** (mmol of BHA/mg fw)	**DPPH (%)**	**Catalase** (µmol/min/mg protein	**Peroxidase** (µmol/min/mg/ protein)	**Ascorbate Peroxidase** (µmol/min/mg/protein)
M. indica (CNR)	585.36 i	50.82 hi	0.343 e	151.74 h	19.93 h
M.latifolia(BC-259)	632.03 j	54.85 ij	0.66 f	178.46j	29.90 j
M. indica (V1)	569.42 i	52.216 i	0.16 d	105.96g	21.85 h
M. sinensis(MR2)	636.25 j	58.53 j	0.38 e	161.66 i	24.73 i
M. indica(M5)	464.51 g	46.71 g	0.041 a b	30.58 a	11.82 d e
M.australis	423.50 f	44.45 d-g	0.13 b- d	152.48 h	6.027 b
M.laevigata	396.11 e	47.28 g h	0.10 a-d	88.89 f	12.013 d e
M.multicaulis	457.18 g	36.98 b c	0.08 a-d	52.28 b	7.381 b c
M.macroura	338.64 c	30.08 a	0.032 a b	62.67 c d	1.650 a
M.cathayana	333.76 b c	45.607 f g	0.02 a	83.12 f	3.065 a
M. indica (DD)	326.04 b c	43.926 d- g	0.107 a-d	36.80 a	16.290 g
M. indica (C2038)	500.38 h	44.82 e -g	0.0628 a- c	66.54 de	8.334 c
M. indica (S54)	462.07 g	40.16 b-e	0.047 ab	67.73 d e	15.030 f g
M. indica (S36)	474.89 g	39.716 b-d	0.039 c	5.30 b	19.93 h
M. indica (Vishava)	257.52 a	36.19 b c	0.165 d	7.10 bc	24.731 i

M. indica (Suvarna)	314.47 b	35.59 b	0.094 a b d	70.31 d	13.579 e f
M. indica G4	261.38 a	40.93 c- f	0.069 a-d	56.13bc	19.83 h
M. alba (S1635)	359.21 d	40.386 b-e	0.03d	65.44 de	12.02 de
M. alba (TR-4)	466.82 g	44.65 d-g	0.071 a-d	11.22 d	21.851 h
SE	8.689	2.067	0.015	3.390	0.951
CV(%)	2.422	5.768	13.33	4.876	8.951

[x]valuesare mean of three replicates(n=3); [y]Different lower- case letters within the column are statistically significant by DMRT at $p < 0.05$

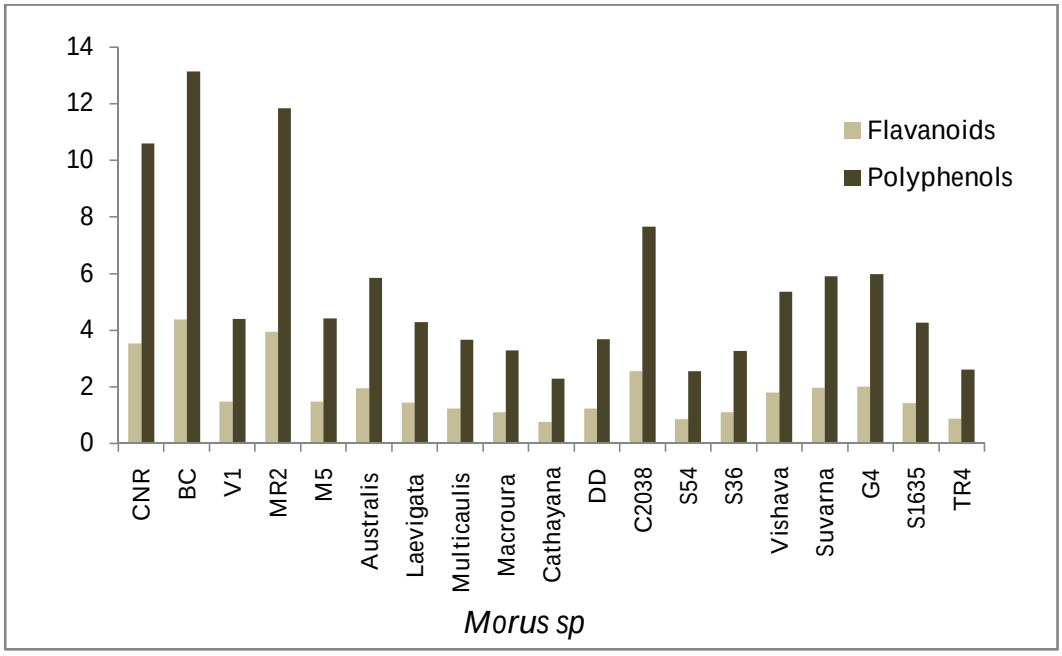

Figure 1.Polyphenol and flavonoid content among morus gergermplasm

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RESEARCH ARTICLE

Investigation of Chemical and Microstructure Properties of Natural Fiber Composite

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ABSTRACT

In this research work removal of lignin and hemicelluloses for untreated coir fiber reinforced composites was accomplished by suitable chemical treatment namely new method at optimum fiber loading such as 25% and 30%. To avoid the problem of untreated coir fiber, chemical treatment was done. Final treated fiber extraction process was gone through new method. It results in treated cellulose composite at 25% and 30% lignin and hemicelluloses was found to be decreased was revealed in Fourier transform infrared spectroscopy analysis, it shows defibrillation, de-polymerization in treated composite was revealed in scanning electron microscopy analysis, and shows high oxygen content percentage than carbon content percentage about 22.06% which demonstrates removal of lignin in treated composite, was revealed in element detection analysis.. The main objective was accomplished by the removal of lignin and hemicelluloses of natural fiber through suitable chemical treatment and at optimum fiber loading.

Keywords : Lignin, Hemicelluloses, Scanning Electron Microscopic, Fourier Transform Infrared Spectroscopy, Element Detection Analysis

INTRODUCTION

Now a day's composites are used in wide variety of applications because of its constituent material can regain their original properties even after mixing. In polymers many research under gone through synthetic because of their promising properties. In this study natural Fiber reinforced polymer was investigated and chosen material was coir

fiber because it is natural, low cost, low density, high specific strength and environmental friendly. The drawback of natural fiber has high moisture absorption capacity, and it consists large amount of lignin , hemicelluloses , pectin and wax which was amorphous in nature, due to which it possess low strength, and poor dynamic characteristics.. Thus in this study various chemical extraction processes was carried out namely New Method[1] and cross and Bevan method [2] to remove lignin, pectin, hemicelluloses content , reducing wear and to improve adhesion between fiber and matrix.

Finally Treated cellulose fibril is extracted from New Method because large quantity of treated cellulose was obtained from new method, and in new method fiber was subjected to mild chemical treatment it does not affect cellulose content which tends to reduce lignin and hemicelluloses contents alone. Thus further extraction process gone through new method for 800 grams. After Extraction process obtained treated cellulose was 350 grams, for which composite sheets were made at different weight percentage such as 25% and 30% in case of both treated and untreated fiber by compression molding .To reduce moisture content and to improve strength reinforcement is done and matrix used for reinforcement is epoxy. For obtained composite sheets morphological analysis was carried out in scanning electron microscopic (SEM), for chemical composition analysis FT-IR (Fourier Transform Infrared Spectroscopy) analysis was carried out, and finally element detection analysis was carried out by EDX Technique to check weight percentage of carbon and oxygen. The microscopic analysis results at treated cellulose sample defibrillation, De-polymerization occurs, and material becomes soft comparison to untreated sample. In FT-IR absence of lignin and hemicelluloses was observed in both 25% and 30% of fiber loading, in element detection analysis it reveals that oxygen content is larger than carbon in case of treated composite ,it proves that amount of lignin content was reduced.

Thus due to chemical treatment it demonstrates removal of lignin and hemicelluloses in case of treated cellulose composite at 25% and 30% of fiber loading comparison to untreated composite. The main theme of this research was to remove lignin and hemicelluloses content of untreated coir fiber reinforced composites through suitable chemical treatment namely new method [1] thus it results high removal Rate at 25 % and 30 % of treated cellulose composite.

MATERIALS AND METHODS

Coconut fiber is extracted from the outer shell of a coconut. Brown fibers are thick, strong and have high abrasion resistance. Density 1.4 g/cc Single fiber, Breaking Elongation 30%, Moisture regain at 65% RH 10.5%.

Methods for the Extraction of Treated Cellulose

In this research two extraction processes were carried out namely cross and Bevan method [2] and new method**.**

New Method

Coir fiber is chopped for 3-4 mm length and it is soaked for 2% caustic soda (NaOH) in the fiber liquor ratio of 1:10 and kept for 6 hrs at a room temperature.Then fiber is washed several times with distilled wasted to remove sticking of NaOH in fiber thus weight reduction was achieved [1]. Steam exploded treatment is done for mercerized fiber for 1 hrs around 200-250 degree Celsius in pressure Cooker thus removal of lignin content and hemicelluloses content is observed weight loss is Obtained after steam explosion. Then it is washed thoroughly with water, dried and weighted. During this process heat is applied around 200-250 degree Celsius [1]. Bleaching process is done for steam exploding Fiber using Naclo2 (Sodium chlorite) at ph. 2.3 [1]. The ph. of water is 7 is reduced to low ph at 2.3 value using Concentrated sulphuric acid (H2SO4) and by using sodium chlorite ph. is maintained at ph. 2.3. Heated for 1 hr. at 50- 60 degree .Then it is washed with distilled water to reduce the effect of sodium chlorite.Oxalic acid treatment is subjected to bleached fiber for mild acid treatment .Oxalic acid 5% is taken for 800 grams of coir fiber and heated for 1 hrs at 50-60 degree followed by steam exploded treatment for 1 hrs at 200-250 degree [1] maintained

in pressure cooker. Then fibers are then washed thoroughly with water and dried. Thus due to mild chemical treatment cellulose content of fiber may not decrease [1]. Finally after acid treatment the dried fiber is subject to constant stirring process on Remi motor at constant speed of 200 rpm for further cellulose. Extracted treated cellulose from new method was shown in Figure 1.

Cross and Bevan Method

In this step chopped coir fiber is first boiled with 1% of NaOH for 30 min. after that it is washed with water until moist chlorine gas is removed and it is dried, weight reduction is achieved . Then after the fiber is treated with 2% of sodium sulphite and heated it for 5 min. Finally it is washed with water and then it is dried and weighted.Fiber after sodium sulphite treatment is subjected to bleaching process by maintaining ph. at 4 thus ph. value is reduced using sulphuric acid and it heated with of potassium permanganate for 1 hrs. At 50-60 degree thus low molecular weight components partially get reduced. Thus it is washed with water again and again. Finally it is dried and weighted . The obtained treated cellulose from cross and Bevan method was shown in Figure 2

Composite sheet fabrication

The matrix used in this process is epoxy because it is able to improve adhesion between fiber and matrix and low cost. The epoxy matrix and epoxy hardener is mixed in ratio of 10: 1, in slow manner to avoid bubble formation in prolonged Duration [10]. The coir fiber of 25% and 30%of both treated and untreated by weight was spread over mold cavity. The fiber is placed in mold impression and mixture of resin and hardener is also placed in mold impression [10]. The other half of mold plate is placed to complete whole setup. Force is applied at the rate of 2 ton per square inch which squeezes the fiber and resin mixture which is able to take mold impression shape and it is kept at constant pressure at 85-95 degree for 3-4 hrs [10]. And then it is cooled for 9-12 degree for 4-5 hrs, to avoid hardener effect in composite plate [10]. After the whole process is completed sheets are withdrawn from mold impression [10]. The fabricated composite sheets were shown in Figure 3 and 4.

Scanning Electron Microscopic(SEM)

Morphological analysis of samples at different weight percentage such as 25% and 30% in case of both treated and untreated composite were evaluated by scanning electron microscope Carl Zeiss EVO 18 at 20Kv. The samples are made for 1mm x 1mm x 3mm .for the prescribed size of sample morphology analysis were carried out for both treated and untreated composite.

Fourier Transform Infrared Spectroscopy(FT-IR)

Fourier transform infrared spectroscopy (FT-IR) of the samples was recorded by an IR-tracer 100 Schimadzu spectrophotometer. About 1mm x 1mm sample was placed into small particles of liquid nitrogen. The samples were mixed with kbr and pressed into small disc about 1mm thickness.

Element Detection Analysis

Energy-dispersive X-ray spectroscopy (EDS, EDX, or XEDS), sometimes called energy dispersive X-ray analysis (EDXA) or energy dispersive X-ray microanalysis (EDXMA), is an analytical technique used for the elemental analysis or chemical characterization of a sample. It relies on an interaction of some source of excitation. In this study element detection analysis were carried out for both treated and untreated sample at both 25% and 30% weight in the dimension of 1mm x 1mm x 3mm sample size. Element detection analysis was carried out in SEM along With EDX technique to determine chemical composition in which able to determine weight percentage of carbon and oxygen.

RESULTS AND DISCUSSION

Scanning Electron Microscopy(SEM)

The results showed in Figure 5 and 6 as untreated raw sample, and chemically treated fiber sample. It shows that rough surface and little porous in case of untreated raw sample at both 25% and 30% weight of fiber loading, and shows strong binding of cementing components lignin and hemicelluloses in untreated raw with average diameter of 100μm in case of 25% untreated and 30% untreated samples. In general alkali treatment at 2% remove lignin, and hemicelluloses content in fiber further steam explosion and bleaching process removes lignin, hemicelluloses, pectin, and tannin from inner part of fiber by De-polymerization and defibrillation , lignin oxidized by bleaching agent which allows to lignin degradation that leads to formation three functional groups such as hydroxyl, carbonyl, and carboxylic that access lignin to be soluble in alkali solution . From the above study it demonstrates that in untreated raw sample it reveals rough surface, porous and shows impurities such as lignin and hemicelluloses at both 25% and 30% of weight in Figure 5. In case of treated composite it demonstrates removal of lignin and hemicelluloses due to alkali treatment followed by stream explosion, bleaching and oxalic acid treatment it allows to defibrillation and De-polymerization that reveals removal of lignin, hemicelluloses, wax and pectin that contained in fiber. Each elementary fiber shows a compact structure; exhibiting an alignment in the fiber axis direction with some non-fibrous components in the fiber surface. It shows that chemically treated fiber shows removal of lignin and hemicelluloses with micro fibrils average diameter around 20-200μm shown in Figure 6

Fourier Transform Infrared Spectroscopy(FT-IR)

The results shows that lignin and hemicelluloses content wave number was absent in case of both treated cellulose at 25% and 30% weight comparison to untreated composites. The composite material is composed of alkaline, esters, ketone, alcohol; aromatics with different oxygen functional group same as observed in literature. An infrared red transmittance spectrum with some main observed peaks for different weight percentage in case of both treated and untreated was shown in Table 1. All samples should be dry because it is difficult to extract due to cellulose water interaction. Table 1 shows that the characteristics peak wave number 1753.2 - 1755.22 cm-1 is responsible for hemicelluloses and lignin content and it was presented in both untreated composite at 25%and 30% weight. At chemical treated composite it was chiefly absent in both weight percentage. The wave number from 1600-1690 cm-1 is responsible for moisture absorption capacity. It shows that increasing to decreasing range from untreated to treated sample at different weight percentage in the range 1610.56- 1600.34 cm-1.

From the Table 2 shows that the characteristics peak wave number 1200-1390 cm-1 is responsible for hemicelluloses and lignin content and another possibility carboxyl adsorption , it was presented in both untreated composite at 25% weight and aromatic ring vibration was presents in 30% untreated. At chemical treated composite it was chiefly absent in both weight percentage. The wave number from 1000-1080 cm-1 is responsible for cellulose to cellulose interaction. It shows that decreasing range to increasing range from untreated to treated sample at different weight percentage in the range 1053.10 – 1072.42 cm-1. From FT-IR we can conclude that lignin and hemicelluloses binding components were removed in chemical treatment during NaOH treatment it breaks hydrogen bond, thus it reduces OH Concentration, further steam explosion, bleaching process it leads to formation of three function groups that access lignin and hemicelluloses to be soluble in chemical treatment, thus raw fiber have this characteristics peak 1700-1760 cm-1 and 1200-1390 cm-1 chiefly responsible for lignin and hemicelluloses. Above results were shown as graph with respect to wave number Vs Infrared transmittance (%), in Figure 7 & 8.

Element Detection Analysis

The results shows that at untreated sample at 25% and 30% weight of fiber loading it shows higher carbon content weight percentage than oxygen it clearly demonstrates due to higher lignin content, because lignin which was aromatic in nature with high carbon content comparison to cellulose and hemicelluloses. Thus during chemical

treatment cellulose will be isolated from impurities which shows higher weight percentage of oxygen than carbon content. At 25% and 30% of fiber loading higher weight percentage of oxygen was observed at 30% of treated sample, but both 25% and 30% of treated sample demonstrates lignin content elimination and results higher oxygen percentage comparison to untreated samples at both 25% and 30% weight. The results of weight percentage of oxygen and carbon content was shown in Figure 9.

CONCLUSION

The main theme of this research was to remove lignin and hemicellulose content of untreated coir fiber reinforced composites and it was accomplished by suitable chemical treatment namely new method thus it possess low amplitude level, and high damping factor in treated composites at both 25% and 30% of fiber loading. Microscopic Analysis Such as Scanning Electron Microscopic, Fourier Transform Infrared Spectroscopy, and Element Detection Analysis were Carried out for Treated and Untreated Composite ,it Shows Defibrillation And Depolymerization in Case of Treated Cellulose at 25% and 30% was recorded in SEM. The Absence of Lignin, Hemicelluloses, Pectin, and Some wax Content was observed in Treated Cellulose at 25% and 30% Comparison to Untreated. In Element Detection Analysis Weight Percentage of Oxygen is higher than Carbon at 30% of Treated Cellulose, Secondly at 25% of Treated Cellulose Comparison to Untreated Composites. So we conclude that removal of lignin and hemicelluloses was observed in Case of Treated Composite Comparison to Untreated Composite at 25% and 30% of weight. In future scope at 30% of treated composite, damping characteristics can be enhanced.

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Table1: Infrared Transmittance spectrum (%) for the different weight percentage in case of both treated and untreated composite.

FT-IR(CM-1) Spectra(%T) [1]	O-H Stretch [1]	C-H Vibration [1]	C=O Stretch [1]	Absorbed Water [1]
25% Untreated	3969.5	2731.2	1753.29	1608.63
30% Untreated	3975.2	1865.17	1755.22	1610.56
25% Untreated	3980.9	2106.27	-	1608.46
30% Untreated	3983.3	-	-	1600.34

Table 2: Infrared Transmittance spectrum (%) for the different weight percentage in case of both treated and untreated composite.

FT-IR (cm-1) Spectra (%T) [1]	C-H Stretching [1]	Aromatic ring Vibration of lignin [1]	C-C Stretching [1]
25% Untreated	1365.60	-	1060.13
30% Untreated	-	1276.88	1053.10
25% Treated	-	-	1068.13
30% Treated	-	-	1072.42

Figure 1. Final Stage Extracted Treated Cellulose and after Stirring Process

Figure 2. Obtained Treated Cellulose from Cross Bevan method

Figure 3. Untreated Composite Sheets

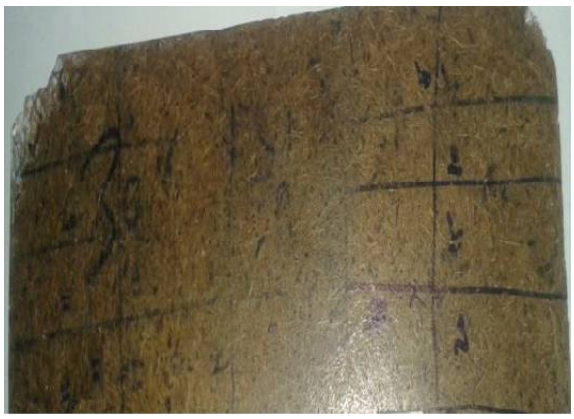

Figure 4. Treated Composite Sheets

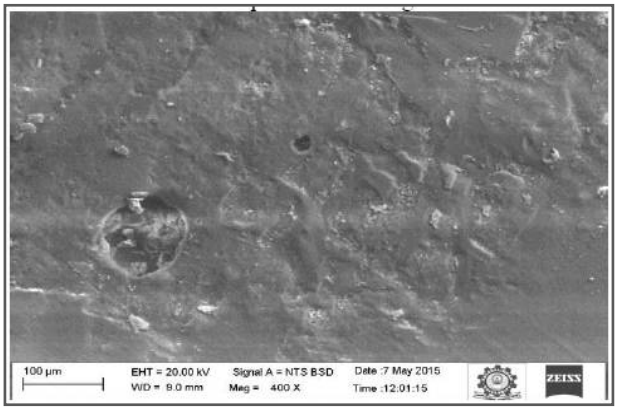

Figure 5.Untreated coir fiber SEM Image

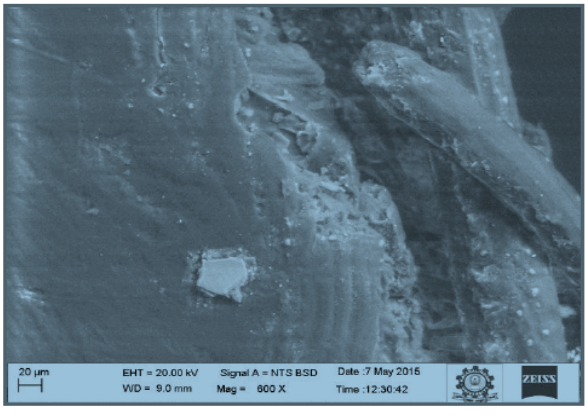

Figure 6. Treated coir fiber SEM Image

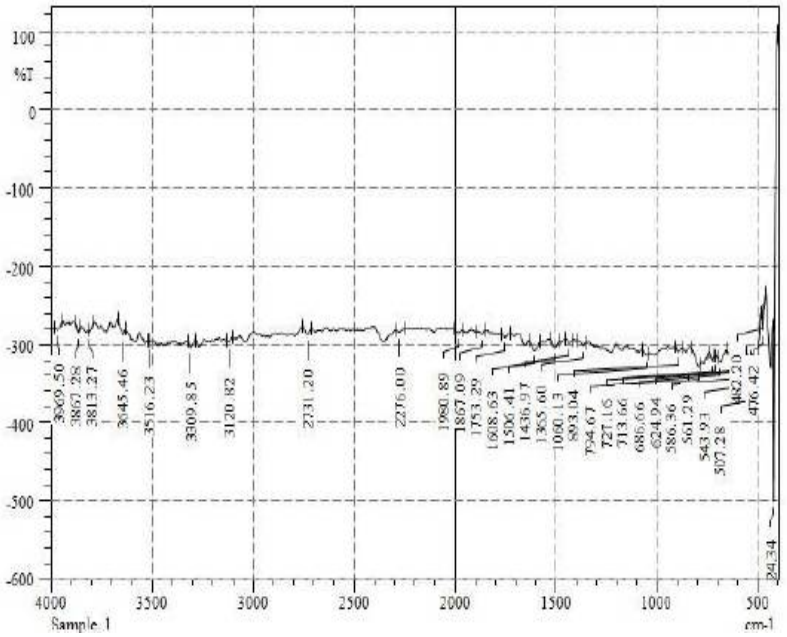

Figure 7. Graph Shows For Untreated 25% Weight Sample Chemical Composition With Respect To Infrared Transmittance (%)

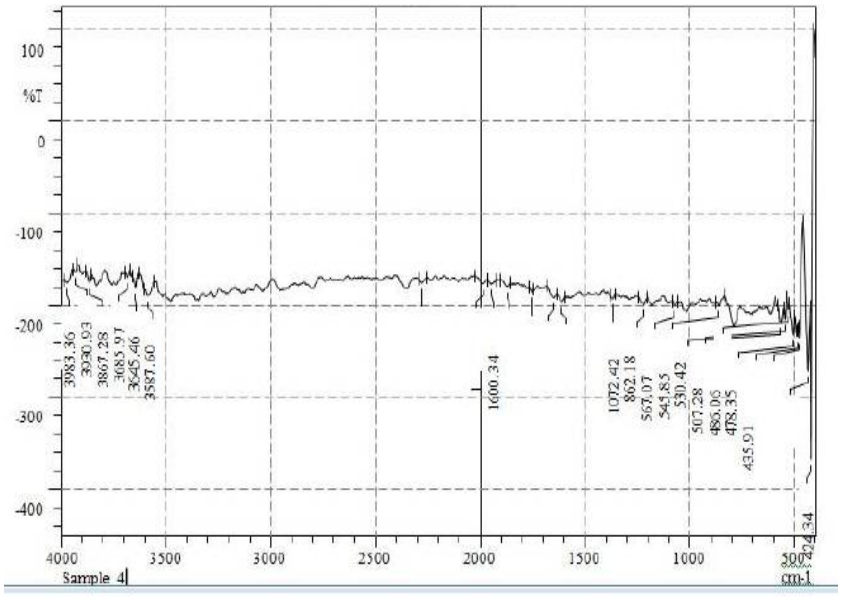

Figure 8. Graph Shows For Treated 30% Weight Sample Chemical Composition With Respect To Infrared Transmittance (%)

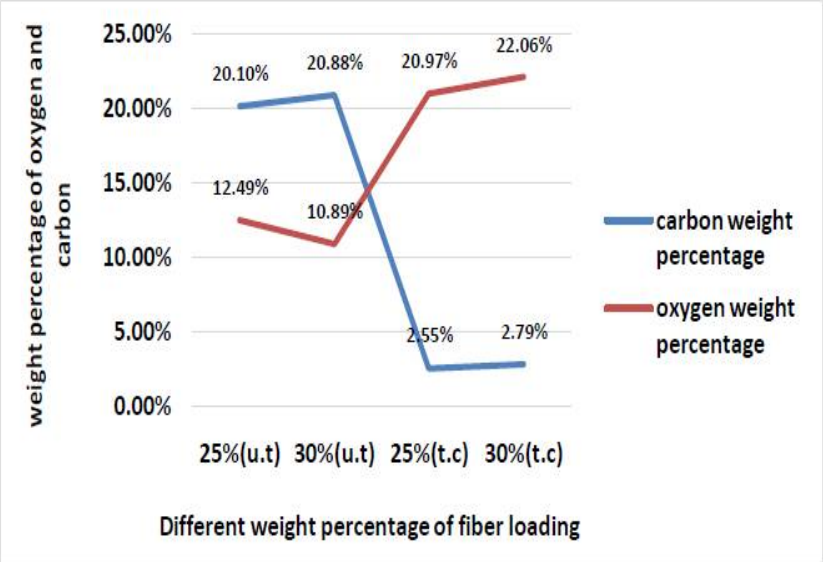

Figure 9. Weight Percentage of Carbon and Oxygen Vs Fiber Loading

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RESEARCH ARTICLE

Implementing Aquaponics in Nilgiris

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ABSTRACT

Aquaponics is a concept of cultivation of plant with soilless media through integration of fish which has attracted worldwide attention in recent years and is considered as an alternative technology for conventional aquaculture. Aquaponics helps to meet the growing demands of present day scenario of food requirement of the world. In this work, common carp (*Cyprinus carpio L.*) was cultured, and attempts were conducted indoor to evaluate aquaponics system performance and characterize three various sources of water emerging from Nilgiri district. The aim of this work was to create awareness on the importance of water quality management of soilless culture in growing plants and fish for food. The integration of aquaculture with hydroponics appears to be an excellent way of saving water, disposing aquaculture wastewater reduced plant diseases and zero pesticides utilization; the modularity of the system uses wide range of purposes like urban agriculture, people resilience in developing countries with marginal land exploitation. The objective were to determine the production of various plants and Common Carp in simplified Personal sized raft aquaponic system which vary greatly in design, but perform all key functions of recirculating aquaponics system, removal of suspended solids and bacterial nitrification. This study determined that the physio-chemical parameters of water is essential for balancing an aquaponic unit and has an impact on fish, plants and bacteria with temperatures 16-26°C, pH 6.2-8.4, DO5.6-11.2 ppm ammonia and nitrite <3mg/L, and nitrate <300mg/L. The results presented in the current study represent some novel data regarding unionized ammonia (UIA).

Keywords : Raft Aquaponic system, Water sources, Plants growth, Common Carp, Physicochemical parameters.

INTRODUCTION

Aquaponics is the integration of aquaculture and hydroponics, a soil less system for crop production. The recirculating aquaculture research community introduced the idea of aquaponics in the mid-1970s [1,2 and 3] Aquaponics is an integrated multi-trophic system that combines elements of recirculating aquaculture and hydroponics, wherein the water from the fish tanks that is enriched in nutrients is used for plant growth. [4] Using fish waste as fertilizer for crops is an ancient practice. In the late 70s and early 80s, researchers at the New Alchemy Institute North Carolina State University (USA) developed the basis of modern aquaponics [5] A survey shows that aquaponics has been receiving growing interest since then [6] which underpins its increasing significance for society as an innovative response for food security. Expanding interest in sustainable agriculture and producing food, closer to urban centers, have simulated involvement from a small but growing aquaponic industry. As far back as 1150-1130 CE,chinampas, often referred to as "floating gardens", were documented as the signs of sustainable agricultural systems [7].

Bacteria in the gravel and bacteria associated with the roots of the plants have a critical role in the cycling of nutrients; without which, the system would stop functioning [8,9,10] Drastically reduced water and nutrients compared with soil grown crops, no chemicals, reduced growing area (limited space required),free from soil diseases and pests, no problem of weeds or digging, yield organic vegetable products all year round adds on additional advantages to aquaponics[11].

MATERIALS AND METHODS

Aquaponics System design: Tank dimensions:

Rearing tanks: Rectangle shape with size-45cm breadth x 33cm length x 27cm height
Volume of water-40 litres
Aquarium air pump specification-220-240 voltage
Power-5 watts
Output-6 litre/minute
Frequency-100 Hz

The above aquaponics system was setup at indoor Coonoor, Nilgiris, Tamil Nadu, India. The system was operated with fish and plants for six months (starting from November 2013) Water from three different sources are collected in and around Coonoor with (CR-pykara river; CL-Wellington lake; CS-Vannarpetai Stream).Three systems were installed in a controlled environment, comprising of river water stored in the fish tank as (CR) and the other with lake water (CL) and stream water (CS) which are the aquaculture components. 15 fishes of carp juveniles weighing an average of 10.0 g each, 9cm in length were acclimated and transferred to a 40 L polystyrene tank. Standard feed of 2% of its body weight was fed twice a day. An aerator was installed in the unit. The systems employed raft cultures for vegetables using carp. Holes are cut in the sheet to accommodate small plastics pots. The bottom and sides of the pots are perforated sufficiently to permit easy movement of the plant roots into the water. Five plant pots are inserted into the holes of the Styrofoam sheets. The pots are filled to a height one inch below the top with small size gravel, which acts as the media for the hydroponics component.

It was started with the introduction of fishes into the aquaculture tank and placing the raft on top of the tank in such a way that the bottom of the pots remains immersed in the water. Leafy green vegetables of three weeks growth purchased from a local nursery are then planted in the pots on the Styrofoam raft. Stocking density in tank culture was measured in units of fish biomass per volume of water [12].The common carp (*Cyprinus carpio L.)* has been one of the oldest domesticated species of fish for food [13] Vegetables selected for this experiment include Celery, Lettuce, Cabbage, Brocolli and Beans.

Stocking density

The stocking density is an important aspect for fish growth. Fish health depends upon the stocking densities [14]. Aeration and water exchange renew dissolved oxygen supplies, remove waste and increase the growth of bacteria in the system.

Fish Health

Maintenance of fish health in aquaponics systems is more challenging than in fish of aquaculture. Maintaining good water quality is an inevitable part in keeping fish healthy. Fish gulping for air at the water surface often indicates low oxygen in the system, sudden decrease in feeding activity, darkening or color change, slow movement, hanging or swimming in an unusual position, spinning, lying on the bottom, torn (or) eroded fins and lumps are the symptoms of abnormal fish behavior [15].

Feed

The aqua feed industries have recommended a fish meal [16] and have been researching and developing alternative protein ingredients, particularly plant derived proteins [17] Therefore, in the present experiment aqua feeds containing plant based proteins was used for fish and system performance.

Fish growth parameters

In the present experiment, considering percentage weight gain and specific growth rate, the highest biomass of carp was obtained in (CR) followed by CL and CS. (Table-1) and measurements of growth and yield [18].

Measurement of growth and yield

At the end of the experiment 180 days, fish biomass and the entire growth of the plant and its yield within their respective harvest period were determined. Fish growth performance was assessed using the following equations:
Weight gain (W) = Final weight (Wt) - initial weight (W0) (g)
Food conversion ratio (FCR) =Total feed (F)/ Total weight gain (w) (g/g)
Specific growth rate (SGR) =In mean final weight- In mean initial weight/ Culture days /initial weight X 100
Relative growth rate (RGR) = (Wt-W0)/ t /BW) (g/kg/.d)

Plant growth parameters

Plant growth had been evaluated by considering the plant height, leaf length, height gain, circumference of crown and yield. The combined effects of environmental factors like water temperature, pH and TDS affected the growth and yield of the vegetables. [19] The highest plant growth was observed in (CR). The analyses of the datas obtained are tabled below (Table-2a, 2b and 2c) and it is observed that the growth of the plant is proportionate to the growth of the fish.

Water quality parameters

All water quality parameters were measured (Table 3a, 3b and 3c) and analysed according to methods described in [20, 21]. Monitoring of water quality was done daily at 6.00am to 7.00am in the morning, the best time to collect data since photosynthesis stops at night while respiration continues [22]. PH and DO concentration were determined using Hach Model HQ40D with digital pH sensor. Other parameters such as alkalinity, ammonia, nitrite, nitrate and phosphorus were analyzed using the standard methods [23]. The chemical composition is complex because of a large number of dissolved ions and organic substances resulting from the release of excretory compounds as a product of fish metabolism and feed digestion. The interaction between the main ions in solution can influence the chemical composition of aquaponics nutrient solutions. The rate of change in nutrient concentration can be influenced by

varying the ratio of plants to fish [24] Oxygen availability is the most critical parameter for fish. Minimum concentration that fish can tolerate is about 4 to 5 mg/L of oxygen. Oxygen consumption of fish varies on temperature, bacterial population, stress on the fish, fish species, fish size, etc.

Temperature and pH are the most important regulation factors for aquaponics systems, and it is needed to be balanced for fish, plants and microbes. Usually, recommended pH for plant cultivation was slightly acid (5.5-5.8) [25], while the optimum pH for nitrification was 7.5 to 8.0 [26] The optimal pH was different for different fish species. [27, 28] Maintaining high DO levels is extremely important for aquaponics systems with high loads. If DO is deficient, then root respiration decreases. This reduces water absorption, decreases nutrient uptake, and causes the loss of cell tissue from roots resulting in reduced plant growth. [29].In healthy tanks, ammonia levels should be maintained as zero. Presence of ammonia is an indication of systems imbalance. Therefore ammonia level should be reduced for the better growth and survival of the fish. Unionised ammonia (UIA is NH3) is toxic to fish and the toxicity begins at the level as low as 0.05mg/L. Fraction of UIA can be obtained by multiplying TAN with appropriate factor from the table values of temperature and pH [30].

Mineral composition of systems

The formations of dense network of roots from the perforated pots were able to draw minerals from the aquaponic water. Variations in the mineral composition of the water were due to excretion of fish, mineral absorption by plants and addition of water to compensate water loss by evapo transpiration. Ca, Mg, So4, Po4 in water increased after one month of cultivation and its concentration subsequently stabilized, Mg concentration continued to increase because of fish excretion [31]. Concentration of iron in (CR and CL) varied to a slight extent. Fluoride was constant with minute quantities. Table (4a, 4b and 4c).

RESULTS AND DISCUSSION

The 100% survival results for fish and plants indicates that aquaponics can adapt to a wide range of temperature and pH In the entire studied period, water temperature and DO were maintained between 16-26°C and 5.6-11.2 ppm in all the three systems. This experiment proved that aquaponics had self regulation ability to cope with temperature and pH changes [32] referred dissolved oxygen as pulse of an aquatic ecosystem which influences major role in biochemical changes and effects metabolic activities of organisms. The respective values for Ca, Mg, Fe, Mn, free ammonia, Cl, Fl, So4, P-Po4. are summarized in Table-4.

It was found that plant biomass increase in yield was attributed to higher nutrient concentration in water and higher water temperature. pH had significant effect on aquaponics production Fish biomass increase was recorded in (CR) when the pH was 7.0-8.4 followed by (CL)6.5-7.7 and (CS) 6.2-8.2, indicating that slightly alkaline environment was favorable for the growth of carp, which was consistent with the work of [33], Under optimal environment there will be increased physiological activity which enhances digestion of feed. Increased feeding frequency significantly promoted the fish growth rate. In the present experiment, a rapid increase in the level of pH (7.5-8.4) in CR was observed at the early stages due to high NH4 + - N in the beginning later on favorable pH was maintained throughout the experiment. Reduction in PH was due to the production of CO2 in water as a result of respiration of fish, bacteria and plant roots in aquaponics system. The CO2 released during respiration reacts with water producing carbonic acid (H2Co3). If sufficient DO is present then CO2 concentration is not highly toxic to fish [34]. Direct NO4 absorption provided sufficient nitrogen nutrition for all the plants which was earlier reported by [35].The functioning regime was still stable owing to an equilibrium between nitrogen supply and requirement. Plant growth was persistent as long as fish were fed and NH4 excreted leading to sufficient bacterial population in all the three systems.

Aquaponics are closed-loop facilities that retain, treat and reuse the water within the system. In the systems design we have determined various parameters like size of the tank, biofilter and choice of the aerator. Thus continuous aeration facilitated nitrification during the experiment. Although various types of vegetables were cultivated to evaluate the performances of aquaponics, fish effluents rich in nutrients can supplement organic fertilizer for vegetable production without affecting fish growth. With the benefits of efficient use of water, limited waste, organic-like management, collocation for producing two agricultural products of plants and fish, increased density of crop production addresses a growing interest in locally grown food. [36] These benefits outweigh aquaponics to be economically viable for the farmer, environmentally sustainable, and beneficial for the society.

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Table-1 Fish Growth Parameters

TANK	Fish Stocked/ recovered	Mean Weight (g)		Fish biomass (kg)			Feed input (kg)	FCR	SGR %/g/d	RGR/g/g/d
		Start	End	Start	End	Gained				
CR	15/15	150	630	10	42	32	72	2.3	1.8	0.018
CL	15/15	150	573	10	38	28	72	2.6	1.6	0.016
CS	15/15	150	546	10	37	27	72	2.7	1.5	0.015

Table-2a. Plant Growth Parameters in River Water (Cr)

S.No	Plants	Initial length Dec 15	Jan (1)	Jan (15)	Feb (1)	Feb (15)	Mar (1)	Mar (15)	Apr (1)	Apr (15)	May (1)	May (15)
1	Celery	3cm	6	16	25	34	42	50	55	-	-	-
2	Lettuce	4cm	8	14	19	24	29	-	-	-	-	-
3	Cabbage	3cm	5	8	15	21	26	30	32		-	-
4	Broccoli	2cm	7	15	24	31	38	43	-	-	-	-
5	Beans	3cm	34	72	103	134	-	-	-	-	-	-

Table-2b. Plant Growth Parameters in Lake Water (Cl)

S.No	Plants	Initial length Dec 15	Jan (1)	Jan (15)	Feb (1)	Feb (15)	Mar (1)	Mar (15)	Apr (1)	Apr (15)	May (1)	May (15)
1	Celery	3cm	5	14	21	33	48	53	-	-	-	-
2	Lettuce	3cm	6	12	18	24	27	-	-	-	-	-
3	Cabbage	2cm	5	7	15	20	26	29	30	-	-	-
4	Broccoli	2cm	7	15	22	29	35	41	-	-	-	-
5	Beans	4cm	30	64	96	131	-	-	-	-	-	-

Table-2c. Plant Growth Parameters in Stream Water (Cs)

S.No	Plants	Initial length Dec 15	Jan (1)	Jan (15)	Feb (1)	Feb (15)	Mar (1)	Mar (15)	Apr (1)	Apr (15)	May (1)	May (15)
1	Celery	3cm	5	12	19	20	34	40	46	-	-	-
2	Lettuce	4cm	6	10	18	23	24	-	-	-	-	-
3	Cabbage	3cm	5	9	16	20	23	26	27	-	-	-
4	Broccoli	3cm	7	16	21	27	31	34	-	-	-	-
5	Beans	3cm	27	60	92	124	-	-	-	-	-	-

Table- 3a. physicochemical parameters of CR

MONTHS	Temp	pH	Do	No2	No3	TAN	UIA
Dec	16	7.5	7.9	0	1.0	1.38	0.013
Jan	19	8.4	7.3	0.1	1.0	1.66	0.141
Feb	20	7.0	6.9	0.03	4.0	1.78	0.007
Mar	22	7.1	6.6	0.02	5.2	2.05	0.014
Apr	24	7.2	6.4	0.01	6.3	2.24	0.019
May	26	7.3	6.1	0.01	6.6	2.36	0.029

Table- 3b. physicochemical parameters of CL

MONTHS	Temp	pH	Do	No2	No3	TAN	UIA
Dec	16	7.7	11.2	0	4.0	1.49	0.022
Jan	19	7.0	9.3	2.2	4.0	1.94	0.007
Feb	20	6.8	8.1	1.9	8.0	3.78	0.008
Mar	22	6.7	7.5	1.4	8.8	3.61	0.006
Apr	24	6.6	6.8	0.9	9.8	3.45	0.005
May	26	6.5	6.3	0.7	10.0	3.31	0.002

Table- 3c. physicochemical parameters of CS

MONTHS	Temp	pH	Do	No2	No3	TAN	UIA
Dec	16	8.2	7.6	0	5.0	1.58	0.070
Jan	19	6.8	7.4	0.50	6.0	4.35	0.010
Feb	20	6.5	7.3	0.40	4.0	3.55	0.006
Mar	22	6.4	6.8	0.38	7.1	2.54	0.004
Apr	24	6.3	5.9	0.35	8.8	1.86	0.002
May	26	6.2	5.6	0.30	9.4	1.38	0.002

Table- 4a. Mineral composition of CR

MINERALS	DEC	JAN	FEB	MAR	APR	MAY
Calcium	14	26	37	50	69	78
Magnesium	11	23	32	45	57	69
Iron	1.48	0.17	0.74	0.98	1.21	1.38
Manganese	0.16	0.35	0.42	0.34	0.31	0.29
Free ammonia	1.38	1.66	1.78	2.85	5.64	9.49
Chloride	6.0	11	21`	38	46	69
Fluoride	0.3	0.3	0.3	0.3	0.3	0.3
Sulphate	2.0	6.0	10	16	19	21
Phosphate	0.16	0.94	3.95	8.52	12.1	17.9

Table- 4b. Mineral composition of CL

MINERALS	DEC	JAN	FEB	MAR	APR	MAY
Calcium	18	25	34	42	55	63
Magnesium	4	8	15	21	28	33
Iron	0.35	0.28	1.24	1.48	1.96	2.02
Manganese	0.21	0.84	1.32	1.65	2.09	2.3
Free ammonia	1.49	2.94	3.78	5.26	7.85	9.4
Chloride	48	42	36	44	52	65
Fluoride	0.3	0.3	0.3	0.3	0.3	0.3
Sulphate	7	9	11	14	17	20
Phosphate	0.28	3.45	7.55	12	15.3	17

Table -4c. Mineral composition of CS

MINERALS	DEC	JAN	FEB	MAR	APR	MAY
Calcium	14	25	36	48	60	73
Magnesium	8	19	25	34	46	58
Iron	0.2	0.6	0.8	1.1	1.18	1.20
Manganese	0.11	0.35	0.91	0.76	0.43	0.23
Free ammonia	1.58	4.35	3.55	5.67	7.82	9.8
Chloride	18	24	36	41	53	65
Fluoride	0.3	0.3	0.3	0.3	0.3	0.3
Sulphate	8	11	13	14	17	19
Phosphate	1.9	3.34	5.38	8.92	12.5	16

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RESEARCH ARTICLE

Economic Impact of Ghee Residue Inclusion in Concentrate Diets of Large White Yorkshire Grower Pigs

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ABSTRACT

An experiment was designed and conducted to study the effect of inclusion of ghee residue on Weight gain and economics of feeding ghee residue in Large White Yorkshire pigs. The study was conducted in pigs for a period of 140 days from 61 days to 200 days of age. The 24 selected weaned piglets were randomly divided into four groups comprising of six piglets in each group.The body weight gain at the end of 140 days by incorporation of graded levels (0, 5, 10, and 15 per cent) of ghee residue in Large White Yorkshire pigs were 83.25, 82.58, 81.83 and 77.41 kg, respectively. Average daily weight gain was 521, 517, 517 and 478 g, respectively. Weight gain at the end of 140 days period was 73.00, 72.44, 72.47 and 66.98 kgs, respectively. Feed conversion ratio observed was 3.30, 3.34, 3.18 and 3.59, respectively in the four treatment levels. There was maximum reduction in feed cost of Rs.18.16 in pig diets supplemented with ghee residue up to 10 per cent level followed by Rs.13.98 and Rs.6.15 in 15 and 5 per cent ghee residue supplemented. From the study it is concluded that incorporation of 10 per cent of ghee residue in concentrate diets were more economical in attaining body weight of Large White Yorkshire pigs.

Keywords : Ghee Residue, Concentrate feed, Economics, Weight gain, Large White Yorkshire pigs.

INTRODUCTION

Among livestock species, pigs have a high production potential next to chicken. The large litter size, increased weight gain with high feed conversion efficiency makes pig forming a profitable venture. Earlier, pigs were reared only by socio-economically poorer sections of society to get profit with low/no input, i.e. scavenging system of feeding with no intensive system of concentrate feeding.Ghee residue, the charred light to dark brown residue is a by- product of ghee industry and is obtained on the cloth strainer after the ghee is filtered and is available at cheaper cost. It is not only a good source of protein and energy, it is rich in source of minerals especially calcium and phosphorus. About 33 per cent of total milk produced in India is being utilized for ghee preparation (Dairy India, 2007) [1] and the average yield of ghee residue is calculated as one tenth of ghee produced. In India, a total of 42.20 million tons of ghee is obtained every year from the total milk production of 140.00 million tons. On an average, ghee residue produced in India is 4.20 million tons (Varma and Narender Raju, 2008) [2]. Ghee residue could be used as a potential alternate unconventional feed ingredient in pig rations. Ghee residue is available at a cheap cost throughout the year.

MATERIALS AND METHODS

The experiment was carried out at Pig Breeding Unit of Post Graduate Research Institute in Animal Sciences, Kattupakkam,Kancheepuram district in Tamil Nadu. The study was conducted in pigs for a period of 140 days from 61 days to 200 days of age. The 24 selected weaned piglets were randomly divided into four groups comprising of six piglets in each group shown in Table 1.

The ghee residue was included at graded levels to prepare experimental diets on iso-caloric and iso-nitrogenous basis. The diets were formulated as per BIS (1986) [3]. The pigs were fed *ad libitum* with grower diets from 61 to 150 days of age and finisher diets from 151 to 200 days of age, respectively. The economics of feeding with graded levels of ghee residue in Large White Yorkshire pigs were calculated by taking prevailing cost of different feed ingredients, feed consumed, feed conversion ratio and total weight gain.
The feed cost per kg live weight gain was calculated as follows:

$$\text{Feed cost per kg live weight gain} = \frac{\text{Cost per kg feed X Total feed consumed}}{\text{Total body weight gain}}$$

RESULTS AND DISCUSSION

The feed cost per kg weight gain (Table 2) were Rs.90.05, 83.90, 71.89 and 76.07 respectively in 0, 5, 10 and 15 per cent ghee residue supplemented groups. Maximum reduction in feed cost by Rs. 18.16 was observed in 10 per cent supplemented group followed by a reduction of Rs.13.98 and Rs. 6.15 respectively in 15 and 5 per cent ghee residue supplemented groups. Barman *et al.* (2011) [4] and Elanchezhian *et al.* (2014) [5] observed similar reduction in feed cost in pigs by feeding unconventional protein and fat rich sources. However, Cherryl *et al.* (2013) [6] recorded only a slight reduction of Rs.1.94 while supplementing protein rich azolla in pigs. The economics of feed cost worked out suggests that there is a saving of Rs. 6.15, 18.16 and 13.98, respectively in 5, 10 and 15 per cent ghee residue supplemented pigs than in control pigs. It suggests that supplementing ghee residue up to 10 per cent is more cost effective than supplementing ghee residue at 5 and 15 per cent levels.

CONCLUSION

A maximum saving of Rs. 18.16 in feed cost per kg body weight gain could be observed in pig diets containing ghee residue at 10 per cent level. From the study it is concluded that incorporation of 10 per cent of ghee residue in concentrate diets were more economical in attaining body weight of Large White Yorkshire pigs.

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Table 1: Piglets in Four Groups

	T-1	**T-2**	**T-3**	**T-4**
Ghee residue	0 %	5 %	10 %	15 %
No. of animals	6	6	6	6

Table 2: Economics of feed cost per kg body weight gain in Large White Yorkshire pigs fed control and experimental rations containing graded levels of ghee residue

Parameters	**Ghee Residue**			
	0% (T1)	**5% (T2)**	**10% (T3)**	**15% (T4)**
No. of Animals	6	6	6	6
Initial body weight (kg)	10.25	10.15	9.36	10.43
Final body weight (kg)	83.25	82.58	81.83	77.41
Body weight gain (kg)	73.00	72.44	72.47	66.98
Total body weight gain (kg)	438.00	434.60	434.80	401.90
Total feed consumed (kg)	1449.35	1451.75	1380.89	1445.45
Cost per kg feed (Rs.)	27.21	25.07	22.57	21.14
Feed conversion ratio	3.31	3.44	3.19	3.60
Feed cost per kg live weight gain (Rs.)	90.05	83.90	71.89	76.07

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RESEARCH ARTICLE

Constraint Analysis of Swine Farming in North-Eastern Agro-Climatic Zone of Tamil Nadu

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ABSTRACT

The study was conducted in North-eastern districts of Tamil Nadu State with the objective identifying the constraints of swine production in the study area. The data regarding swine farming were collected from a random sample of 45 farmers selected from the study area, which were post stratified into small, medium and large farms based on the sow maintained in the farms. The data were collected by personal interview method with the help of pre-tested questionnaire and pertained to the year 2013-2014. The sample farms consist of 36 small farmers, 6 medium farmers and 3 large farmers. Garret ranking technique was used to identify the constraints in pig farming. From the results it could be observed that Inadequate credit facilities, Lack of government support through schemes/subsidies and disease outbreak were most important production constraint in study area. Non-existence of organized markets, less demand in the state and exploitation by middle-man were the most important marketing constraints in the study area. Hence, government may implement suitable policy like providing institutional credit, subsidies for pig production, vaccination programmes for endemic diseases and Organized markets for

piggery sector may implemented to rectify the most rated Production and marketing constraints in the study area.

Keywords : Swine farming, Garett ranking, Production Constraints, Marketing Constraints

INTRODUCTION

Among livestock, pig production has high potential to contribute to high income gain. This is because, pigs have the highest feed conversion efficiency that they produce more live weight gain from a given weight of feed than any other class of meat producing animals except broilers. The pig can utilize wide variety of feed stuff like grains, forages, damaged feeds and garbage and convert them into valuable nutritious meat. They are prolific breeders with short generation interval. A sow can be bred as early as 8-9 months of age and can farrow twice a year. They produce 6-12 piglets in each farrowing. Nowadays due to urbanization most of the people start to consume pigs. Hence, most of the farmers and entrepreneurs are attracted towards the swine farming because of economic traits. Eventhough demand of pork increases day by day farmers faced many constraints because of social ignorance on pigs. So this study aims to identify the constraints in pig farming in study area.

MATERIALS AND METHODS

For the study, North-eastern zone of Tamil Nadu was purposively selected which is comprised of seven districts viz., Chennai, Thiruvallur, Kancheepuram, Thiruvannamalai, Vellore, Villupuram and Cuddalore.Details of selected districts in North-eastern zone of Tamil Nadu. As per *Integerated sample survey report 2012-13*[1], Among the seven agro-climatic zones of Tamil Nadu, north-eastern zone of Tamil Nadu has contributed 29.57 per cent (84,094 heads) of pig population to the overall state population (2,84,324 heads) in 2012-13. Apart from this, rapid urbanization of this zone makes changes in the consumption pattern of the people,which leads to increase in pork consumption. This changing pattern leads to increase in demand for pork consumption in this zone. Hence the people adopted more number of pig farming activities in this zone than the other zones of Tamil Nadu.Due to the above reasons, north-eastern zone of Tamil Nadu was selected for this study.

A sample of 45 swine farms were selected from the study area by simple random sampling procedure, which were post stratified into small, medium and large farms based on the sow maintained in the farms based on the study of Sharma *et a.l* (1997) [2] and Jain and Pandey (2000) [3].The data were collected by personal interview method with the help of pre-tested questionnaire and pertained to the year 2013-2014. The sample farms consist of 36 small farmers (1-8 sows), 6 medium farmers (9-16 sows) and 3 large farmers (> 16 sows). The data collected are tabulated and analyzed with a view to achieve the objectives of the study. Specific tools of analysis appropriate to analyze the data with reference to objectives were applied.

Garret's ranking technique

Garret's ranking technique was used to analyse the problems faced by the farmers in pig rearing in the study area. The pig farmers were asked to rank the factors that they felt as the constraints in their farming activity. The order of the merits given by the respondents was changed to ranks (Garret and Woodworth, 1971) [4] using the following formula:

$$\text{Percent position} = \frac{100(R_{ij}-0.5)}{N_j}$$

Where,

R_{ij} = rank given for the i^{th} factor by the j^{th} farmer

N_j = number of factors ranked by the j^{th} farmer

The percent position of each rank was converted into scores by referring the table given by Garret.Then for each factor or problem, the scores of the individual respondents were added together and divided by the total number of respondents for whom scores were given and thus based on the mean scores, the ranks were given. These mean scores for all the factors were arranged in descending order and the most important factors was ranked first and the least important problem was ranked as the last.

RESULTS AND DISCUSSION

Data regarding the constraints in swine farming were collected from the sample farmers in the study area and were compared for their order of importance based on Garrett mean score values.The ranks obtained for different production and marketing constraints with their respective Garrett's mean score are presented in Table 1 and Table 2 respectively.

Production constraints

The farmers have rated Inadequate credit facilities for pig farming as first and foremost important constraint because in the study area pig farmers are facing a lot of difficulties to get institutional credit from financial institutions, because financial institutions are not ready to furnish loan for pig farmers as like other livestock farmers.The farmers rated lack of government support through schemes/ subsidies as second most important constraint, Disease outbreak was the third most important constraint rated by the farmers. Hence, the farms were more prone to disease outbreaks. Apart from top three constraints, other important constraints ranked by the swine farmers in the study area are piglet mortality, inadequate technical knowledge, high wage rate for labours, difficulty in accessing swill feed and pig farming seen as an socially ignorant occupation.

Marketing constraints

Non-availability of organized market was the first and foremost marketing constraints rated by the sample farmers. Hence, without authorized price fixing agency for pigs, farmers encountered lot of problems in selling the pigs in various seasons. Farmers rated less demand within the state as the second most important constraint, because they mostly depend on nearby states for marketing. Exploitation by middle man was rated as the third most important constraint in study area, non-availability of markets and no proper price fixation for products are the major reason for entering of middleman in the market and they exploit the farmers by fetching lower price for products. Apart from the above constraints unremunerative price for pigs and price fluctuation over different seasons were the other constraints rated by the farmers.

CONCLUSION

From the results it could be observed that Inadequate credit facilities, Lack of government support through schemes/subsidies and disease outbreak were most important production constraint in study area. Non-existence of organized markets, less demand in the state and exploitation by middle-man were the most important marketing constraints in the study area. Hence, government may implement suitable policy like providing institutional credit, subsidies for pig production, vaccination programmes for endemic diseases and Organized markets for piggery sector may implemented to rectify the most rated Production and marketing constraints in the study area.

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Table 1: Production constraints in pig farming

Sl. No	Production Constraints	Mean score	Rank
1	Inadequate credit facilities	81.11	I
2	Lack of government support schemes/ subsidies	57.80	II
3	Disease outbreak	53.88	III
4	Piglet mortality	49.33	IV
5	Inadequate technical knowledge	48.00	V
6	Inadequate labour/ high wage rate	47.42	VI
7	Difficulty in accessing swill feed	40.71	VII
8	Socially ignorant occupation	28.86	VIII

Table 2: Marketing constraints in pig farming

Sl. No	Marketing Constraints	Mean score	Rank
1	Non-availability of organized market	61.35	I
2	Less demand in the state	57.55	II
3	Exploitation by middle man	56.62	III
4	Un remunerative price	54.73	IV
5	Price fluctuation over different season	48.93	V

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RESEARCH ARTICLE

Internal Obturator Muscle Transposition and Autogenous Fascia Lata Graft for Perineal Herniorrhaphy in Dogs

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ABSTRACT

The present study was carried out to evaluate the efficiency of internal obturator muscle transposition and autogenous feascia lata graft for perineal herniorrhaphy. The routine clinical examination and radiographic studies were conducted to confirm the hernia and evaluate the organs that were herniated. Twelve cases were selected and randomly divided into two groups of six animals each. In group I, perineal herniorrhaphy was performed by internal obturator muscle transposition and in group II, perineal hernioplasty was performed using autogenous fascia lata graft harvested from the lateral thigh region. Pain, inflammation, and defecation were graded on the 1st, 3rd and 7th day post-operatively in both the groups based on the subjective scoring system. The post-operative complications encountered were recorded. All the dogs were uncastrated male dogs. Hernia was unilateral in 81.25% (in which the right side was affected in 69.23% and left side in 30.76%) and bilateral in 18.75%. In the present study, the clinical signs observed were perineal swelling, tenesmus, rectal prolapse, faecal impaction, anuria and altered tail carriage. Pain score and inflammation score were found to be elevated in group II dogs on 3rd and 7th day. The defecation score did not have significant difference between the groups and gradually

improved on the 7th day. The mean days for complete cure in group I dogs (12 ± 0.57) were lesser than that of group II dogs (14 ± 0.54). Post-operative complications were minimum in group I. In group II one dog developed wound infection, one dog developed wound dehiscence and three dogs had lameness which resolved after 2 days. Perineal hernia repair using internal obturator muscle transposition proved to be a better technique than fascia lata graft with reduced pain, inflammation and minimal post-operative complications with no recurrence rate up to a maximum of 12 months.

Keywords : Perineal herniorrhaphy, Muscle transposition, Autogenous fascia graft, Small animals, Hernioplasty, Small animal surgery.

INTRODUCTION

Perineal hernias are characterized by disruption of the pelvic muscles and protrusion of abdominal viscera, probably a result of the weakening of muscles and separation of the fascia of the pelvic diaphragm, which favor the displacement flow of anatomical structures leading to perineal swelling (Ferreira and Delgado, 2003). They occur in old uncastrated male dogs in which, the causes are neurogenic muscular atrophy and senile pelvic diaphragm are predominant factors, suggesting the involvement of hormones (Gilley *et al*., 2003). Numerous surgical techniques have been proposed for the treatment of perineal hernia, appropriateness for presenting each case, with advantages and disadvantages. The two techniques most used are rapprochement or traditional anatomical and technical transposition of the internal obturator muscle (Sjollema and Van Sluijs, 1989).The other techniques for herniorrhaphy includes semitendinosus muscle transposition (Chambers and Rawlings, 1991), placing synthetic mesh (Matera *et al*., 1981) or biological (Daleck *et al*., 1992) or a combination of techniques. Based on studies of Bilbrey *et al*. (1990), when evaluating the use deferentopexy of treating perineal hernia in obtaining good results, pexies of abdominal organs have been proposed in order to allow repositioning visceral organs and correction of disorders caused by the projection of the organs in the pelvic diaphragm and a more accurate assessment of the viscera involved.

The bladder repositioning, achieved by deferentopexy with or without the colopexy held prior or in parallel with perineal hernia, was nominated for minimizing pressure on the pelvic diaphragm and prevent further displacement flow of the viscera, reducing the risk of relapse (Bilbrey *et al*., 1990; Barreau, 2008). In parallel, the colopexy also proved effective in reducing deviations, rectal diverticulam, reducing the diameter of rectum and restoring the linear format of the cervix (Brissot *et al*., 2004). Existing repair techniques do not adequately satisfy these objectives. The internal obturator transposition causes less tension on sutures, less deformity of the anus and creates a ventro-lateral muscle sling for closure of ventral region of the hernia. The fascia lata graft can also be used for augmentation when the internal obturator muscle is thin, friable, or reduced in size or as a salvage procedure when recurrence occurs after muscle flap transposition failure.

MATERIALS AND METHODS

Selection of Cases and Design of Study

Dogs presented to the Small Animal Surgery Out-patient Unit of Madras Veterinary College Teaching Hospital with history and clinical signs suggestive of perineal hernia were subjected to detailed clinical and radiographic examination to confirm the hernia. Twelve animals with perineal hernia and free from concurrent diseases were selected and randomly divided into two groups of six animals each. The details of the cases selected for the present study are given in Table 1.

Group I: Perineal herniorrhaphy was performed by Internal obturator muscle tranposition .

Group II: Perineal hernioplasty was performed using autogenous fascia lata graft harvested from the lateral thigh.

Preparation

In all the dogs food and water were withheld for 12 hrs and 6 hrs respectively prior to surgery. The impacted faeces in the rectum evacuated manually before surgery. The perineal region, base of the tail, groin area and the rear legs were clipped and shaved. The entire thigh was clipped and shaved in cases where the fascia lata graft had to be harvested. Purse string sutures or gauze tampon was placed in the anus. The surgical site was scrubbed with 5% W/ V Povidone iodine[1].

Premedication and anaesthesia

The dogs were premedicated with atropine sulphate [2] at the dose rate of 0.04 mg / kg and xylazine[3] at the rate of 1 mg / kg body weight intramuscularly. Induction was achieved with a combination of diazepam [5] at the dose rate of 0.2 mg / kg intravenous and ketamine [4] at the dose rate of 5 mg / kg by intravenous route and anaesthesia was maintained using a combination of ketamine [4] and diazepam[5] at half the induction dose. Very old age dogs were premedicated with atropine sulphate [2] at the dose rate of 0.04mg/kg intramuscularly and acepromazine[6] at the dose rate of 0.1mg/kg body weight intravenously. For lumbosacral epidural analgesia tramadol [10] at the dose rate of 2mg/kg and 0.5 percent bupivacaine [9] was taken in the same syringe and from that total volume, 0.2 ml/kg was administered. Induction was done with diazepam[5] at the dose rate of 0.2 mg / kg and propofol[7] at the dose rate of 4mg/kg body weight by intravenous route and anaesthesia was maintained using isoflurane[8].

Surgical correction using internal obturator muscle transposition (Group I)

For transposition of the internal obturator muscle, an incision of the, internal obturator muscle was made along the dorsocaudal border of the ischial tuberosity and a periosteal elevator was used to elevate the muscle subperiosteally as far as the caudal limit of the obturator foramen. Then suturing of the coccygeal muscle and sacrotuberous ligament, external anal sphincter and internal obturator muscle was carried out. Three to four sutures were placed between the coccygeal muscle and the sacrotuberous ligament as well as the external anal sphincter. Two sutures were placed from the internal obturator muscle to the external anal sphincter and another two sutures from the internal obturator to the coccygeal muscle. The suture material used for reconstruction of the perineal diaphragm was polypropylene No.1 for dogs weighing more than 20 kg and polypropylene No.1-0 for dogs weighing less than 20 kg. Single interrupted sutures were first preplaced but not tightened. Additional sutures were placed after palpation of diaphragm if necessary. Upon placement of the last suture, progressing from dorsal to ventral, the sutures were tightened (Vnuk *et al.,* 2008)

[1] Providone iodine Solution I.P., Stedman Pharmaceuticals Ltd., Tamil Nadu, India.

[2] Atropine Sulphate Injection I.P., [4]Ketamine hydrochloride.,[5]Diazepam., Tamman Titoe pharma Pvt. Ltd., Tamil Nadu, India.

[3] Xylaxin., Indian Immunologicals Ltd., Andhra Pradesh, India.

[6] Ilium Acepril., Troy Laboratories Pvt. Ltd., Australia.

[7] Neorof., Neon Laboratories Ltd., Mumbai.

[8] Forane., Abbott India Ltd., India.

[9] Anawin., Neon Laboratories Ltd., Mumbai.

Surgical correction using autogenous fascia lata graft (GroupII)

Fascia lata graft (FLG) harvest

A small craniolateral cutaneous incision was made over the thigh region. Subcutaneous tissues were then bluntly dissected to visualize the entire surface of the graft and then fascia lata graft was excised. The margins of excision were proximally the tensor fascia lata muscle, cranically the sartorius, caudally the cranial border of the biceps femoris and distally the distal third of the femur.The margins left was 0.5-2 cm of fascia lata in the donor site for closure. The fascia lata graft was stored in a sterile gauze soaked with 0.9% sodium chloride until grafting. After saline lavage the defect was closed in a simple continous manner using polyglycolic acid 1/0 followed by apposition of the skin using silk (1/0) in simple interrupted manner.

Hernioplasty

A curvilinear incision 1-2 cms lateral to the anus and extended from the base of the tail to the ischial border, and then perineal fascia was incised to open and expose the contents of hernia. The hernia contents were then reduced. The fascia lata graft was then sutured to the pelvic diaphragm muscles using polypropylene (1/0) in a simple interrupted manner. The graft was placed with its proximal margin oriented ventrally and the cranial margin apposed to the external anal sphincter. The first suture was placed ventrally between the graft and ischiourethralis muscle, sutures were placed between the periosteum covering the caudodorsal ischial border and graft. The graft was sutured medially to the ventral part of levator to the sacrotuberous ligament. Dorsally, sutures were placed between the graft and the caudal part of external anal sphincter medially and between the coccygeus and levator ani muscle laterally. The graft was sutured under tension to provide a "sail effect" in the pelvic diaphragm. The subcutis was closed with polyglycolic acid (1/0) in simple interrupted pattern (Bongartz *et al* ., 2005). The skin apposed using silk.

Post-operative management

All the animals were observed for any post-operative complications. Dressing was done every alternate day. Tramadol [10] at the rate of 2 mg / kg intravenously was administered at 24 hrs interval for two days. Parenteral antibiotic (Cefotaxime[11] @ 20 mg / kg) was administered for seven days. Lactulose was advised orally at the rate of 3 ml twice daily as stool softener to prevent straining during defecation. The sutures were removed on the tenth day following surgery.

Post-operative evaluation

Pain score

Pain was graded on the 1st, 3rd and 7th day in both the groups. Pain was graded by assigning scores (Stoll *et al.,* 2002). It was based on the dog's willingness to ambulate and sit, vocalization and physiological variables like temperature, pulse and respiratory rate.

0 : No pain
1 : Mild pain
2 : Moderate pain
3 : Severe pain

Inflammation score

Inflammation assessment was based on the subjective scoring system assessed on the 1st, 3rd and 7th post-operative day (Stoll *et al.,* 2002).

0 : No redness or swelling
1 : Mild redness or swelling

2 : Moderate redness or swelling
3 : Severe redness or swelling

Defecation pattern

The pattern of defecation was subjectively assessed on the 1st, 3rd and 7th post-operative day (Bongartz *et al.,* 2005).

0 : Normal defecation
1 : Mild difficulty, no pain expression
2 : Moderate difficulty and pain expression
3 : Severe difficulty and pain

Post-operative radiography

Contrast radiography was performed after the surgical repair to evaluate the correction of the hernia defect. The organs that were herniated were examined for their location inside the abdominal and pelvic cavities.

Time taken for return to normalcy and post-operative complications

Time taken for complete cure from the day of surgery to return to normalcy was recorded in all cases. The animals in both groups were evaluated and the post operative complications following surgery were recorded.

Statistical Analysis

The data were analyzed statistically based on the methods described by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Surgical correction by internal obturator muscle transposition technique

In all the cases a curvilinear incision was made from the base of the tail to the lateral borders of the ischium. In dog No. 2 some difficulty was encountered in identification of the caudal gluteal vessel, perineal nerve and transposition of the internal obturator muscle due to large belly of the muscle. In dog No. 6 difficulty was encountered in repositioning of the organs back in to the normal position through the hernial ring due to adhesions. In dog No. 2 some difficulty was encountered in closing the dorsal defect due to atrophy of the levator ani and coccygeus muscle. In all other animals suturing of the coccygeal muscle and sacrotuberous ligament, external anal sphincter and internal obturator muscle was carried out easily. Additional sutures were placed after palpation of diaphragm if necessary. The suture material used for reconstruction of the diaphragm was polypropylene. In all the dogs perineal fascia was also sutured after herniorrhaphy using polyglycolic acid. No recurrence was noticed during the study period.

Surgical correction using autogenous facia lata graft

1. Fascia lata graft harvest

In all the dogs the fascia lata graft was harvested easily through an incision on the craniolateral thigh region. All the dogs were initially positioned in lateral recumbency and then repositioned in sternal recumbency for herniorrhaphy.

2. Hernioplasty

The fascia lata graft that was harvested was stored in sterile gauze soaked in 0.9% saline until herniorrhaphy. The graft was placed with its proximal margin oriented ventrally and the cranial margin apposed to the external anal sphincter. Dorsally sutures were placed between the graft and the caudal part of external anal sphincter medially and between the coccygeus and levator ani muscle laterally but in dog No. 3 of group II, difficulty was encountered

in closing the dorsal defect due to atropy of both levator ani and coccygeus muscles. The placing of the sutures on the ventral aspect of hernia was found to be easier. Polypropylene was used as the suture material in all the dogs.

Post-operative management

Lactulose administration was found to reduce post operative straining. Most of the dogs exhibited pain during defecation on the immediate post-operative day.

Castration

In dog No. 2, 4 of group I and dog No. 1 of group II castration could not be performed due to the owner's unwillingness for surgery.In all other animals castration was performed by the prescrotal method.

Post-Operative Evaluation

1. Pain score

The pain score was given based on the subjective scoring system on 1, 3, 7 days post- operatively. Dog No. 3 of group I had severe pain and dog No. 4 had mild pain on the immediate post-operative day. All other dogs of group I had moderate pain and pain score was markedly reduced and absence of pain on 7th post-operative day except in dog No. 3 had mild pain. In group II, dog No. 1 and 5 had severe pain and all other dogs had moderate pain and pain score gradually reduced and except in dog No. 2 and 6 all other dogs had mild pain on 7th post-operative day (Table 2).

2. Inflammation score

In group I, dog Nos. 1, 2, 3 and 6 had moderate inflammation while dog No. 4 and 5 had mild inflammation and inflammation score reduced moderately and no inflammation in all the dogs except dog No. 6 which had mild inflammation on the 7th post operative day. In group II, dog No. 3 had severe inflammation, dog No. 6 had mild inflammation and all other dogs had moderate inflammation and inflammation score gradually reduced and mild inflammation was not noticed in dog No. 1, 3 and 4. All other dogs had no inflammation on 7th post-operative day (Table 2).

3. Defecation pattern

In group I, dog No. 3 had moderate difficulty and pain expression while defecation, all other dogs had mild difficulty and pain expression on 1st post-operative day and all the animals had pain free normal defecation on 7th post-operative day except dog No. 3 which had mild difficulty and pain expression. In group II dog No. 1, 2 and 5 had moderate difficulty and pain expression while defecation and all other dogs had mild difficulty and pain expression on 1st post-operative day and all the animals came to normal defecation on 7th post-operative day except dog No. 2 and 5 which had mild difficulty and pain expression (Table 2).

4. Post-operative radiography

Post-operative radiography revealed normal anatomical location of the herniated organs in all the animals except in dog No.1 of group II had mild rectal dilatation.

5. Days taken for return to normalcy and post-operative complications

The mean days taken from the day of surgery to return back to normalcy in group I was 12 ± 0.57 and in group II was 14.16 ± 0.54.The days taken were prolonged to about 16 days in dog No. 4 of group II. The dog No. 3 of group I developed a perianal fistula following third day of surgery. Dog No. 1 of group II developed wound infection on the suture site which was discharging seropurulent fluid. Dog No. 4 of group II developed a wound dehiscence on 12th

day after removing the sutures on the 10th day. In all other dogs in group I and group II the surgical site healed without any complications.

Follow Up

The dogs were evaluated at regular intervals and the outcome was graded. In group I, the outcome was graded as excellent in dog No. 1, 2, 4, 5 and 6 whereas outcome was good in dog No. 3 which had occasional difficulty in passing faeces and rectal prolapse was encountered even before the surgery and was managed with lactulose. In group II, the outcome was excellent in dog No. 3 and 4 whereas, outcome was graded as good in dog No. 1, 2, 5 and 6. Per rectal examination was conducted to evaluate the intactness of the pelvic diaphragm of the operated and contralateral side. The herniorrhaphy side was found to be intact in all the dogs. The anal sphincter tone was found to be reduced in dog No. 3 of group I and dog No. 5 of group II. Mild weakening of the perineal muscles was found in dog No.3 of group I and dog No. 1, 2 and 4 of group II. (Table 3).

CONCLUSION

Surgical correction by internal obturator muscle transposition

In the present study a dorsoventral skin incision was performed over the hernia extending from the spot just lateral to the tail base down to the medial angle of the ischial tuberosity as described by Vnuk *et al*. (2008) and this incision was helpful in the visualization of the full part of the hernial ring and the muscles of the pelvic diaphragm. The skin incision was done with caution as the herniated organs especially the bladder would be present immediately beneath the skin resulting in puncturing during incision. In case where adhesions had developed between the hernial contents and the hernial sac there was difficulty in freeing the adhesions and repositioning of the contents and the findings concurred with that of Krahwinkel, (1983)

In some cases intensive bleeding occurred on incising internal obturator muscle which concurred with the findings of Vnuk *et al*. (2008). Dorsal aspect of the hernia was closed by suturing the levator ani and coccygeus muscles to the external anal sphincter and the caudal gluteal vein was avoided during transection of the tendon as reported by Hardie *et al*. (1983). In the present study polypropylene suture was used for the reconstruction of perineal diaphragm as recommended by Vnuk *et al*. (2008). Polypropylene was selected due to its greater strength and duration of tensile strength and also being a monofilament material resulted in less risk of suture sinus formation or potentiation of infection as reported by Pratschke (2002). In the present study no recurrence has been noticed following internal obturator muscle transposition during the study period.

Surgical correction using autogenous fascia lata graft

1. Fascia lata graft harvest (FLG)

FLG harvesting was performed with the dog in lateral recumbency whereas Bongartz et al. (2005) harvested fascia lata graft with rear of the dog twisted about 45 degree ventrally to perform herniorrhaphy simultaneously. The harvest of the graft was found to be easy because of its superficial position with no complications encountered during harvesting in the study as reported and the findings were similar to that of Bongartz et al. (2005).

2. Hernioplasty

The harvested graft was oriented in the hernial defect with proximal margin oriented ventrally and the cranial margin apposed to the external anal sphincter, as it was wider in the proximal aspect. The hernial funnel has a wide base hence more width of the graft was essential on the ventral aspect and moreover as width of the graft increased the strength of the graft increased the findings concurred with that of Robertson (1984) and Thomas et al. (1998). In the present study polypropylene was used to secure the graft to the perineal defect whereas, Bongartz et al. (2005)

used polydioxanone. Polypropylene had greater strength and duration of tensile strength as reported by Pratschke (2002).

The harvested graft was stored in sterile saline (0.9%) soaked in gauze and the graft was trimmed to make the margins regular. The graft was sutured to the perineal muscles under tension to provide a sail effect in the pelvic diaphragm which retained the abdominal and pelvic organs. The use of graft prevented the excessive stretching of the perineal muscles especially in cases where there was severe atrophy of the muscles. There was no recurrence observed during the study period. The findings concurred with that of Bongartz *et al*. (2005).

Post-Operative Management

The dogs were fed with low residue diet to reduce the post-operative straining as mentioned by Bojrab and Toomey (1981). Stool softeners were also advised to reduce the straining and to prevent the disruption of the perineal sutures. Lactulose was given orally in the present study as stool softener and was found to be effective in the easy passage of stool. Administration of Meloxicam was done to provide a good analgesic effect and to prevent straining in the post-operative period as observed by Pratschke (2002).

Castration

All the dogs in the present study were intact males and castration was performed in nine animals, at the time of herniorraphy as described by (Bilbrey *et al*., 1990; Fossum, 1997; Aliabadi and Deghani, 2007). Mann *et al*. (1989) reported that there was no significant difference in the serum testosterone concentration, serum estradiol 17-β concentration among sexually intact male dogs with perineal hernia and clinically normal sexually intact male dogs. Three dogs in the present study had prostatomegaly and Harvey (1977) reported that castration was indicated if prostatic disease, testicular disease or perianal adenomata coexisted with hernia. No recurrence was observed in any of the dogs both castrated and uncastrated during the study period. However Hayes *et al*. (1978) reported the risk for recurrence among uncastrated dogs was 2.7 times greater than among castrated males.

Post-Operative Evaluation

1. Pain score

In group I, five animals exhibited moderate pain and mild pain in one animal on the immediate post-operative day and the pain score was gradually reduced and no pain was exhibited by five animals except one which had mild pain on 7th post-operative day. This might be due to less tension on sutures, less deformity of the anus and created a ventral patch or sliding for the defect and the findings concurred with that of Aliabadi and Dehghani (2007). In group II, two animals had severe pain and all other dogs had moderate pain and pain score was gradually reduced and except two animals all other dogs had mild pain on 7th post-operative day. This can be attributed to excessive tension of the sutures on the friable muscle fibres and stretching of the fibres and the findings were similar to that of Robertson (1984).

2. Inflammation score

In group I, four animals had moderate inflammation and two animals had mild inflammation on immediate post-operative day and inflammation score reduced moderately with no inflammation in all the dogs except one animal which had mild inflammation on the 7th post operative day. This might be due to exact anatomical dissection and transposition of muscle and created a ventral patch or sliding for the defect as reported by Aliabadi and Dehghani (2007). In group II one animal had severe inflammation, one had mild inflammation and all other dogs had moderate inflammation and inflammation score was gradually reduced and mild inflammation was not noticed in 3 dogs on 7th post-operative day. This could be due to focal perivascular lymphocytic infiltration with soft tissue reaction as observed by Bongartz *et al*. (2005). Acute pain exhibited by few animals in group II may be due to manipulation of

soft tissues in perineal region resulting in pain and inflammation. Findings of this study concurred with that of Bongartz *et al*. (2005); Semiglia *et al*. (2011).

3. Defecation pattern

The defecation was found to be difficult with pain expression during the first post-operatively in both the groups. This might be due to pain inflicted by surgical trauma. The defecation score was found to improve in both the groups and the findings concurred with that of Bongartz *et al*. (2005). This indicated that the rectal deformity was corrected in both the groups.

4. Post-operative radiography

Follow up barium studies were conducted and no rectal deviation was recorded in any of the cases but rectal dilatation was diagnosed in one case in group II, which concurred with the findings of Hosgood *et al*. (1995).

5. Days taken for return to normalcy and post-operative complications

The average days taken for return to normalcy was less in group I dogs as compared to group II, wherein two dogs developed post-operative complications thereby prolonging the overall time.In group II, one of the dogs developed wound infection and one developed wound dehiscence. This might be due to the infection of the surgical wound due to contamination of the site with faecal matter. Wound infection and wound dehiscence were managed by placing the animal on antibiotic therapy and wound management. In group I, one dog developed a perianal fistula which was cured by wound management for ten days the findings concurred with that of Robertson (1984).In three dogs in which fascia lata was harvested showed mild lameness during the initial two days of harvest. It might be due to pain in the surgical site following harvest and the findings were similar to that of Bongart *et al*. (2005).

Follow Up

In follow up studies, per rectal examination proved to be useful in assessing the intactness of the pelvic diaphragm and the tone of the anal sphincter.Follow up studies revealed that no breakdown in the integrity of the pelvic diaphragm in both the groups in herniorrhaphy side and in group I one dog and in group II three dogs had weakened perineal muscles on the contralateral side. In two dogs, anal sphincter was observed to be relaxed and the finger could be inserted in to the rectum with ease as reported by Bray (2001). In these dogs tenesmus was observed following surgery. This could be due to damage to the pudendal nerve which supplied external anal sphincter as stated by Burrows and Harvey, (1973). The outcome of the surgery was graded as described by Szabo *et al*. (2007). In group I, the outcome in most of the animals was excellent based on minimal post-operative evaluation and complication when compared to group II.

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Table 1.Details of dogs with perineal hernia in group I and group II

Group	Dog No.	Breed	Age	Sex	Castrated/intact	Side affected
Group I	1	ND	13yrs	Male	Intact	Left
	2	Boxer	9 yrs	Male	Intact	Left
	3	Spitz	11 Yrs	Male	Intact	Right
	4	GSD	6 yrs	Male	Intact	Right

	5	ND	7 yrs	Male	Intact	Left
	6	Spitz	10 yrs	Male	Intact	Left
Group II	1	Spitz	11 yrs	Male	Intact	Right
	2	ND	7 yrs	Male	Intact	Bilateral
	3	Spitz	11 yrs	male	Intact	Right
	4	Spitz	9 yrs	Male	Intact	Right
	5	Spitz	6 yrs	Male	Intact	Bilateral
	6	Spitz	7yrs	Male	Intact	Right

Table 2. Post-operative evaluation

Group	Animal No.	Pain score			Inflammation score			Defecation score			Complications
		Day 1	Day 3	Day 7	Day 1	Day 3	Day 7	Day 1	Day 3	Day 7	
Group I	1	2	1	0	2	1	0	1	0	0	None
	2	2	1	0	2	1	0	1	1	0	None
	3	3	2	1	2	2	0	2	1	1	Perianal fistula
	4	1	1	0	1	1	0	1	0	0	None
	5	2	1	0	1	1	0	1	0	0	None
	6	2	1	0	2	1	1	1	0	0	None
Group II	1	3	2	1	3	2	1	2	1	0	Wound infection
	2	2	1	0	2	1	0	2	1	1	None
	3	2	2	1	2	2	1	1	0	0	None
	4	2	2	1	2	2	1	1	0	0	Wound dehiscence
	5	3	2	1	2	1	0	2	1	1	None
	6	2	1	0	1	1	0	1	1	0	None

Table 3. Follow up

Group	Animal No.	Anal sphincter tone	Per rectal examination		Outcome
			Herniorrhaphy side	Opposite side	
Group I	1	Tone present	Intact	Intact	Excellent
	2	Tone present	Intact	Intact	Excellent
	3	Relaxed sphincter with tone reduced	Intact	Weakening of perineal muscles	Good
	4	Tone present	Intact	Intact	Excellent

	5	Tone present	Intact	Intact	Excellent
	6	Tone present	Intact	Intact	Excellent
Group II	1	Tone Present	Intact	Mild Weakening	Good
	2	Tone present	Intact	Weakening of perineal muscles	Good
	3	Tone present	Intact	Intact	Excellent
	4	Tone present	Intact	Intact	Excellent
	5	Reduced sphincter tone	Intact	Weakening of perineal muscles	Good
	6	Tone present	Intact	Intact	Good

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RESEARCH ARTICLE

Evaluation of Response of Mustard (Brassica juncea Coss) Varieties to Varying Levels of Nitrogen

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ABSTRACT

Though the country has entered an era of self sufficiency in food grains,still there is a lag behind its oil seed production. Seeds are known by different names in different places (e.g.) Sarson,rai or raya,loria or lahi.Rai(Brassica juncea L.) is one of the oil seeds introduced from china into North Eastern India contains 36 Chromosomes. The total agriculture production rose at an average rate of 2.81% as compared to a poor performance of oil seed at a rate of about 1.62%.Total oil seed acreage in country is revolving around 18 million ha.Over past few years and its production is 128 million tones.Country needs about 38 lakh tones of edible oil. An experiment was undertaken on the evaluation of the response of mustard varieties to varying levels of nitrogen in the Agronomy Research plot,Allahabad Agricultural Institute,Allahabad. Variety RH-30 gave the highest yield amongst the varieties tested in the trial hence for maximising the mustard yield varieties RH-30 may be grown.

Keywords : Agronomy Research, Allahabad Agricultural Institute, Allahabad, Chromosomes.

INTRODUCTION

The indigenous production of all the oil derived from cultivated and non cultivated plant resources is about 26 lakh tonnes.Thus the gap of10 lakh tonnes of edible oils which at present is being imported.Thus there is an urgent need to increase the production of oil seeds particularly the cultivated ones to reduce our dependency on other countries as also to onserve our precious foreign as also to exchange resources.India is the largest producer of rape seed-mustard in the world i.e. it produces about 1.86 million tonnes seeds from the area of 3.56 million hectares.Seeds are known by different names in different places (e.g.) Sarson,rai or raya,loria or lahi.Rai(Brassica juncea L.) is one of the

oil seeds introduced from china into North Eastern India contains 36 Chromosomes.Nitrogen is an important and indispensible nutrient for the rape seed mustard.It is required in a large quantity and is absorbed throughout the growth period.Indian soils are generally poor in nitrogen content which makes it a limiting factor for the proper growth and development of rape seed mustard plants.It promotes the vegetative growyh,flowering and fruiting and also adds in setting of siliqua.It also helps in increasing the size of siliqua.When nitrogen is better than single dose as it checks the unnecessary losses of nitrogen from the soil.Among the different sources of nitrogen,Ammonium Sulphate and Urea have been found to give better results than others.Therefore the determination of affective source of nitrogen is one of the important considerations in the new technology of mustard production.

In the view of the above,the present investigation was under taken to evaluate the Response of different varieties of mustard to nitrogen levels.The main objectives of the investigation are as follow:

1. To study the effect of nitrogen levels on the plant growth.
2. To determine the crop response regarding seed yield as influenced by different levels of nitrogen.
3. To determine the optimum doses of nitrogenous fertilizers as economic yields.
4. To study the relationships between yield and yield contributing factors.

Indian mustard (Brassica juncea coss.) is highly responsive to nitrogen application (Sandhu and Singh 1960).Holmes 1980 has mentioned that rape seed mustard requires high doses of nitrogen,needing ore than is provided by most of the soils.This has also states that maxium total uptake by winder rape(excluding roots) might be over 250kg per hectare of nitrogen,although these could be great variation associated with difference in dry matter production,the rest with nitrogen content.Majumdar(1965) has reported that a crop of brown sarson variety dichotna,yielding 1.05 tonnes of seed per hectare removes 29kg of nitrogen per hectare.

MATERIALS AND METHODS

Experimental Site

The experiment was conducted during the rabi season of 1991 in the plot no.100 of Agronomy Research Farm,Allahabad Agricultural Institute,Allahabad which is situated six kilometers away from Allahabad city beside Yamuna river.The experimental site is located in the sub-tropical region as 25.57 North latitude,81.5 East longitude and 98 meters above the sea level.

Soil

Before conductivity the experiment soil samples from different locations of the plot were randomly collected from a depth of 12 to 15 cm before disturbing the soil profile.

Design and Treatments

The experiment was conducted in a two factor factorial in randomized block design with four varieties of mustard and three levels of nitrogen being combined into twelve treatments and replicated three.Treat merits were randomly arranged in each replication dividing the block into twelve plots in breadth wise direction.The particulars of the treatments and their combinations are shown in table respectively.

Planting Material

Indian mustard varieties evolved(Varuna,Pusa Bold,RH-30,T-151) were used as the experimental crop.These varieties were relased earlier by central variety release committee,but they are bring tested under different field trials in various agno-climatic zones of country.As per recommendation,these varieties are suitable for rainfed condition

and are early maturing.These varieties were compared with varuna of Allahabad Agricultural Institute,Agronoy Department,Allahabad.

Sampling Technique

To study the growth character,five plants per plot were randomly selected and tagged for all the preharvest observations throughout the investigation. Post harvest observations were recorded plot wise from the harvested plants from in area of three square meters.

Study of Growth Characters

The influence of the observant treatment combination on the following growth character essecting the yield of the crop was observed.

1.Pre Harvest Observation:

i. Germination of seeds
ii. Number of plants per running row meter
iii. Height of the plant
iv. Stem diameter of the plant
v. Number of branches per plant
vi. Number of siliqua per plant

2.Post Harvest Observation:

i. Seed yield
ii. Test weight of the seeds

Statistical methods

Analysis of Data

The observation which provided quantitative data were tabulated and statistically analysed by Analysis of Variance Technique.The Fisher's 'F' test method was followed to test the significance of the experimental means at 5% and 1% levels.Calculated 'F'(variance ratio) was compared with the table value of 'F' at 5% and 1% level of significance.If the calculated value was more than that of the table value of 'F' at 5% level of significance the effect was considered to be significant.Further it was compared with table value of 'F' at 1% level of significance to see wheather it was highly significant.

To compare the effect of different levels of treatment,critical difference between the treatment means was calculated at 5% and 1% level of significance by the following formula,

$$\text{Critical Differnce (C.D)} = \frac{2\text{xMESS}}{\text{No.of replications}} \text{ X at error df}$$

Where,
MESS is Mean Error Sum of Square
t at 5% or 1% level of significance
df is degree of freedom.

RESULTS AND DISCUSSION

Crop Emergence

The observation taken on the 5th day after sowing was on the crop emergence.Irrespective of the treatments there was uniform emergence of mustard plants in all the plots.Germination of mustard seed was not affected by the application of different levels of nitrogen.

Number of plants per Running Row Meter

Average number of plants per running row meter under different treatments is presented. Based on the results obtained it may be stated that test weight remained un affected by nitrogen levels.Among the varietiese,maximum test weight was recorded 6.52g by T-151 followed by 5.62g by RH-30m,4.95g by pusa bold,4.29g by varuna.Although variety RH-30 recorded maximum yield but the test weight was found to be minimum in comparision to varuna.The variation in test weight in varieties may be attributed due to varieties character.

Observations

The results of different pre-harvest observations are observed: Height of the Plant, Stem diameter of the plants, Number of branches per plant, Number of Siliqua per plant. The results of different post-harvest observations are observed: Yield,Test weight of seeds(g).

CONCLUSION

An experiment was undertaken on the evaluation of the response of mustard varieties to varying levels of nitrogen in the Agronomy Research plot,Allahabad Agricultural Institute,Allahabad in Rabi season of 1991.The experiment was laid out in a Two Factor Factorial and Treatments were arranged in randomized block design with three replications.The treatments combinations were 3 levels of nitrogen(0,40 and 80 kg/ha) as urea and 4 varieties of mustard viz.Vauna,Pusa Bold,Rh-30 and T-151.The intra quantity of phosphorous potassium and half of the total quantity of nitrogen irrespective of all treatments were applied as basal dose.The remaining half of the total quality of nitrogen irrespective of treatments were top dressed at the early vegetative growth stage(47 DAS)the crop was sown on 16th November 1991 in rows at 50cm apart.Thining of the plants was done at 25 days after sowing one hand weeding was done with Khurpi at 26 days after sowing followed by 2nd weeding at 37 days after sowing.The seed rate was taken 5 kg/ha.One irrigation was given at 39 days after sowing.Thiodan was sprayed at a concentration of 0.1% at 60 days after sowing, subsequently followed by another application of Rogar 0.2% at 82 days after sowing,as a preventive measures against aphid.The crop stand was very good,the stems were thick varied with different treatments and the plant grew to a height of 167.07cms.

The crop was harvested on 3rd March 1992.The general results of the findings are summarized below:

1. Nitrogen application to the mustard crop significantly increased the plant height,stem diameter,number of branches per plant,number of siliqua or pods per plant.The best dose of nitrogen was found to be 80kg/ha in bringing the highest value of the above mentioned character.
2. Nitrogen applied at the rate of 80kg/ha was found to produce bolder seed.
3. The height of mustard plant was also influenced by levels of nitrogen.Variety T-151 achieved the maximum height of the plant followed by RH-30.
4. The variety of RH-30,recorded maximum seed yield followed by T-151.
5. The variety T-151 had the maximum test weight followed by RH-30.

The aforesaid results indicate that mustard responds well to higher doses of nitrogen.Therefore a dose of 80kg N/ha may be recommended.Variety RH-30 gave the highest yield amongst the varieties tested in the trial hence for maximising the mustard yield varieties RH-30 may be grown.

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Table 1.Result of Soil Analysis

Particulars of the soil analysis	Result	Methods followed
A.Mechanical Analysis		
i. Sand	60%	Bouyoucos hydroeter Method (1927)
ii. Silt	26%	
iii. Clay	14%	
iv. Soil texture	Sandy loam	
B.Chemical Analysis		
i. Organic carbon	0.21	Walkly and Black Jackson (1973)
ii. Available Nitrogen	210kg/ha	Alkali Permanganet method (ADAC 1960)
iii. Available Phosphorous	14.45kg/ha	Olson Colorimetric method(Olsn 1954)
iv. Available Potassium	220.4kg/ha	Flame photometer method (Jackson 1973)
v. pH of Soil	7.5	Bcukmen pH meter

Table.2: Details of Treatments

Treatments	Dosage of N/ha varieties of mustard	Symbol used
I. Level of Nitrogen(Main treatment)	0kg.N. as Urea(control)	N_1
	40kg.N. as Urea	N_2
	80kg.N. as Urea	N_3
II. Varieties of mustard (Sub treatment)		
	Varuna	V_1
	Pusa Bold	V_2
	R.H.30	V_3
	T-151	V_4

N=Nitrogen;V=Variety

Table 3. Average number of plants per running row meter recorded at 30 days after sowing as influenced by nitrogen and variety

Treatments Varieties	Nitrogen Levels(kg/ha)			Mean varieties
	0	40	80	
Varuna	12.9	12.0	12.6	12.2
Pusa Bold	12.3	12.3	11.3	11.9
R.H. 30	11.0	11.6	11.6	11.4
T-151	11.0	12.0	11.0	11.3
Mean Nitrogen	11.6	11.9	11.6	0.8

Test	Nitrogen	Varieties	Interaction
C.D	N.S	N.S	N.S

Table 4: Average test weight(g) as influenced is different levels of N and different varieties

Treatments Varieties	Nitrogen Levels(kg/ha)			Mean varieties
	0	40	80	
Varuna	4.17	4.32	4.38	4.29
Pusa Bold	4.30	5.26	4.95	4.95
R.H. 30	5.34	5.19	6.34	5.62
T-151	6.26	6.54	6.54	6.52
Mean	5.01	5.64	5.64	5.34

Nitrogen	Variety	Interaction	C.D.(N)	C.D.(V)	C.D. int.
S.	S.	N.S.	.128	.085	

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RESEARCH ARTICLE

Impact of Organic Farming Programme Implemented by World Vision India on Behavior Aspect of Farmers of Chhattisgarh

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ABSTRACT

Organic farming is one of the several approaches found to meet the objectives of sustainable agriculture. Many techniques used in organic farming like inter-cropping, mulching and integration of crops and livestock are not alien to various agriculture systems including the traditional agriculture practiced in old countries like India. However, organic farming is based on various laws and certification programmes, which prohibit the use of almost all synthetic inputs, and health of the soil is recognised as the central theme of the method.Projects based on organic agriculture are more subtle than chemical agriculture and therefore, situation specific. The experience of organic agriculture projects run by World Vision India in 3 districts of Chhattisgarh is the particular focus of this study.

Keywords : sustainable agriculture, organic farming, chemical agriculture, Chhattisgarh.

INTRODUCTION

The importance of agriculture for food security and rural livelihoods cannot be understated. In recent decades, agriculture and farmers' welfare have both been thrown into jeopardy in India. The alarming farmers' suicides have recently acquired some visibility, but these are only symptoms of the larger crisis - a deep, widespread distress among farmers. Many farmers have been led into high-input high-risk farming where the increasing prices of fertilizers, pesticides, seeds and water have caused a debt trap. The chemical intensive agriculture has led to depleted soils leaving crops more vulnerable and prompting farmers to apply even more inputs. Low-input Sustainable Agriculture: The aim is two-fold - to make agriculture remunerative to farmers and to make it sustainable year after year by enhancing the quality of the farm and the soil. This requires moving away from the Green Revolution

paradigm of dependence on chemical fertilizers, pesticides and seeds from the market."Organic agriculture is a production system that sustains the health of soils, ecosystems and people. It relies on ecological processes, biodiversity and cycles adapted to local conditions, rather than the use of inputs with adverse effects. Organic agriculture combines tradition, innovation and science to benefit the shared environment and promote fair relationships and a good quality of life for all involved."—International Federation of Organic Agriculture Movements.

Organic farming is one of the several approaches found to meet the objectives of sustainable agriculture. Many techniques used in organic farming like inter-cropping, mulching and integration of crops and livestock are not alien to various agriculture systems including the traditional agriculture practiced in old countries like India. However, organic farming is based on various laws and certification programmes, which prohibit the use of almost all synthetic inputs, and health of the soil is recognised as the central theme of the method.Projects based on organic agriculture are more subtle than chemical agriculture and therefore, situation specific. There is then a need for technique adaptation within the project zone. Projects should have a developmental component to them. Successful organic agriculture is 'knowledge intensive' requiring more design and management from the outset, as opposed to the 'just in time' approach of chemical agriculture. Training, extension and demonstration are perhaps even more critical here than with conventional projects. Benefits from organic agriculture may not be immediate. Small farmers will require considerable support or incentive over the initial years if the system is to gain momentum and be maintained. Some agro-ecological situations will convert more easily to organic systems than others.

Farmers appear to resist conversion to organic agriculture when they have been heavily exposed to the chemicals, operate in high input, high output systems, involve in relatively mechanized production along with situation where labour costs are high or labour is not available. However, farmers appear more receptive to conversion to organic agriculture when they have not been exposed to the chemical message, their farming system is traditional or nil input, previous extension services have not been effective production is relatively labour intensive, labour costs are low or labour is readily available and the concept is developed by them or with them.An appropriate national agriculture policy, giving a prominent place to organic farming addressing the issues related to its coverage, financial support during the conversion period, creation of linkages among the farmers, processors, traders and consumers, inspection and certification of organic products and increasing the public awareness of the benefits of organic agriculture along with the ill effects of the conventional system, should be designed. This must be followed by concrete action on the ground if we do not want to miss the far reaching changes all over the world heralded by the organic farming movement.

Objectives

1. To ascertain and compare the socio-economic profile of beneficiaries and non beneficiaries under World Vision India.
2. To determine and compare the knowledge of beneficiaries and non beneficiaries about Organic farming.
3. To ascertain and compare the knowledge & attitude of beneficiaries and non beneficiaries towards organic farming concept being implemented by World Vision India.
4. To evaluate the level of adoption of organic farming by the beneficiaries.
5. To find out the problems and constraints faced by the beneficiaries and seek their suggestions to overcome them.
6. To develop suitable strategies for greater adaptation of organic farming based on the findings.

Significance of the Study

World Vision's Agriculture Strategy's Overall Goal is to "ensure productive and sustainable agriculture systems that will contribute to improved nutrition, food security, health and over all wellbeing of children and their families" One of the Principles is Being stewards of God creation and making a difference & ensure the natural resource based for

future generation by adopting sustainable agriculture system. Priorities are to enable farmers to adopt proven technology to enhance production & ensure conservation of natural resources.Through organic agriculture, farmers learn healthy, sustainable farming practices, and are regaining the wisdom of their agricultural heritage. Organic agriculture enriches the soil season by season. It increases the long-term yield, nutrient value and potency of their crops. It allows for a naturally clean water supply, and provides overall richness, health and well-being of their families, livestock, farmlands and communities. Organic agriculture requires the elimination of commercial fertilizers, herbicides, pesticides and other dangerous chemicals. This in turn affects the whole community and creates a significantly healthier working and living environment for the farmers, their families and livestock, as well as the local wildlife.

It was felt necessary that organic projects must be at least as rigorously identified, designed, implemented, monitored and evaluated as any other development project with strong stakeholder participation. The organic context does not make the project immune to the potential problems with project implementation from misidentification of issues and weak institutional support. Extra emphasis should be placed on human resource and institutional development, recognizing that organic farming is knowledge intensive rather than input intensive.Taking the above facts in contingence, this study is planned as one component of a wider review to examine the contribution organic agriculture projects can make towards the goal of sustainable rural livelihoods. The experience of organic agriculture projects run by World Vision India in 3 districts of Chhattisgarh is the particular focus of this study.

MATERIALS AND METHODS

Locale of the study

Chhattisgarh, a 21st century State, came into being on November 1, 2000. Larger than Tamil Nadu, it is just the right size, and is also fortunate to have a low population density. 12% of India's forests are in Chhattisgarh, and 44% of the State's land is under forests. Identified as one of the richest bio-diversity habitats, the Green State of Chhattisgarh has the densest forests in India, rich wildlife, and above all, over 200 non-timber forest products, with tremendous potential for value addition.

Selection of the District

Out of 18 districts of Chhattisgarh state, World Vision India operates in 6 districts. Purposively 3 districts namely Rajnandgaon, Durg, Bilaspur will be selected for the present study as the Organic farming programme is being implemented in these target areas as well as the researcher is well acquainted with the area.

Selection of the block

Rajnandgaon districts have 6 blocks. World Vision India is operating in one block ie.Rajnandgaon. So, Rajnadgaon block will be selected purposively for the present study.
Durg districts have 12 blocks.World Vision India operating in one block, ie Gunderdehi. So, Gunderdehi block will be selected purposively for the present study.
Bilaspur districts have 10 blocks.World Vision India operating in one block, ie, Kota. So, Kota block will be selected purposively for the present study.

Selection of Village

Rajnandgaon block has 70 no. of villages, and World Vision India operates in 50 villages. Out of these 50 villages, five villages will be selected purposively for the present study as these villages considering the maximum numbers of beneficiaries are covered under the organic farming programme.Gunderdehi block has 163 villages, and World

Vision India operates in 49 villages. Out of these 49 villages, five villages will be selected purposively for the present study as these villages considering the maximum numbers of beneficiaries are covered under the organic farming programme.Kota block has 162 villages, and World vision India operates in 68 villages. Out of these 68 villages, five villages will be selected purposively for the present study as these villages considering the maximum numbers of beneficiaries are covered under the organic farming programme.

Selection of Respondents

Fifteen (15) beneficiaries form five (5) villages from three (3) blocks will be selected randomly with the help of tippet random number method for the present study. Similar number of non-beneficiaries will also be selected for study from the different villages within the project area which are located in distant location where the organic farming programme is not implemented. Hence, a total of 450 respondents will be selected. [225 beneficiaries and 225 non-beneficiaries] Personal interview method will be undertaken and the data will be collected with the help of pre-structured interview schedule.

Statistical Methods

Depending upon the nature of the data and objectives of the study suitable statistical tools will be used mainly 1. Inferential and differential statistics will be used.

Descriptive Statistics

The statistical method which are used in describing the distribution of a characteristics among a series of varying units are called descriptive statistics which will be used in this study the percentage and mean.

Inferential Statistics

This deals with analysis of several aspects of association or relationship between the distributions of two or more characteristics among the same group of units. Thus they consciously describe the existence direction, degree and nature of association between two or more characteristics enumerated or measured.

RESULTS AND DISCUSSION

This section is devoted to the description and comparison of selected socio economic characters (factor) of beneficiaries and non beneficiaries.It is evident that as we go from lower to higher intervals there was an increase trend in the number of respondents in both the categories.The maximum beneficiaries 44.44 per cent were concentrated in the age group 40-50 years the maximum number of non beneficiaries 51.11 per cent were concentrated in the age group of 40-50 years.It is evident that as we go from lower to higher intervals there was an increase trend in the number of respondents in both the categories. The maximum beneficiaries 97.77 per cent were concentrated in the Hindu religion whereas the maximum number of non beneficiaries 72.44 per cent were concentrated in the hindu religion.It is eviden that as we go from lower to higher intervals there was an increase trend in the number of respondents in both the categories. The maximum beneficiaries 67.11 per cent were concentrated in the OBC caste whereas the maximum number of non beneficiaries 69.33 per cent was concentrated in the OBC caste.

It is clear from that 51.11 per of beneficiaries had educational level illiterate (Can read only) whereas highest number of none beneficiaries 52.49 per cent were illiterate (Can read only). The majority of the beneficiaries 64.00 per cent were having nuclear family system and majority of non beneficiaries 56.89 were having nuclear family system. From the beneficiaries 36.00 per cent of them were having joint family system and 43.11 percent non beneficiaries were having joint family system. It is clear that majority of the beneficiaries (82.22 per cent) possessed the member of

family up to 5 member where majority of non beneficiaries (93.33 per cent) possessed the member of family more than 5 members.It is evident from the above table that majority of beneficiaries and non beneficiaries were engaged in occupation. Data incorporated that 88.00 per cent beneficiaries and 88.44 per cent non beneficiaries were having main farming as their occupation, 6.67 per cent beneficiaries and 4.44 per cent non beneficiaries were having farming business as their occupation. 3.11 per cent beneficiaries and 4.44 per cent non beneficiaries were having farming occupation as their occupation.The finding presented in clearly reveals that 41.78 per cent beneficiaries had semi-pucca type of house followed by the beneficiaries i.e. 32.44 per cent who possessed pucca type of house and 25.78 per cent beneficiaries posses kaccha type of house.

The finding presented in clearly reveals that 95.11 per cent non-beneficiaries had kaccha type of house followed by the non-beneficiaries i.e. 2.67 per cent who possessed pucca type of house and 2.22 per cent non-beneficiaries posses semi pacca type of house.The findings clearly shows that majority of the beneficiaries 73.78 per cent had annual income up to 20,000 followed by the respondent 16.89 per cent had annual income between 20,000 – 40000. In case of non beneficiaries, the majority of them 77.78 per had annual income Up to 20,000 followed by the respondents 20.00 per cent who had annual income between Rs. 20,000 – 40,000.It is evident that majority of beneficiaries and non beneficiaries both are 97.78 per cent had land holding from Marginal< 1 ha.It is clear from that majority of the beneficiaries 41.33 per cent were having dug well irrigation source and majority of the non beneficiaries 68.00 per cent were having canal irrigation source. From the beneficiaries 31.56 of them were having canal irrigation source and 32.00 per cent non beneficiaries were having dug well irrigation source.

It is clear that majority of the beneficiaries 92.89 per cent were having bullock cart farm power and majority of the non beneficiaries 93.78 per cent were having bullock cart farm power.It could be observed that 47.11 per cent beneficiaries and 49.78 non beneficiaries had cow. 32.44 per cent beneficiaries and 30.67 non beneficiaries had goat.It could be observed that 84.00 per cent beneficiaries and 84.44 non beneficiaries had cycle. 7.56 per cent beneficiaries and 12.00 non beneficiaries had bullock car.

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RESEARCH ARTICLE

Effect of Xylazine – Ketamine Anaesthesia on Sleep Time and Toe Reflex in Sprague Dawley Rats

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ABSTRACT

Use of experimental animals in the field of biomedical research is gaining importance as it is essential to study the nature of human diseases and to device effective treatment modalities (Hedenqvist P, 2008). The experimental animals are also being used to understand the host response to natural and artificial scaffold materials. Rodents like rats are commonly used in biomedical research.The field of experimental rodent surgery has grown significantly as transgenic technology and provided numerous rodent models suitable for surgical investigation (Parritz L, 2007).To reduce pain and suffering during experimental surgery, suitable anaesthetic protocols should be adopted. It can be either inhalant or injectable anaesthesia and the adopted protocol should be reliable and safe. In the current study, injectable anaesthesia was adopted using a combination of xylazine and ketamine during experimental surgery. It was seen that injectable anaesthesia is more reliable compared to inhalant anaesthesia, because the dose of the anaesthetic can be calculated based on body weight and the depth of the anaesthesia can be easily monitored.

Keywords : Experimental Animals, Biomedical Research, Anaesthesia, Scaffold Materials.

INTRODUCTION

Natural and synthetic biological scaffolds are now being extensively used in correcting tissue defects in both animals and human beings. Before being implanted in human beings, such scaffolds are undergoing rigorous research in experimental animals.The tissue response of the host to the implanted biomaterial, inflammatory response, neovascularisation and neo collagenisation etc. are assessed in such studies. In the current study, differences in the sleep time and pinch reflex are compared between groups. The biological scaffold used was bovine pericardium and it was implanted subcutaneously in adult Sprague Dawley rats.Firstly inhalant anaesthesia using isoflurane was attempted to anaesthetise the rats. But the depth of the anaesthesia could not be controlled. As a result, the animals showed strugglingand breathing difficulties after anaesthesia.To alleviate the effects of inhalant anaesthesia, injectable anaesthesia was adopted using a combination of xylazine and ketamine based on body weight. The Sprague Dawley rats were having body weights in the range of 300-500g. Xylazine was administered at the rate of 2mg/kg body weight and ketamine at the rate of 50mg/kg body weight. Both of them were taken in a single syringe to make a cocktail and administered intra peritoneally before surgery. It produced sufficient depth of anaesthesia during surgery and the rats made an uneventful recovery after surgery. Many researchers have used xylazine and ketamine in combination and xylazine and/or ketamine along with other anaesthetic agents (Hsu *et al* 1986, Welberg *et al* 2006).

MATERIALS AND METHODS

Thirty two adult Sprague Dawley rats of either sex were selected randomly for conducting the experiment. The biomaterials (decellularized and glutaraldehyde treated bovine pericardium) were implanted subcutaneously in rats. Half of the rats received decellularized bovine pericardium and the other half received glutaraldehyde cross linked bovine pericardium.

Experimental design

The rats were weighed prior to anaesthesia and the weights in gram were recorded. The rats with body weights in the range of 300-400 g were assigned to first group and animals with body weights in the range of 400-500 g were included in the second group.

Anaesthesia and preparation of animals for surgery

The anaesthetic dose of xylazine and ketamine are calculated based on theirweights. Both the groups received anaesthesia at the same dose rate (xylazine 2mg/kg, ketamine 50 mg /kg). The hind quarter of the rats were raised by lifting them at the tail region and providing grips for them on the top of the grilled cage. In this position the viscera of the rat will be moved forward and it will not puncture any of the internal organs while giving intra peritoneal injection of anaesthetics. When the rats are properly anaesthetised the surgical site will be prepared aseptically.

Aseptic surgery

The right lateral side of the rats were selected for implantation of the biomaterial.The area was shaved and aseptically prepared by applying povidone iodine. A subcutaneous tunnel of 5cm X 1cm was created to correctly fit the biomaterial of the same size into the tunnel. During surgery whenever required or whenever the animals showed signs of pain sensation, additional volumes were given for maintenance of anaesthesia. Both ends of the tunnel was closed by simple interrupted sutures using proloene 6/0.

RESULTS AND DISCUSSION

Sleep time between groups

There sleep time of two groups were comparable and did not show much difference. The first group (300-400g) showed 20-40 minutes sleep time and the second group showed 20-30minutes sleep time.

Toe pinch reflex

Toe pinch reflex was analysed to determine the depth of anaesthesia. This parameter also was comparable as majority of the rats responded to toe pinch reflex in both groups. In the first group (300-400g)out of the 16 animals 10 animals showed toe pinch reflex and in the second group 12 out of the 16 responded to toe pinch.Struck M.B.*et al* (2011) conducted similar studies in rats. In his experiment one group was fasted for 3 hours. And the other group did not undergo fasting. He observed that there was no significant difference in toe pinch reflex and sleep time between the two groups.Hsu W.H. *et al* (1986) studied the reversal of xylazine-ketamine anaesthesia using yohimbine in adult male Sprague Dawely rats. Xylazine and ketamine were administered in different combinations in two experiments. In both the experiments the sleep time could be considerably decreased after the administration of yohimbine.The findings of the current study was in agreement with the findings of Struck *et al*(2011) with respect to toe reflex and sleep time where both experiments showed no significant difference between groups. In the current study both the groups received the dose of anaesthetics.Even though the individual animals showed variability in responses. Flecknell (1996) reported that individual variability can occur in rats during xylazine –ketamine anaesthesia.

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RESEARCH ARTICLE

Feasibility Evaluation of Solar Desalination System Coupled with Vaccum Tubes

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ABSTRACT

Water is an important renewable energy resource having several advantages for human use. The availability of clean and pure water is very important need for humans in many countries. Hence solar still is the renewable energy device to obtain pure water using solar energy. So this paper presents the thermal analysis of a solar still integrated with evacuated tube collector in natural mode. It will be helpful to predict the behavior of solar still in different climatic condition. The internal heat transfer coefficients evaluated using Dunkle's model for 0.03 m water depth in basin. Higher value of evaporative heat transfer coefficient 153.42 $W/m^2\ ^0K$ is obtained at 14:00 h which decreases with decrease in water and glass cover temperature. The respective values of convective and radiative heat transfer coefficient are found to be in the range of 1.04 to 3.81 and 6.47 to 9.72 $W/m^2\ ^0K$ throughout the day. The integration of evacuated tube collector with solar still increases water temperature as well as distillate yield.The daily yield obtained is 8 liters for 0.03 m basin water depth.

Keywords : Evacuated Tube Collector, Solar Still, Distillate Yield

INTRODUCTION

Water is fundamental to human life on earth for survival and good health. Access to safe water is a major challenge in many communities in developing countries. As world population and social-economic growth, societies are challenged to provide fresh water to meet those needs for all of their people. Water is the basic necessity for human along with food and air. There is almost no water left on Earth that is safe to drink without purification [1].Solar stil

is a device which is used for desalination purpose. Solar still are of two types namely passive solar still and active solar still. Generally passive solar still employs only solar radiation to evaporate water for the production of distillate output, where as active solar still requires the addition some mechanical source in the form of collector with solar energy. Solar water distillation system also called "Solar still". Solar Still can effectively purify seawater & even raw sewage. Solar Stills can effectively removing Salts/minerals {Na, Ca, As, Fe, Mn}, Bacteria {E.coli, Cholera, Botulinus}, Parasites, Heavy Metals & TDS.Availability of drinking water is one of the major challenges faced by our modern society. There are certain locations throughout the globe where there is scarcity of fresh drinking water but availability of brackish or saline water. This available water resource can be used for harnessing usable water to meet the present drinking water requirement [2].

MATERIALS AND METHODS

Experiments were conducted at the department of Unconventional Energy Sources and Electrical Engineering, Dr. PDKV, Akola. Akola is located at latitude 20.7˚North and longitude 77.07˚East. Akola has tropical savanna climate. During experimentation, properly calibrated thermocouples were fixed at the inner and outer surfaces of condensing covers and inside the water to measure the condensing cover and water temperature. One thermocouple is hung between water and condensing cover to measure vapor temperature. The basin is then filled with required quantity of water, one day before the start of experiment to attain steady state condition. The condensing covers are cleaned properly before the start of experiment. Experiments were conducted at the water depth of 0.03 m.

Experimental Observations

The temperatures of water, glass cover and water vapor were recorded with the help of calibrated copper constantan thermocouples in combination with a digital temperature indicator. The ambient temperature is measured by calibrated mercury thermometer. The distillate from the still was measured using measuring jar. The blackish was supplied to the flat plate collector using tap valve. The solar radiation on inclined plane facing east and west were measured using a solarimeter [3].

Measurement of Temperature

Thermocouples were used to measure water, water vapor and condensing cover temperature. Thermocouples used in the experiment are properly calibrated with the help of thermometer (standard thermometer). The ambient air temperature is recorded with the help of a calibrated mercury thermometer having a least count of 1°C.

Measurement of Distillate Yield

The condensed water is collected in a galvanized iron channel fixed at the lower end side of both the glass covers. The distillate collected is continuously drained through flexible pipe and stored in a jar placed outside on both side. The collected distillate yield has been measured using graduated cylinder with least count of 1 ml.

Measurement of Solar Radiation

The sun emits an electromagnetic radiation with different wavelengths and with a peak centered in the visible spectrum. This radiation is arrive to the earth ground must go through the earth atmosphere, where suffer absorptions, refractions, reflections and emissions that work in selective way. Every element in the atmosphere in fact reacts in different way to the various electromagnetic radiation wave lengths that is every component absorbs and emits the radiation to a different wavelength (absorbing the radiation it heats in accordance with the reached temperature and emits a different one). This fact provokes that the solar radiation to the ground level has a spectrum much different from the extra atmospheric level. The solar intensity was measured with the help of a calibrated solarimeter.

Design and Fabrication of Solar Still Coupled with Evacuated Tube Collector

The solar still coupled with evacuated tube collector was designed for distillating water. Solar still coupled with evacuated tube collector consisted of following components:

1. A rectangular shape basin of solar still
2. A evacuated tube

The fabrication of solar still coupled with evacuated tube collector was done as per the design specifications through fabricator. All the materials required for fabrication was purchased from local market.

Performance Evaluation of the System

Performance of the system evaluated in terms of distillate yield of water sample from the solar still.Also by measuring temperature at different locations of the system through digital temperature indicator by calibrating thermocouples at proper locations.

RESULTS AND DISCUSSION

Fig. 1 shows the variation of temperature and solar radiation with respect to time for sample 1.It has been observed that as intensity of solar radiation increased the temperature also increased and it was found to be maximum of about 43˚c at 14.00 h [4].Average temperature of evacuated tube was found to be 68.08˚C with corresponding average ambient temperature of about 39.25˚C and solar radiation of about 541.72 W/m^2. As temperature in evacuated tube were measured to determine the useful energy from the collector, similarly the temperature of inner glass surface and outer glass surface was found to be in the range of 36.1 to 87.5 ˚C and 35.1 to 81.9, respectively [5].The temperature of water in the basin was found to be in the range of 38.8 to 91.70˚C as it is the temperature which is responsible for evaporation of water in the basin (Table 1).

Fig. 2 shows the variation of distillate yield and solar radiation with respect to time. Performance of still mainly depends on the intensity of solar radiation absorbed by absorber plate and hence increased in distillate yield due to increased difference between temperature of water and glass cover temperature [6]. It was observed that performance of still directly affected by solar radiation particularly at 14 h when maximum production was observed i.e 950 liter with corresponding maximum solar radiation of 862.4 W/m^2. Increased in solar radiation resulted in increase of water mass temperature, hence it would cause evaporation at faster rate. Therefore, the decreased solar radiation intensity would lower the system distillate yield. The solar radiation curve followed the same path as that of the distillate yield [7].

CONCLUSIONS

Water is a most important and abundant commodity in nature and its requirement increasing day by day. Pure water is not only essential for mankind but also for the plants and animals. The distillate yield from solar still depends on the temperature difference between the water in basin and inner glass cover. Higher the difference, the greater is the yield. The integration of evacuated tube collector with solar still increased the water temperature as well as distillate yield. The daily yield of 8 litres for 0.03 m water depth in basin was obtained in normal sunny days of summer season.

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Table 1 Variation of temperatures and solar radiation with time for water sample

Time (h)	T_{Amb} (^{0}C)	T_b (^{0}C)	T_{gi} (^{0}C)	T_{go} (^{0}C)	T_e (^{0}C)	T_w (^{0}C)	Solar radiation (W/m^2)
7.00	34	36.2	36.1	35.1	37.60	38	250.6
8.00	34	39.5	42.1	37.7	41.00	46.2	363.2
9.00	35	43.2	50.2	40.3	43.23	54.2	567.6
10.00	38	54.7	60.7	55.1	54.50	64.4	683.0
11.00	40	65.4	71.5	61.3	65.83	74.9	860.6
12.00	41	79.4	76.1	69.9	76.10	79.8	836.8
13.00	42	90.8	82.1	72.4	82.10	85.8	913.7
14.00	43	87.0	85.4	72.7	88.17	89.2	862.4
15.00	43	89.1	87.5	74.9	90.30	91.6	542.0
16.00	41	85.2	84.6	81.9	87.30	87.1	397.3
17.00	40	76.5	74.3	60.8	78.40	76.2	142.8
18.00	40	71.6	69.2	58.0	18.00	70.5	80.6
Average	**39.25**	**68.22**	**68.32**	**60.01**	**68.08**	**71.49**	**541.72**

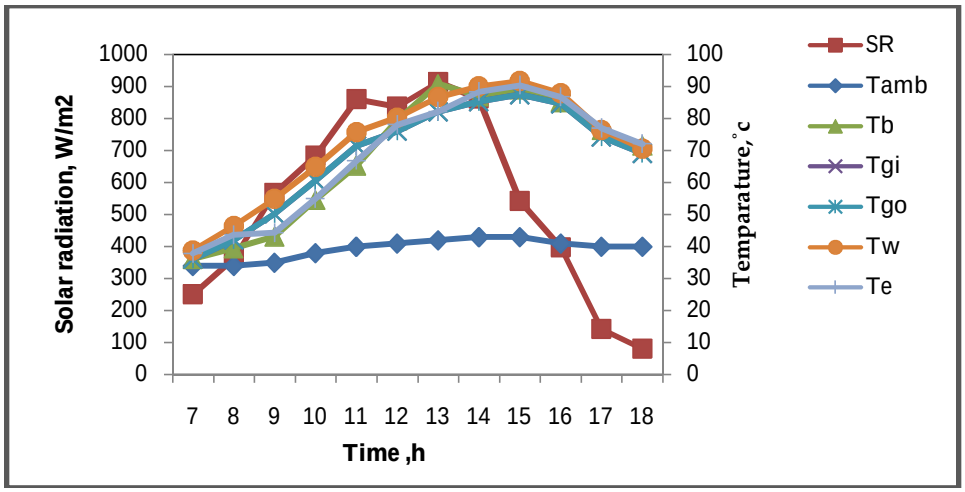

Fig. 1.Hourly variation of temperature and solar radiation with respect to time for water sample

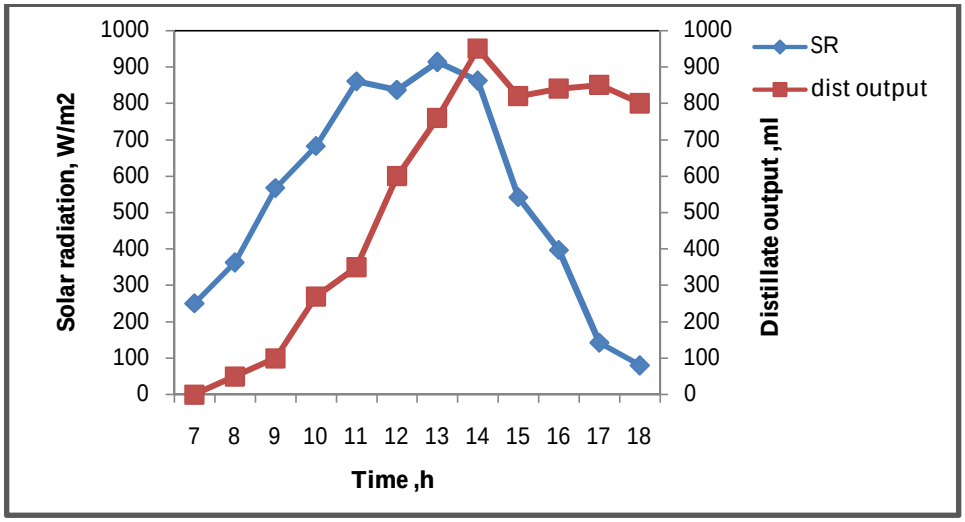

Fig. 2. Variation of distillate yield and solar radiation with respect to time for water sample

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RESEARCH ARTICLE

Experimentation on Drying Kinetics of Aonla Candy in Mini Solar Tunnel Dryer

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ABSTRACT

A mini solar tunnel dryer was designed and fabricated for drying. Experiment was conducted to test the performance of the dryer for Aonla candy. The results indicated that drying in solar tunnel dryer was faster as compared to open sun drying. It was found that the system could maintain an adequate temperature in the range of 50 to 60^0 C required for drying. In order to select the appropriate drying model, seven mathematical drying models were fitted to the experimental data. Result indicated that the drying took place in the falling rate period. Considering the statistical criteria (R^2, χ^2, SSE & RMSE) Logarithmic model was found to fit well to describe the drying behaviour of Aonla candy having different proportions.

Keywords : Solar Tunnel Dryer, Moisture Ratio, Drying Time, Drying Rate, Thin Layer Model

INTRODUCTION

Aonla (Pyllanthus emblilica), is the richest source of vitamin C Ascorbic acid) among all the fruits. Every 100g of amla contains nearly 700 – 750 mg of vitamin C. Aonla is also rich in vitamins and minerals like phosphorous, iron, calcium, carotene and vitamin B complex . The spherical, six lobed, light greenish yellow aonla fruit is sour, bitter and astringent to taste and is fibrous. It is found to have strong antioxidant properties. It is highly nutritive and has plenty of medicinal properties that made it more popular.

Many Ayurvedic and Unani preparation use aonla as a major constituent as it rejuvenates all the organ systems of the body and promotes health and wellness. Aonla helps in curing a number of ailments like fever, anemia, indigestion, liver disorder, piles, heart complaints and urinary problems. It has got antibacterial property and anti-ageing property.

A solar dryer is an enclosed unit, which keep the food safe from damage, birds, and unexpected rainfall. The food is dried using solar thermal energy in a cleaner and healthier way. Studies undertaken so far have clearly indicated that initial cost of solar dryers are high and the life time cost of drying is only a third of dryers based on conventional fuels. Different dryers have been developed and used to dry agricultural products in order to improve shelf life [1]. Solar tunnel dryers utilize the energy of the sun and wind to dry agricultural products, preparing them for proper storage, processing and export. The crop is spread in an even layer on tables or drying racks inside the tunnel. The air below the semi-transparent collector is heated by the sun and spreads throughout the tunnel. The increased temperature decreases the relative humidity of the air, thereby allowing the air to more efficiently dry the crop [2].Thin-layer drying models have been used to describe the drying process of several agricultural products and medicinal plants such as black pepper [3] [4].

MATERIALS AND METHODS

The schematic diagram of the natural convection walk-in type solar tunnel dryer is shown in fig.1. It is essentially consisted of a hemi cylindrical walk-in type metallic frame structure covered with UV stabilized semi-transparent polyethylene sheet of 200 micron thickness. To create natural draft inside the dryer, chimneys are provided on the top of dryer which operates by increasing the buoyancy force to aid the airflow through a structure [5]. This buoyancy force is directly proportional to the difference between the mean air density within the chimney and the density of outside air. Adequate provisions were made to reduce heat losses from the floor and the northern side of the tunnel is also provided to boost up natural convection. The dryer was designed for drying turmeric on large scale at moderate temperature in Indian conditions. Such a system has been commissioned at the Department of Unconventional Energy Sources and Electrical Engineering, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola.

Firstly 5 kg Aonlas were taken and washed with the help of water .Then for steaming process Aonlas were kept in the pressure cooker for 15 to 20 minutes and steaming of sample was done at 800 ºC . After steaming process sample kept for the cooling and then Aonla seed was removed and Aonla separated or cut in pieces manually. In three large plates the pieces of Aonla was divided in equal parts and sugar poured on it in the 1:1 , 1:0.75, 1:0.5 proportion respectively and the sample kept for fully absorption of sugar in Aonla pieces for 2 days. After this process all three samples were divided in to two equal part each part having 100 gram Aonla pieces and all samples are taken for drying in solar tunnel dryer

Study of drying characteristics

Moisture content

Initial moisture content of sample was determined by the hot air oven drying method as recommended by [6]. Samples were taken and weighed using electronic weighing balance of least count 0.01g. The samples were placed in hot air oven at 70 ± 0.5 °C for 24 h. Following formulae were used

$$\text{M.C.(wb)\%} = \frac{(W_1 - W_2)}{W_1} \times 100$$

$$\text{M.C.(db)\%} = \frac{(W_1 - W_2)}{W_2} \times 100$$

Where, W_1 - weight of sample before drying, g W_2- weight of bone dried sample, g

Moisture ratio

The moisture ratio of the produce was computed by following formula.

$$\text{Moisture Ratio (M.R.)} = \frac{(M - M_e)}{(M_0 - M_e)}$$

Where,

M - Moisture content (db), %

M_e- EMC, (db), %

M_0 - IMC, (db), %

Drying rate

The drying rate of produce sample during drying period was determined as follows:

$$\text{Drying rate } (D_R) = \frac{\Delta W}{\Delta t}$$

Where, ΔW = Weight loss in one hour interval (g $100g^{-1}$of bdm)

Δt = Difference in time reading (h)

Thin layer drying models

The moisture content of samples during thin layer drying were expressed in terms of moisture ratios (MR) and calculated from the equation given by [7]. Seven thin layer-drying equations were tested (Table 2) to select the best model for describing the drying curve equation of Aonla. Non-linear regression analysis was performed for the drying data by using STATISTICA-11. The Models were tested on the basis of coefficient of determination (R^2) chi-square (χ^2), and mean bias error (MBE) and root mean square error (RMSE). R^2 value should be higher for quality fit, whereas χ^2, MBE and RMSE values should be lower.

RESULTS AND DISCUSSION

The different thin layer drying models were applied for the variation of moisture ratio with respect to the drying time using STATISTICA-11. The best ranked thin layer drying model with higher coefficient of determination (R^2) and lower chi square (χ^2) and root mean standard error (R_{MSE}) value was selected. The result obtained from statistical analysis to identify the best ranked thin layer model is summarized in Table 3 to 5 .It was observed that, the best ranked thin layer drying model applicable for drying of Aonla candy for different proportions was Logarithmic model in the form of,

$$M.R. = a.exp(-b.x)+c$$

Where, a, b and c are constants.

The dying constants for the best fit equations for Aonla candy samples with minimum value off chi square (χ^2) and root mean slandered error (R_{MSE}). The value of (R^2) was found maximum and more than 0.99 revealed the best fit equation and good correlation between the attributes of moisture ratio and drying time of Aonla candy sample in solar tunnel dryer as depicted in Fig 2.

CONCLUSION

The drying behaviour of the Aonla candy was investigated in a thin layer in solar tunnel dryer and the drying of samples occurred in the falling rate period. Drying air temperature affected the drying rate and time. The minimum drying time was found 600 min in solar tunnel dryer at 50 ^oC temperature. The drying rate increased with increase in

the drying-air temperature. Logarithmic model was adequate for describing the thin-layer drying behaviour of Aonla candy samples having different proportions.

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Table 1: Technical specifications of Solar tunnel dryer

S.No.	Particulars	Specifications
1.	Aperture area, m^2	3.9
2.	Length of dryer, m	1.6
3.	Width of dryer, m	1.06
5.	Height of tunnel, m	0.9
6.	Number of trays	2
7.	Plastic cover, UV stabilized	200 micron
8.	Height of Chimney, m	0.35

Table 2: Mathematical Models applied to solar drying curves

S.No.	Model Name	Model
1.	Lewis	M.R. = $exp(-a.x)$
2.	Page	M.R. = $exp(-a.x^b)$
3.	Henderson-Pabis	M.R. = $a.exp(-b.x)$
4.	Logarithmic	M.R. = $a.exp(-b.x)+c$
5.	Two term	M.R. = $a.exp(-b.x)+c.\ exp(-d.x)$
6.	Modified Henderson and Pabis	M.R.= $a.exp(-b.x)+c.\ exp(-d.x)+e.exp(-f.x)$
7.	Wang and Singh	M.R. = $1+a.x+b.x^2$

Table 3: Results of thin layer drying model STASTICA 11.0 for Aonla Candy Sample having proportion of 1:1 dried in mini solar tunnel dryer

S.No.	Model name	Model	Coefficients	R^2	χ^2	MBE	RMSE
1	Lewis model	$y=exp(-a.x)$	0.00384	0.9650	0.00849	0.000157	0.0557
2	Page model	$y=exp(-a.x^b)$	0.0034 1.4007	0.9676	0.0021	0.0461	0.01195
3	Henderson & Pabis	$y=a.exp(-b.x)$	1.1159 0.0042	0.9735	0.0064	0.0062	0.0486
4	**Logarithmic**	***y=a.exp(-b.x)+c***	**1.5912** **0.0019** **-0.5332**	**0.9953**	**0.0016**	**0**	**0.0244**
5	Two term	$y=a.exp(-b.x)+c.\ exp(-d.x)$	0.5580 0.0042 0.5580 0.0042	0.9735	0.0064	0.0062	0.0486
6	Modified Henderson & Pabis	$y=a.exp(-b.x)+ c.\ exp(-d.x)+e.exp(-f.x)$	0.3719 0.0042 0.3719 0.0042 0.3719 0.0042	0.9735	0.0065	0.0061	0.0476
7	Wang and Singh	$y=1+a.x+b.x^2$	0.0018 0.0010	0.9782	0.0053	0	0.0441

Table 4: Results of thin layer drying model STATISTICA 11.0 for Aonla candy sample having proportion of 1:0.75 dried in mini solar tunnel dryer

S.No.	**Model name**	**Model**	**Coefficients**	**R^2**	**χ^2**	**MBE**	**RMSE**
1	Lewis model	$y=exp(-a.x)$	0.0039	0.9677	0.0078	0.00062	0.0535
2	Page model	$y=exp(-a.x^b)$	0.000 1.7497	0.9824	0.0019	0.0441	0.01102
3	Henderson & Pabis	$y=a.exp(-b.x)$	1.1107 0.0043	0.9754	0.0059	0.0063	0.0467
4	**Logarithmic**	***y=a.exp(-b.x)+c***	**1.5244** **0.0021** **-0.4695**	**0.9940**	**0.0014**	**0**	**0.0230**

5	Two term	*y=a.exp(-b.x)+c. exp(-d.x)*	0.55538 0.0043 0.5553 0.0043	0.9754	0.0059	0.0063	0.0467
6	Modified Henderson & Pabis	*y=a.exp(-b.x)+ c. exp(-d.x)+e.exp(-f.x)*	0.3696 0.0043 0.3703 0.0043 0.3707 0.0043	0.9754	0.0059	0.0063	0.0467
7	Wang and Singh	$y=1+a.x+b.x^2$	-0.0018 0.0010	0.9763	0.0057	0	0.0458

Table 5: Results of thin layer drying model STATISTICA 11.0 for Aonla candy sample having proportion of 1:0.5 dried in mini solar tunnel dryer

S.N.	Model name	Model	Coefficients	R^2	χ^2	MBE	RMSE
1	Lewis model	*y=exp(-a.x)*	0.002914	0.9234	0.0214	-0.0068	0.0086
2	Page model	$y=exp(-a.x^b)$	0.001 1.5040	0.9432	0.0019	0.0198	0.0436
3	Henderson & Pabis	*y=a.exp(-b.x)*	1.2166 0.0035	0.9526	0.0134	0.0069	0.0702
4	**Logarithmic**	***y=a.exp(-b.x)+c***	**1.5912** **0.0019** **-0.5332**	**0.9900**	**0.0028**	**0**	**0.0325**
5	Two term	*y=a.exp(-b.x)+c. exp(-d.x)*	0.6082 0.0035 0.6082 0.0035	0.9526	0.00124	0.0070	0.0712
6	Modified Henderson & Pabis	*y=a.exp(-b.x)+c. exp(-d.x)+e.exp(-f.x)*	0.4055 0.0035 0.4055 0.0035 0.4055 0.0035	0.9527	0.00125	0.0070	0.0712
7	Wang and Singh	$y=1+a.x+b.x^2$	0.0019 0.0010	0.9888	0.0032	0	0.0344

Fig.1. Mini Solar Tunnel Dryer used for experimentation

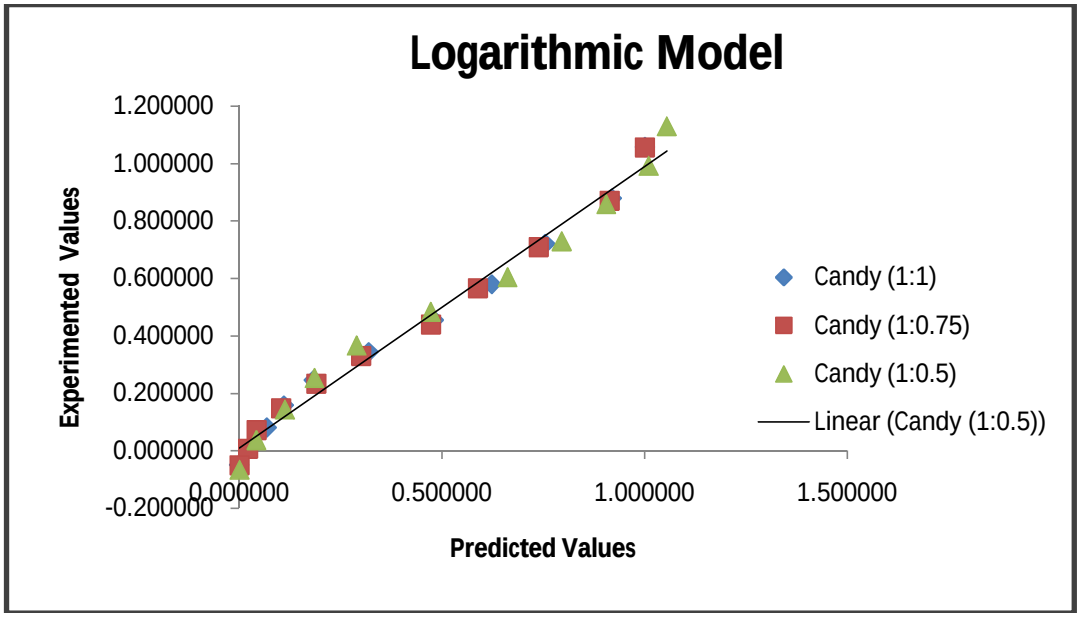

Fig. 2 Experimented vs predicted values of moisture ratio using the Logarithmic model for different Aonla candy samples proportions

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RESEARCH ARTICLE

Experimental Performance of Solar Powered Refrigeration System

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ABSTRACT

Photovoltaic (PV) is a technology that converts sunlight directly into electricity. With the global demand to reduce carbon dioxide emissions A Photovoltaic-powered refrigerator is a cooling appliance that is operated completely with energy harnessed from the sun that has been converted to electricity through photovoltaic (PV). It can store food, medications, and other products that require less temperature. The solar photovoltaic (PV) system generates both electrical and thermal energy from solar radiation. Therefore an energy and exergy analysis of the system was carried out. Energy analysis was concerned only with the quantity of energy use and efficiency of energy processes. Exergy is the maximum work potential which can be obtained from energy. The experimental data were used for the calculation of the energy and exergy efficiencies of the PV systems. The average photovoltaic conversion efficiency and exergy efficiency of thermoelectric refrigerator found nearer to 12.05% and 13.61%, respectively in no load and about 12.05% and 14.20% in full load condition in November month and The average photovoltaic conversion efficiency and exergy efficiency of vapor compression refrigerator found nearer to 12.07% and 14.31% , respectively in no load and about 10.91% and 12.09% in full load condition in November and December months. Hence it is concluded that there was no impact of load on the performance of photovoltaic panel. It was observed that the PV module temperature had a great effect on the exergy efficiency, could be improved by maintaining module temperature close to ambient and that could be achieved by removing the heat from PV module surface. It was concluded that the exergy losses increased with increasing module temperature.

Keywords : Solar photovoltaic, vapor compression refrigeration, thermoelectric refrigerator, exergy efficiency, photovoltaic efficiency.

INTRODUCTION

Renewable energy from sun and wind is an abundant and ubiquitous resource. Although capable of providing plentiful and reliable electricity, these resources are largely untapped. The world's energy consumption and electricity generation is mostly dependent on fossil fuel and in the process of electricity generation by means of these fuels a number of poisonous byproducts are released which affect the natural eco-system[1]. To minimize environmental impacts associated with refrigeration system operation, it is reasonable to evaluate the prospects of a clean source of energy.Photovoltaic (PV) is a technology that converts sunlight directly into electricity. With the global demand to reduce carbon dioxide emissions, PV technology is gaining popularity as a mainstream form of electricity generation[2]. The rate of electrical power capable of being generated by a PV system is typically provided by manufacturers of PV modules for standard rating conditions, i.e., incident solar radiation of 1,000 W/m2 (10 800 W/ft2) and a module temperature of 25°C (77°F).

The energy efficiency of a solar panel, the ratio of the power output to the energy originally delivered to the solar panel, conventionally is used to measure solar PV efficiency. Energy analysis is based primarily on the first law of thermodynamics, as compared with exergy analysis which is based on the second law. Energy analysis is concerned onlywith the quantity of energy use and efficiency of energy processes. Exergy analysis is used to find out the energy utilisation efficiency of an energy conversion system[3]. Exergy analysis yields useful results because it deals with irreversibility minimisation or maximum exergy delivery. To perform energy and exergy analyses of the solar PV, the quantities of input and output of energy and exergy must be evaluated.

MATERIALS AND METHODS

The experiment was carried out in the Department of Unconventional Energy Sources and Electrical Engineering, Faculty of Agricultural Engineering, Dr. PDKV, Akola, which lies at latitude 22°42' N and longitude 77°02' E. In this experimental study, the comparative energetic and exergetic analysis of solar photovoltaic (SPV) modules has been carried out for a November and December month under the different climatic conditions.

Experimental System

A SPV refrigeration system consisted of DC vapour compression refrigerator of 25 liter capacity. Considering the power requirement of its continuous operation, one 80 W SPV panels were used to convert solar energy into electrical energy. A battery of 12 volt was used for this experiment. The panels were kept on fixed masonry structure at 35° (tilt angle) from horizontal, facing south direction. A battery was used so that it could give high starting current required to start the motor of compressor. It consisted of one 12 V – 150 Ah sealed lead batteries connected in parallel. Panels were connected to the battery via charge controller which avoided the battery from deep discharge. Battery supplied DC current to refrigerator as it operated on DC current.The connections are shown in Fig.1,2.

Energy efficiency of the solar panel

Photovoltaic efficiency

The efficiency of the solar panels, defined as the ratio of the electrical power produced to the incident radiation and varies in between 10 to 15% at maximum power conditions for the PV array. If the PV refrigeration system is to operate at high efficiency, it is essential that the voltage imposed on the PV array be close to the voltage that provides maximum power. Photovoltaic efficiency of solar panel was determined at no load and full load condition by using following formula.

$$\eta_{pv} = \frac{P_{max}}{S \times A_{pv}}$$

Where, η_{pv}= Efficiency of photovoltaic system

P_{max} = Maximum power from photovoltaic system (W)

S = Solar irradiance (W/m^2)

A_{pv} = Area of the photovoltaic system (m^2)

Exergy analysis

Exergy is defined as the maximum amount of work that can be done by a system. Unlike energy, exergy is not subject to a conservation law; exergy is consumed or destroyed, due to the irreversibility present in every real process[5].

Photovoltaic exergy

The energy of a PV module depends on two major components--electrical and thermal. In SPV system electricity is generated by the PV effect, the PV cells are heated due to the thermal energy present in the solar radiation. The electricity (electrical energy), generated by a photovoltaic system, is also termed as electrical exergy since it is the available energy that can completely be utilized in useful purpose. Since the thermal energy available on the photovoltaic surface was not utilized for a useful purpose it is considered to be a heat loss to the ambient. Therefore, due to heat loss, it becomes exergy destruction. The exergy output of the photovoltaic system can be calculated as: (Sudhakar and Srivastava 2013)

$$Ex_{out} = V_m I_m - \left(1 - \frac{T_0}{T_{cell}}\right)\left[h_c \times A_{pv}(T_{cell} - T_o)\right]$$

WhereV_m, I_m, h_c, A ,T_{cell} and T_o are the maximum voltage and current of the photovoltaic system, convective heat transfer coefficient from the photovoltaic cell to ambient, area of the photovoltaic surface, cell temperature and ambient temperature (dead state temperature), respectively.The convective heat transfer coefficient from the photovoltaic cell to ambient can be calculated by using correlation

$$h_c = 5.7 + 3.8 \times v$$

Where, v = Wind velocity (m)

The module or cell temperature is used to predict the energy production of the photovoltaic module. Cell temperature is a function of ambient temperature, wind speed and total irradiance. The cell temperature can be determined by the following relationship:

$$T_{cell} = 0.943T_a + 0.028\,Irradiance - 1.528Windspeed + 4.3$$

Exergy input of the photovoltaic system, which is the exergy of solar energy, can be calculated approximately as below

$$Ex_{in} = Ex_{solar} = A_{pv} \times S \times \left[1 - \frac{4}{3}\left(\frac{T_o}{T_{SUN}}\right) + \frac{1}{3}\left(\frac{T_o}{T_{SUN}}\right)^4\right]$$

Where, T_{SUN} = Temperature of the Sun taken as 5760 °K

Exergy efficiency of the photovoltaic system is defined as the ratio of total output exergy (recovered) to total input exergy (supplied). It can be expressed as

$$\psi_{PV} = \frac{Ex_{out}}{Ex_{in}}$$

RESULTS AND DISCUSSION

Performance evaluation of photovoltaic system at no load

The performance of the photovoltaic system was evaluated in terms of photovoltaic energy and exergy efficiencies during no load testing. Solar intensity was measured hourly and the open circuit voltage and short circuit current was measured during the off cycle of the refrigeration system.

No load testing of solar photovoltaic vapour compression refrigerator November month

Figure 3 Indicates the exergy efficiency is more 12.87% at 09:00 and then it decrease with the increase in radiation upto 11.42% at 11:00 and then it starts increasing upto maximum limit of 17.61% at 17:00 and this varies from 11.66 to 17.61%.Photovoltaic efficiency is also varying similar to exergy efficiency. During the morning hours it is 11.30% at 09:00 and then it starts reducing and reaches to its minimum limit 9.64% at 11:00 and then it raises upto maximum limit 14.51% at 17:00. In the morning and evening hours Solar radiation intensity is minimum so the efficiency is more and during the afternoon hours it is more so the efficiency is less. The photovoltaic efficiency varies from 9.64 to 14.51%[6].

No load testing of solar photovoltaic vapour compression refrigerator December month

Figure 4 Indicates the exergy efficiency is more 11.76% at 09:00 and then it decrease with the increase in radiation upto 11.02% at 13:00 and then it starts increasing upto maximum limit of 15.62% at 17:00 and this varies from 11.02 to 15.62%.Photovoltaic efficiency is also varying similar to exergy efficiency. During the morning hours it is 9.82% at 09:00 and then it starts reducing and reaches to its minimum limit 9.81% at 11:00 and then it raises upto maximum limit 14.20% at 17:00. In the morning and evening hours Solar radiation intensity is minimum so the efficiency is more and during the afternoon hours it is more so the efficiency is less. The photovoltaic efficiency varies from 9.81 to 14.20%.

Performance evaluation of photovoltaic system at full load

Full load testing of solar photovoltaic vapour compression refrigerator during November month

Figure 5 Indicates the exergy efficiency is more 14.22% at 09:00 and then it increase with the increase in radiation upto 14.68 % at 11:00 and then it starts increasing upto maximum limit of 15.09 % at 15:00 and this varies from 13.22 to 16.03%. Photovoltaic efficiency is also varying similar to exergy efficiency. During the morning hours it is 11.63 % at 09:00 and then it starts increasing and reaches to its maximum limit 13.91% at 13:00 and then it start decrease upto 12.69% at 15:00. In the morning and evening hours Solar radiation intensity is minimum so the efficiency is more and during the afternoon hours it is more so the efficiency is less. The photovoltaic efficiency varies from 11.63 to 13.91% [7].

Load testing of solar photovoltaic vapour compression refrigerator December month

Figure 6 Indicates the exergy efficiency is more 8.16% at 09:00 and then it decrease with the increase in radiation upto 12.35% at 11:00 and then it starts increasing upto maximum limit of 15.57% at 17:00 and this varies from 8.16 to 15.57%. Photovoltaic efficiency is also varying similar to exergy efficiency. During the morning hours it is 7.17% at 09:00 and then it starts increasing and reaches to its maximum limit 14.65% at 17:00. In the morning and evening hours Solar radiation intensity is minimum so the efficiency is more and during the afternoon hours it is more so the efficiency is less. The photovoltaic efficiency varies from 7.17 to 14.65%[7][8].

CONCLUSION

Performance of photovoltaic system at no load and full load condition were carried out to assess its technical viability. This study indicated the necessity and usefulness of energetic and exergetic techniques to evaluate the performance of the SPV refrigerator.The average photovoltaic conversion efficiency and exergy efficiency of vapor compression refrigerator found nearer to 12.07% and 14.31% respectively in no load and about 10.91% and 12.09% in full load condition in November and December months. This indicated that the full load condition did not affect the PV system. This indicates that the full load condition does not affect the PV system. The photovoltaic and exergy efficiency was found less due to the module temperature hence exergy were destroyed highly in PV. The solar photovoltaic operated DC vapour compression refrigeration system under test was able to maintain the temperature as specified by the WHO for the vaccine preservation (2-8°C).The 80 Wp photovoltaic module and 150Ah battery bank is the least possible configuration required to run refrigerator.

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Fig.1 connections of Vapour Compression Refrigerator

Fig.2 Wire Connection of Panel

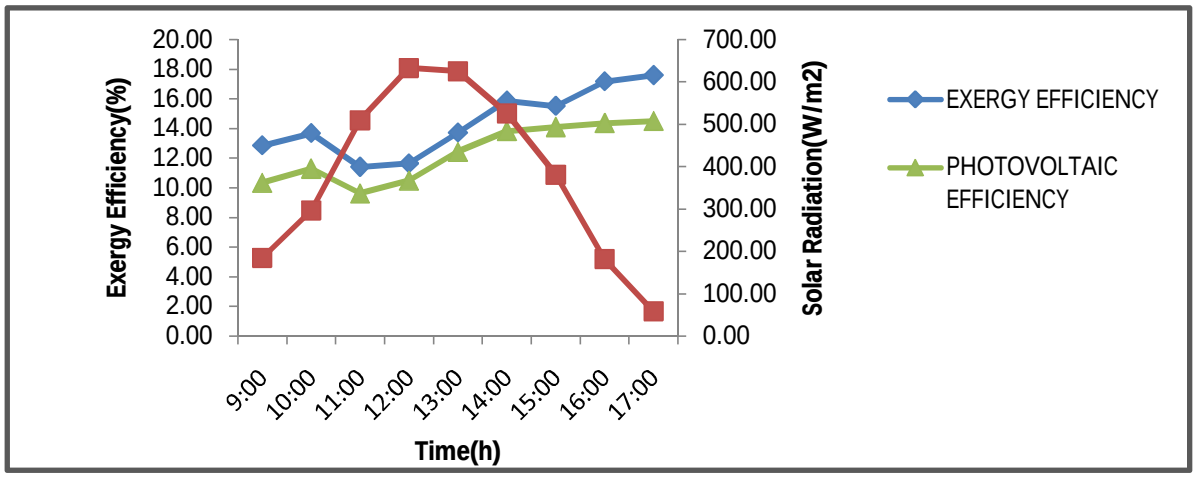

Fig.3.Variation in Exergy efficiency, Photovoltaic efficiency and solar radiation with Time (November)

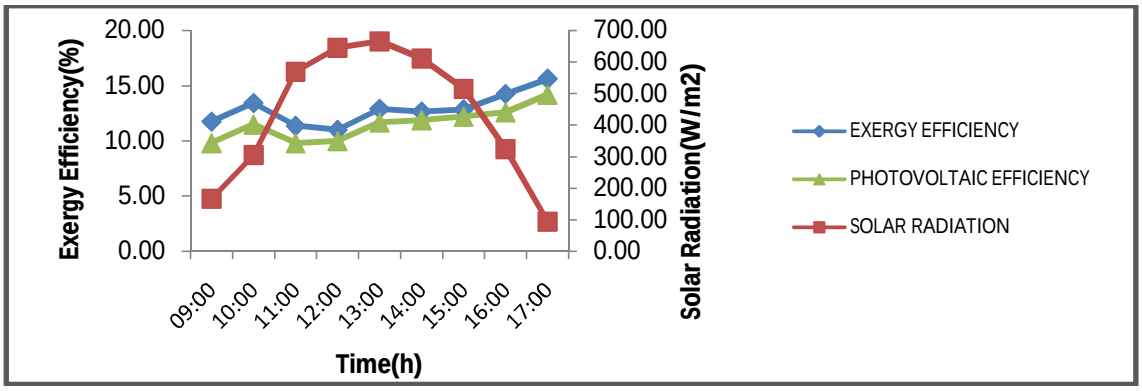

Fig .4 Variation in exergy efficiency, Photovoltaic efficiency and Solar radiation with Time (December)

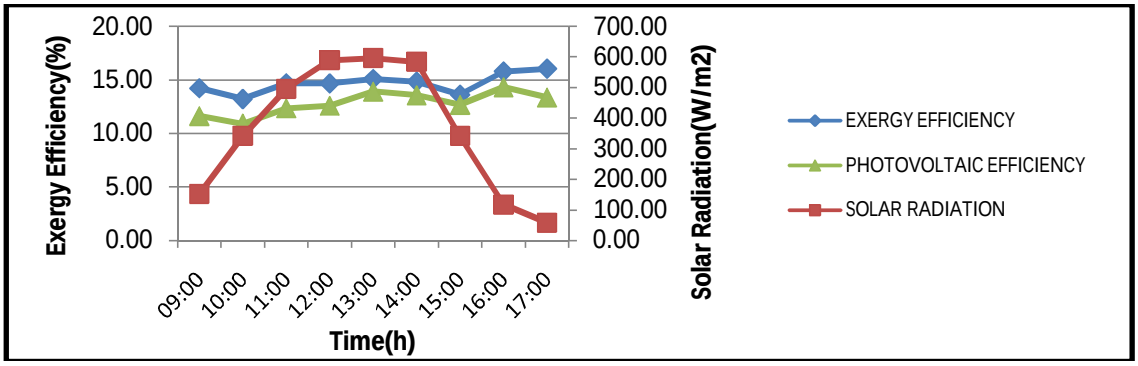

Fig .5 Variation in Exergy efficiency, Photovoltaic efficiency and Solar radiation with Time (November)

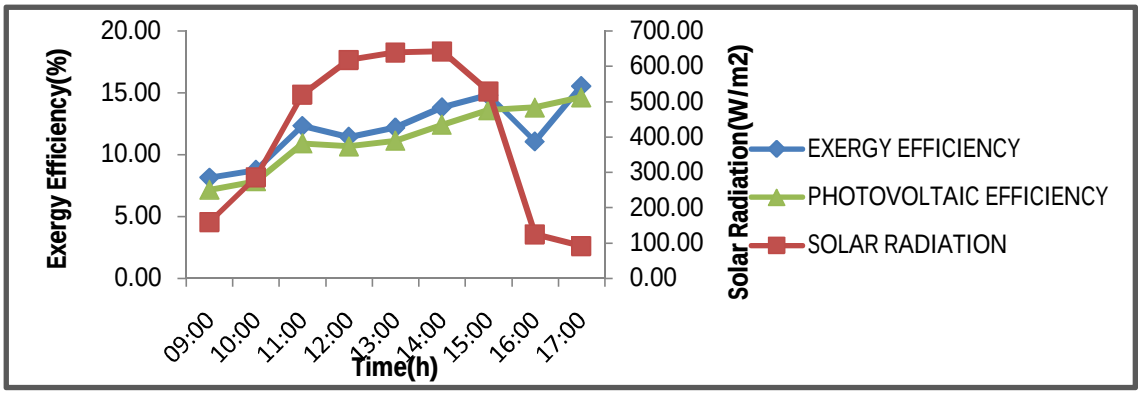

Fig .6 Variation in Exergy efficiency, Photovoltaic efficiency and Solar radiation with Time (December)

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RESEARCH ARTICLE

Evaluation of Mechanical and Microstructure Properties of Al6061 –TiB_2-SiC of Metal Matrix Composite by using Stir Casting Method

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ABSTRACT

Aluminum metal matrix composites are suitable materials for various application such as marine, automotive (pistons, shaft, bearings), aerospace and structural components because they have ductile, lightweight and have a high strength to its weight ratio. In this work aluminium alloy AA6061 was been reinforcement with TiB_2 and SiC by varying the weight percentage composite was prepared by using stir casting method. Sample1 (Al 90%+ TiB_2 5% +SiC 5%), Sample2 (Al85 %+ TiB_2 10% +SiC 5%) & Sample3 (Al80% + TiB_2 10% +SiC 10%).By using pin-on-disc wear tester the wear and frictional properties of the composite materials were studied and the wettability of matrix materials was improved by adding TiB_2 and SiC in composition. The mechanical properties like hardness, Tensile and wear test made by using optical and scanning electron microscope uniform distribution of reinforcement materials have been investigated. The results showed that Sample3 (Al80% + TiB_2 15+SiC 5%) were the increases in the reinforcement materials in Al6061 will have increasing the mechanical properties and wear resistances their Micro structural characterization clearly shows uniform distribution of TiB_2 and SiC in the composition.

Keywords : Al6061-Aluminium 6061 Grade, SiC -Silicon carbide, TiB_2- Titanium diboride, stir casting method, AMMCs- Aluminium Metal Matrix Composites

INTRODUCTION

Metal matrix composites have become as a significant role in the case of materials due to their light weight and high strength, good machinability, low coefficient of thermal expansion (CTE), and improved mechanical properties. Aluminum-matrix composites (AMCs) reinforced with blinking reinforcements are been increased use in military, aerospace, automotive and electricity industries because of their improved and mechanical and physical properties. Among Al 6061 alloy is majorly used in various engineering applications like construction sectors and transport industries for their greater mechanical properties. There are many number of reinforcement materials such as Al_2O_3, SiC B_4C, ZrO_2, SiO_2, TiB_2 and graphite improve the mechanical properties of 6061Al alloy.

Aluminium metal matrix composite is been by three manufacturing techniques like semisolid methods, liquid state methods, powder metallurgy methods. In this paper we had taken liquid state methods were the ceramic particulates are been included in molten metallic matrix and using stir casting method Aluminium metal matrix composite is been done. In liquid state methods, the ceramic particulates are incorporated into a molten metallic matrix composite.

H. Alvandiet al in this work she tell about Wear and Friction behaviour of Al6061 by varying the weight percentage of Silicon Carbide and carbon nanotube through stir casting method. For the prepared specimen wear test was made by using pin-on-Dics wear tester investigation shows that under mild wear condition wear rate has been reduced. However for cruel wear conditions wear rate has been increased friction and wear behaviour of Al–SiC-MWCNT is largely affected by varying the load [1].H.G. Rana, in his experiment Al356-reinforced with SiC and Al2O3 by varying the 3 wt. % and keeping constant investigation is been made. The shape of the reinforcement particles with 12 μm in diameter which make the improvement in wear resistance for the composite materials was become important for specific load up to 1 MPa [2]. Timothy J Harrison et al in his paper he tells about liquid metallurgical technique. A pin on disc wear testing machine is used to carried out tribological tests on the both matrix alloy and composite with load range of 10 to 50N by varying the distance 0.5 to 3km. Al6061 is been reinforced by SiC particles will obtain superior mechanical properties [3]. M. Meischel et al he has noticed that one of most significant in fabrication of aluminum matrix composite because of their compatibility of reinforcement in the matrix materials. In this case Al composite is covered with a thin layer of ox-ide which may block the surface wetting and reacts with ceramic particles .The tribological behavior of aluminium, SiC, graphite hybrid composites with by varying the weight percentage of graphite is prepared by using semi solid powder method. Since the particle are been filled in MMCs there is no significant variance in the mechanical properties.[4] M.Ramesh et alMost of the MMC's was prepared by using stir casting method is made to be compared with other casting method. Stir casting method was easy to manufacturing, high flexible during the machining process. In this method reinforcement is distributed uniformly for the different conventional alloys [5]. C.Ramesh et al. during this conventional process the binding of metal and ceramic is biggest achievement of composite materials. Development of composite material good bonding between the base material and reinforcement material [6]. M.Ramesh et al oxidation is difficult to placing between the matrix material and reinforcement in order to achieve uniform distribution of powder particles. The size and shape of powder material of reinforcement encountered during the manufacturing of metal matrix composites [7].

MATERIALS AND METHODS

Aluminum alloy 6061 was used as the base material and SiC and TiB_2 was used as reinforcement.

Compositions of samples

In this study, AA6061 alloy has been selected as matrix alloy since it has high strength, good toughness, good surface finish, excellent corrosion resistance to atmospheric conditions, good workability and good weldability. The chemical composition and properties of A6061 are shown in Tables II respectively

Silicon carbide (SiC)

The various mechanical, thermal and wear properties is been significantly improve by adding reinforcement materials in MMCs. Silicon carbide are strong bond in crystal lattices is been composed of silicon atoms and tetrahedral carbon. This produces very strong and hard materials.The physical properties of SiC which has high strength, high hardness, low density, high elastic modulus, low thermal expansion and excellent thermal shock resistance [8].

Titanium diboride (TiB_2)

Titanium di-boride is stable for many titanium- boron compounds.Since TiB_2 does not in nature but they may be prepared by synthesis of carbo thermal reduction from B_2O_3 and TiO_2 . As with other covalent bonded materials [9].

Stir Casting Process

By using stir casting techniques the material composition were prepared.The blower furnace has to be preheated before putting AA 6061 were the furnace was too heated at 800°C for twenty five minutes. Drop Aluminium Alloy (Al6061) inside the furnace metal meting temperature has to be kept 750°C .The stirring has to be carried out with the help of drilling machine for a about 15 minutes. Kept the Stirrer Speed in 750 RPM.The reinforcement materials TiB_2 and SiC powder is preheated at 750°C.Preheated reinforcement materials were made to be added manually by the vortex[10].

RESULTS AND DISCUSSION

Hardness Test

The result shows that hardness test results which were conducted by using Brinell Hardness Test.Three samples were made by AA6061 and varying weight percentage of TiB2 and SiC.Where the four mm diameter hardened steel indenting is used for testing the materials at load of 500kg.The load is applied normally 15 to 20 seconds. The diameter made by the indentation left on the test materials is been measured by low powered microscopes.

Tensile Test

To determine the mechanical properties of the composites materials the tensile test was taken by using Universal Testing Machine.Three different specimen were tested.We attain the stress and strain curves which clearly show the strength of the composition. The tensile properties such as % elongation, yield strength and tensile strength can be obtained from it.Where the increases in the reinforcement materials which lead to increases in tensile strength.

Microstructure Test (SEM Test)

Figure show1,2,3 SEM micrographs of the fabricated of three different samples. Where the Sample1 (Al 90%+ TiB2 5% +SiC 5%), Sample2 (Al85 %+ TiB2 10% +SiC 5%) & Sample3 (Al80% + TiB2 10% +SiC 10%) . Since the size of SiC

and TiB2 powder has been resulted in the significant reduction in the diameter. In the other side Size of SiC and TiB2 to be found independent during casting process. Where the sample 3 (Al80% + TiB2 10% +SiC 10%) which shows that the homogeneous distribution of Al6061 & TiB2 along with Sic particulates all through the matrix alloy since it has excellent interface bonding between reinforcements materials and base metal

CONCLUSION

The mechanical, wear properties and microstructure test were conducted with three different samples where Al6061 is reinforced with TiB_2 and SiC were fabricated by using stir casting method.

- The result revels that the sample III (Al80% + TiB_2 10% +SiC 10%) better hardness properties since the reinforcement materials have been made to be increased compare to other two samples.
- Certainly when the increases of reinforcement also increase the strength of the materials.
- Wear test were made for all three different sample by using pin-on-disc wear tester in which sample III has better wear resistance characters and tremendous co-efficient of friction

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Table I. Composition of Three Different Samples

Sample I	Sample II	Sample III
AA 90 % - 90g	AA 85 % - 85 g	AA 80 % - 80 g
TiB_2 5% - 5g	TiB_2 10% - 10g	TiB_2 10% - 10g
SiC 5% - 5g	SiC 5% - 5g	SiC 10% - 10g

Table II.Chemical Composition of AA6061

Elements	Weight %
Fe	0.17
Cu	32
SiC	0.63
Ti	0.02
Mg	1.08
Mn	0.52
V	0.01
Al	Remaining

Table III: Mechanical Properties of AL6061, SiC and *TiB_2*

Material	Tensile strength, σu (MPa)	Modulus of elasticity, E (GPa)	Mass density P (mg/m3)	Melting temperature (°C)
Al6061	310	68.9	2.7	620
TiB_2	280	510-575	4.52	2970
SiC	370	482-522	3.21	2730

Table IV : Brinell Hardness Test

Work pieces	Hardness(BHN)
Reinforcement	**Extruded**
Base Material Al6061	32.08
Sample1 (Al 90%+ TiB_2 5% +SiC 5%),	37.15
Sample2 (Al85 %+ TiB_2 10% +SiC 5%)	38.36
Sample3 (Al80% + TiB_2 10% +SiC 10%)	40.25

Table V : Tensile Test Result

Samples	Yield Strength	Ultimate Tensile Strength	Elongation (%)	Reduction in Cross Section (%)
Base Material	110	123	1.3	1.8
Sample - I	112.5	131.3	1.4	1.9
Sample - II	113.1	134.9	1.6	2.2
Sample - III	113.8	135.6	1.7	2.5

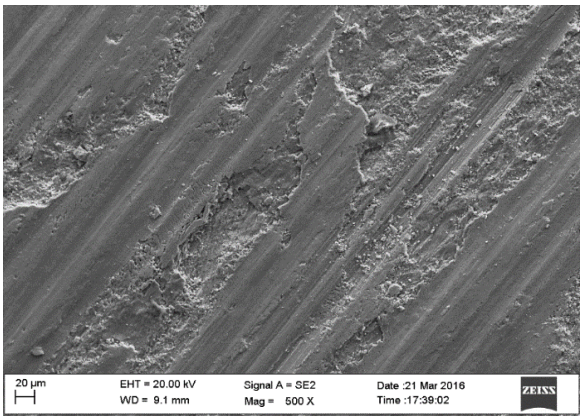

Fig.1:Sample1 (Al 90%+ TiB_2 5% +SiC 5%)

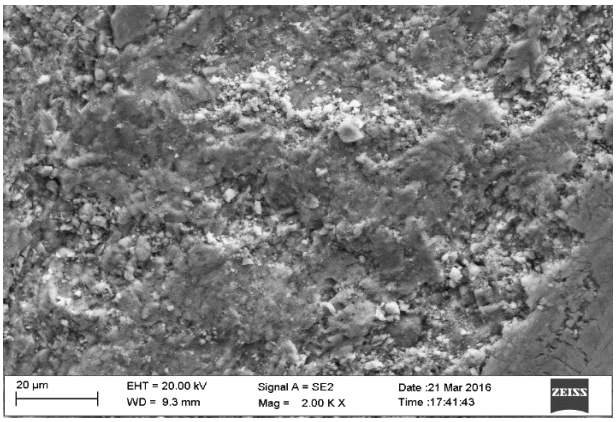

Fig.2 : Sample 3 (al85 %+ tib_2 10% +sic 5%)

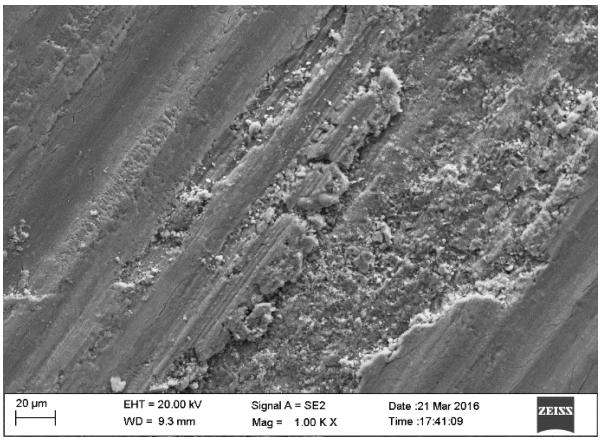

Fig.3: Sample 3 (Al80% + TiB_2 10% +SiC 10%)

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