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RESEARCH ARTICLE

Production and Utilization of Marine Copepods as Live feed for Larval Rearing of Tiger Shrimp *Penaeus monodon* with Special Emphasis on Astaxanthin Enhancement

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ABSTRACT

The industrial development of shrimp culture in recent times has been greatly hampered by vulnerable diseases and lack of suitable feed. Hence the export of shrimps has attained decreasing phase. Shrimps appearing bright are generally considered to be of good quality. So developing a feed that has high pigmentation value is equally essential as a nutritionally good feed. In fact carotenoid pigments (Astaxanthin) are essential substances in the dietary requirement of shrimps. Besides its role in body pigmentation, astaxanthin is a potent antioxidant and has also been suggested to function as a vitamin-A precursor. Copepods are important crustaceans studied because of their key role in ecology, trophic biology, fisheries management, in modeling the flow of energy and matter, ecotoxicology and aquaculture. This paper discusses various aspects of the state of knowledge of copepod culture at large scales and provides the scientific community with ideas and concepts that could improve and quicken the development of copepod mass cultures. The aim of the present study was to gain knowledge on survival and pigmentation effect of copepods on black tiger prawn (*Penaeus monodon*). The results of the present study inferred that the shrimp larvae fed with copepod showed rapid increase in growth, weight and pigmentation.

Key words: Live feed, Copepod, Astaxanthin, Shrimp feed, Pigmentation

INTRODUCTION

Shrimp culture is considered as one of the lucrative industries due to the high market price of shrimp and the unlimited demand for it in the international market. By virtue of its geographical location in the Indian Ocean, India possesses a rich shrimp ground in the sea and offers immense potential for shrimp farming [1]. For a sustained growth of the aquaculture industry, regular supply of adequate quantities of quality seeds is one of the prerequisites. Quality seeds are those that ensure high growth rate, low mortality and can withstand stress during culture [2]. To produce quality shrimp, broodstock selection is very important, while sound management during the larval rearing activities is necessary to produce quality shrimp fry. Major impacts could be documented on improved larviculture outputs, not only in terms of survival, growth and success of metamorphosis, but also with regard to their quality, e.g. improved pigmentation and stress resistance [3]. The importance of live feed in fish/shrimp culture is well documented. The use of live feeds for larval and post larval penaeid are well established with brine shrimp (*Artemia*) and rotifer (*Brachionus plicatilis*) being the most common among them. While brine shrimp are very amenable to commercial culture [4], difficulties in feeding rotifers have been reported because of their small size, nutritional variability, and the susceptibility of rotifer culture to crashing [5]. However, although *Artemia* nauplii are widely used as live food, by no means it is the optimal live food organism in terms of nutritional requirement of fish/shrimp. The biggest disadvantages of *Artemia* are marked variation in cost, physical properties, and nutritional quality among different sources. Hence the production of very small, rapidly developing and highly vulnerable larvae remains a bottleneck in the commercially successful culture of many marine fish species [6].

Nutritional compounds such as n-3 fatty acids, essential amino acids (EAA) and protein content of live feeds are critical factors for the survival and optimal growth of larval finfish and crustaceans. Hence the need for developing copepod gains importance. The marine copepods are considered to be "nutritionally superior live feeds" for commercially important cultivable species, as they are a valuable source of protein, lipid (especially HUFA, 20:5 n–3 and 22:6 n–3), carbohydrates and enzymes (amylase, protease, exonuclease and esterase), which are essential for larval survival, growth, digestion and metamorphosis [7- 11] and a relatively high weight specific caloric content [12]. In addition, the growth stages of calanoids from first nauplius to adult provide a broad spectrum of prey sizes (80 to N900 µm in length and 3–5 µg in dry weight). This makes them suitable prey for a similarly broad range of developing fish sizes [11]. The red pigment astaxanthin is one of the strongest antioxidants in nature [13] and is abundant in crustaceans [14]. Studies on the pigmentation of *Marsupenaeus japonicus* juveniles showed that dietary carotenoids may improve survival and growth. Carotenoids are important antioxidants and often exhibit other biological functions, such as regulatory effects on intra- and intercellular signalling and gene expression [15]. In the present study, the effects of three copepods (*Macrosetella gracilis, Pseudodiaptomus* sp. and *Oithona rigida*) as live food on survival, growth, and pigmentation of black tiger shrimp, *Penaeus monodon* postlarvae were compared with *Artemia* nauplii.

MATERIALS AND METHODS

Collection and identification of copepods

The zooplankton samples were collected from the Muthupet lagoon during night time using scoop (plankton) net with 158µm mesh. The collected samples were immediately transported to the laboratory by providing aeration using battery aerator. The zooplankton samples thoroughly rinsed to reduce the contamination from other zooplankters. From the samples, *Macrosetella gracilis, Pseudodiaptomus* sp. and *Oithona rigida* were identified under

microscope using the key of [16-18]. Based on the key provided by the authors the species was conformed for their taxonomy and used for culture.

Copepod culture

After the species identification, 50 gravid females of *M.gracilis*, *Pseudodiaptomus* sp, and *O.rigida* were isolated and stocked in an oval shaped, flat-bottomed fiberglass tank (0.54m diameter, 0.81m length) filled with 100 litre filtered seawater and vigorous aeration was given. Seawater filtered through a membrane filter (1μm) was used for copepods culture. The water quality parameters such as temperature, salinity, pH and dissolved oxygen were maintained in the ranges of: 26-30°C; 28-34‰; 7.5-8.5; 5.0-7.5 ml/l respectively (during rearing period) fed with a daily ration of microalga *Chlorella marina* in the concentration of 30,000 cells/ml. The cultures were harvested at every 10 days by gentle siphoning. The generation time of *M.gracilis, Pseudodiaptomus* sp. and *O.rigida* under optimal conditions is about 10-12 days at 26-30°C and having 6 naupliar and 6 copepodite stages including the adult. Finally the adult gravid female copepods were used to restart stock culture and to feed the prawn larvae. Water quality parameters such as temperature, salinity, pH, Dissolved oxygen and the population density of nauplii, copepodite and adults of *M.gracilis*, *Pseudodiaptomus* sp. and *O.rigida* were observed daily.

Algal culture

Marine micro algae *Chlorella marina* pure strains were obtained from the Central Institute of Brackishwater Aquaculture, Chennai. The algal stock culture was maintained in air conditioning room. *C.marina was* cultured in 10-L capacity container at a temperature of 23-25°C, salinity 30‰ and 12:12 light and dark conditions using Conway's medium. The seawater was filtered by using filter bag (5 micron), the filtered seawater was sterilized by using autoclave and after cooling water was transferred to the culture flask. All vessels used for algal culture was sterilized properly and dried in an oven before use. Algae in exponential phase were harvested to feed copepods.

Artemia culture

Artemia cysts (OCM Brand, USA) were purchased from the commercial shop. *Artemia* nauplii hatched in 12-L plastic tank stocked at 1.5 g of cysts/L of filtered autoclaved seawater of 30‰ salinity, 27.7°C water temperature, and pH of 8.0-8.5 and aerated vigorously. After a 24-h period, aeration was stopped and nauplii were allowed to school at the bottom (around 15 min). Thereafter, they were siphoned into a fine mesh net (40 μm) and washed with filtered autoclaved seawater. After counting, the *Artemia* nauplii were given as feed to the shrimp post larvae.

Larval rearing of Tiger Shrimp (*Penaeus monodon*)

Two 100-l glass aquarium tanks were used in order to larval rearing of *Penaeus monodon*. Shrimp larvae's were purchased from private hatchery and transported to the laboratory for adaptation and stocking. The post larvae were stocked at a density of 15 nos. per 25 l of seawater (30‰) in each tank. Post larvae were kept under a photoperiod of 12 h light: 12 h dark. Total mean length and weight of post larvae were measured prior to stocking. Aeration was supplied to the aquaria using an aquarium pump. Prior to the experiment, several batches of shrimp post larvae (PL15) were washed carefully with distilled water and then frozen at -20°C for pigment analysis. Fifteen PLs per tank were stocked randomly for each experimental feed. Three larvae from each tank were sampled every 4 days to measure the length, weight and survival parameters. The shrimp larvae were fed 2 times per day. The survival of shrimp larvae was checked daily by manual observation.

Feeding Trial

Two different diets (mixed copepods and *Artemia* nauplii) were used in the experiment. The mixed copepods such *as M.gracilis, O.rigida* and *Pseudodiaptomus* sp. were used to feed at the rate of 50-60nos./100ml. Similarly *Artemia* nauplii was given in the rate of 50-60nos./100ml.

Survival and Growth Rates

The survival rate and specific growth rate (SGR) of post larvae were estimated by following formula [19].

Survival rate (%) = N/N0*100
Where N0 and N are the initial and final number of post larvae.

Gw (/d) = (lnW-lnW0)/D
Where W0 and W are the initial and final mean body weight (mg) of post larvae and D is duration of feeding trial.

Gl (/d) = (ln L-ln L0)/D
Where L0 and L are the initial and final mean body length (mm) of post larvae and D is duration of feeding trial.

SGR weight (%/d) = (Exp Gw-1)*100.

SGR length (%/d) = (Exp Gl-1)*100.
Final weight and length of post larvae determined at end of experiment.

Water Quality Monitoring

Water quality parameters including temperature, salinity, dissolved oxygen, and pH were measured every day before feeding. Water temperature was measured with the help of standard centigrade thermometer. Salinity was measured with the help of hand refractometer (ERMA, Japan). The pH was measured by using pH meter (ELICO Grip pH meter). Dissolved oxygen was measured by Winkler's method [20].

Astaxanthin analysis in live feeds

The astaxanthin in copepods and *Artemia* naupplii was analyzed by the method of [21] with an absorption maximum at 474 nm. Copepods and *Artemia* nauplii pigments were extracted from whole animals using approximately equal numbers of each species with 10 mL of 95% ethanol. In each extraction, 10-15 adult copepods were put whole in 95% ethanol and placed in the dark at room temperature for 24-48 h. Pigmentation intensity (optical density per milligram) was measured as the optical density of the extract at the wavelength of maximum absorption (474 nm) divided by the total copepod dry mass in milligrams. Absorbance readings were calibrated to a 95% ethanol blank. Dry mass was calculated from the length dry mass relationship of [22]. Average visibility deriving from a combination of pigmentation intensity and body size (optical density per animal) was measured as the optical density of the pigment extract per individual without correcting for mass.

Astaxanthin analysis in shrimp larvae

The astaxanthin content of the shrimp larvae was determined as described by the method of [23]. Two shrimp individuals were used for carotenoid analysis and the analyses were run in triplicate. 0.5-1g of sample were taken and homogenized with a homogeniser and then transferred to 10 ml of pre weighed glass tubes. First, 10 ml of dry

acetone was added to the samples, which was followed by about 1-1.5 g of anhydrous sodium sulphate. The solutions were centrifuged at 5000 rpm for 5 min and then stored in a refrigerator at 4° C. After 3 days of incubation in sealed glass tubes, the absorption of the extracts was measured at 476 nm in a spectrophotometer.

RESULTS

Culture of copepods

The favorable result on the total density of *Oithona rigida* was obtained at the temperature of 26-34°C, salinity 26-35‰ and food concentration 30,000 cells/ml of mixed microalgae.Over 12 days culture, the system produced an average of 2126.54 nauplii L^{-1}, 1016.58 copepodids L^{-1} and 624.89 adults L^{-1} on the 12th day. For the entire culture period (35 days) the system produced totally 35701.02 L^{-1} comprising 16,564.35 nauplii, 10,568.45 copepodids and 8569.28 adults L^{-1}. The maximum average density of *O. rigida* was recorded as 4524.9 nauplii L^{-1}, 2900.32 copepodids L^{-1} and 1906.23 adults L^{-1} on 10, 12 and 12th day (s) of culture respectively.

The favourable result was obtained on the total density of *Pseudodiaptomus* sp. Over 12 days culture, the system produced an average of 1485.54 nauplii L^{-1}, 689.52 copepodids L^{-1} and 456.02 adults L^{-1} on the 12th day. For the entire 35 days culture, the total mean production was 29,692.85 L^{-1}, comprising 13.896.5 nauplii, 6232.46 copepodids and 8569.28 adults L^{-1}. The maximum mean density of *Pseudodiaptomus* sp. was recorded at 3385.12 nauplii L^{-1}, 1620.84 copepodids L^{-1} and 1065.34 adults L^{-1} on 10, 12 and 12th day(s) of culture respectively.

The twelve days culture experiment on *M.gracils* produced an average density of 1664.62 nauplii L^{-1}, 610.32 copepodids L^{-1} and 425.78 adults L^{-1} under the temperature of 26-30°C, salinity 28-34‰ and food concentration of 40,000cells/ml. For the entire 45 days culture, the total mean production was 31,963.35 L^{-1}, comprising 14,524 nauplii, 9856.01 copepodids and 7583.87 adults L^{-1}. The maximum mean density of *M.gracilis* was recorded as 3867.69 nauplii L^{-1}, 1754.21 copepodids L^{-1} and 1113.58 adults L^{-1} on 10, 12 and 12th day(s) of culture respectively.

Growth performance and survival of *P.monodon* larvae

The growth trials were conducted without interruption or disease problems. The water quality parameters across all experiments were: salinity, 28–32‰; temperature, 28–30 °C. The present result indicated that the shrimp larvae those fed with the copepod grew faster compared to larvae fed with *Artemia* nauplii. The initial, 5th and final (9th) day length (mm) of copepods fed larvae were 25.66±1.52, 31.33±1.52 and 35.33±1.52 respectively. Whereas the initial, 5th and final (9th day) length of *Artemia* nauplii fed shrimp larvae were 25.66±1.52, 25.66± 2.081, 27.33± 1.52 mm respectively.

The weight gain was also comparatively higher in copepods fed larvae (44 mg on Day 9). Length, weight and overall weight gain were significant in copepod fed larvae. The initial, 5th and final (9th) day weight of copepods fed larvae were 152.66±17.78, 202.66±4.50 and 206±4.58 mg respectively. However, the initial, 5th and final (9th) day weight of *Artemia* nauplii fed shrimp larvae was reported as 152.66±17.78, 159.33±40.67 and 162.66±37.81 mg respectively. Unexpectedly there was no death and a survival rate of 100% was observed in both copepod fed and *Artemia* fed experiment.

Pigment analysis

On conclusion of the feeding experiment there was a significant difference in pigment content between the two groups of feed. The maximum absorbance of astaxanthin was recorded in copepods and minimum in *Artemia* nauplii.

The astaxanthin content of 0.416, 0.362 and 0.317 OD/mg was observed in *M.gracilis*, *O.rigida* and *Pseudodiaptomus* sp. respectively. *Artemia* nauplii showed the low astaxanthin content of 0.225 OD/mg.

The astaxanthin content was comparatively higher in the copepods fed shrimp larvae than *Artemia* fed larvae. Here, the maximum astaxanthin content of 9.282μg/g was recorded in shrimp larvae fed on copepods whereas the shrimp larvae fed on *Artemia* showed very low astaxanthin content of 3.5649μg/g. This suggests that shrimp is capable of accumulating high amount of carotenoid based on the availablity of carotenoid in the feed that it intakes.

DISCUSSION

The present study evidenced that the potentiality of copepods as essential live feeds for shrimp larvae. According to most Southeast Asian shrimp processors and importers, colour besides others is one of the most important, but widely neglected quality criteria for penaeid shrimps. In fact, today poor general pigmentation as well as a sort of blue discolouration also known as the so-called blue shrimp syndrome is one of the most alarming problems plaguing the shrimp industry in that region. The provision of an accurate carotenoid source in the field, therefore, is important in yielding a natural pigmentation acceptable to the consumer as well as to improve the animal's general performance. However, pigmentation of shrimps may be influenced by several factors, such as achievement of an optimal and consistent pigmentation.

Since physical appearance of shrimp provides an important link in increasing the marketing value of the shrimp, this study was undertaken to improve its physical characteristics. In the view of deteriorating effects of synthetic pigments researchers are emphasizing the need for natural source of pigments which can substitute synthetic chemicals. Since the aquaculture industry seeks an environmental friendly pigment source there is a great potentiality for use of natural pigment source. It also paves way for the use of live feed with superior nutritional quality and can also provide shrimps with better pigmentation. The study has comprehensively proved the superiority of copepods over commercially used *Artemia*.

Several recent studies have suggested that carotenoids, including β-carotene, astaxanthin and canthaxanthin, are potent antioxidants in in-vitro membrane models and they work synergistically with vitamin E [24-25] In the present study, feeding shrimp larvae with *Artemia* had apparently no detrimental effects on survival and growth. This can be attributed to Astaxanthin availability in *Artemia* also. One of the clear sign of nutritional differences in this experiment was the observed carapace coloration and significant increase in size of shrimp larvae. The clear band formation in the copepod fed shrimp larvae may be attributed to high amount of astaxanthin content in copepod.

The larval rearing experiment on shrimp post larvae clearly indicated that the astaxanthin present in marine copepods can not only enhance the pigmentation but also its growth. However, the *Artemia* nauplii fed larvae did not show much growth. It might be due to the lack of required nutritional component such as astaxanthin and other fatty acids. It is well known that the nutritional strength that includes pigments (astaxanthin) besides essential fatty acids of copepods may be the reason to obtained higher growth presently [26-27].

Fig. 1 Total Density of *Oithona rigida*

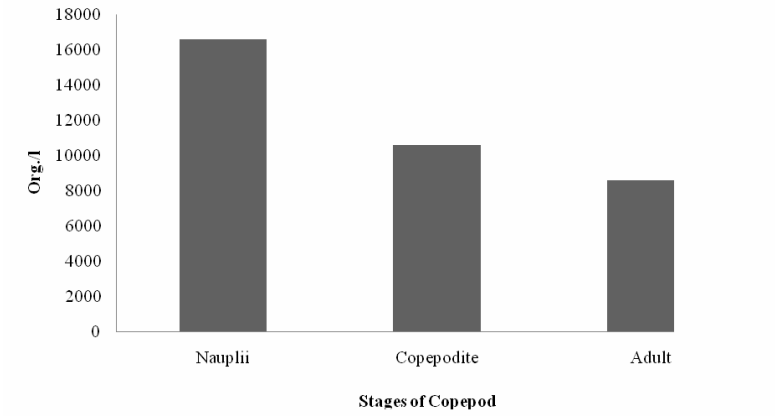


Fig. 2 Total Density of *Pseudodiaptomus* sp.

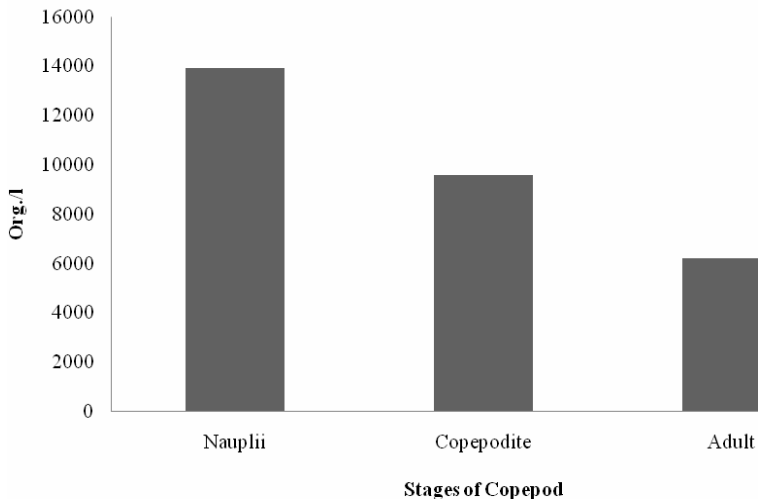


Fig. 3 Total density of *Macrosetella gracilis*

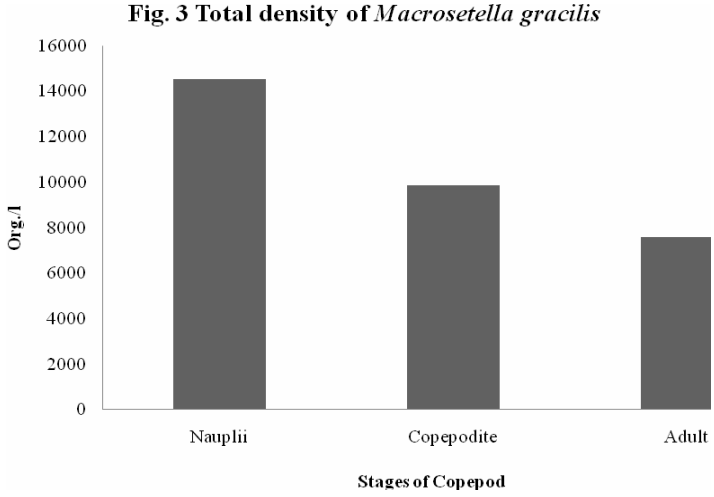


Fig. 4 Length increment of shrimp larvae fed with copepods and *Artemia*

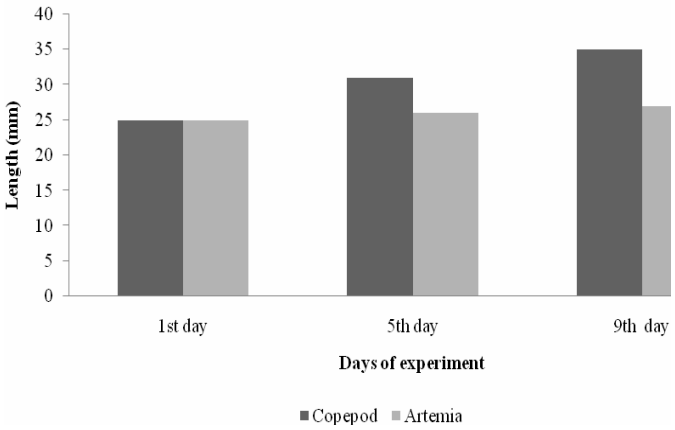


Fig. 5 Weight increment of shrimp larvae fed with copepods and *Artemia*

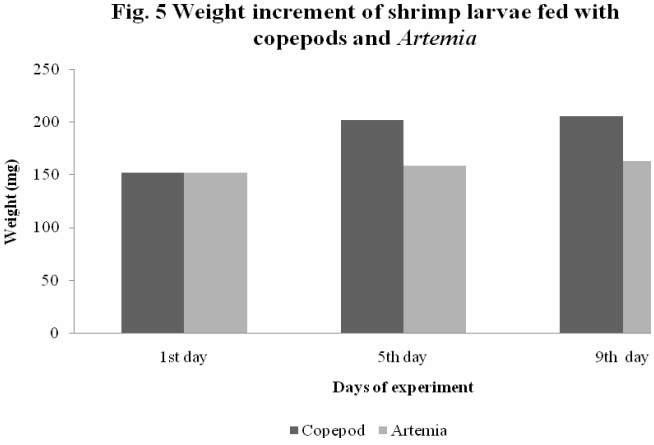
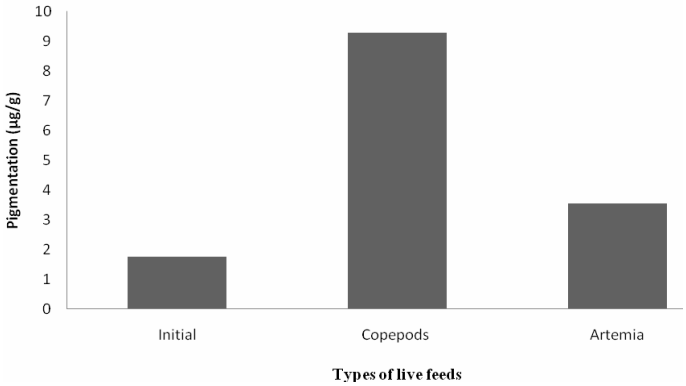


Fig. 7 Comparative Astaxanthin increment in shrimp larvae fed with copepods and *Artemia*



CONCLUSION

From the present investigation, it is clearly understood that the astaxanthin can be an enhancing factor on the growth and survival of tiger shrimp *P.monodon* post larvae. So the present study concluded that the marine copepods such as *Oithona rigida, Pseudodiaptomus* sp. and *Macrosetella gracilis* can be considered as a potential live feeds for successful larval production of tiger shrimp *P.monodon* in aqua hatcheries. Further, the present study gives evidence to convert the tiger shrimp *P.monodon* for ornamental purpose since it grew with maximum pigmentation when fed with copepods.

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RESEARCH ARTICLE

Effect of Growth Regulators on Yield and Quality in Phalsa (*Grewia Sub-Inaequalis* Dc)

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ABSTRACT

A field experiment was carried out during 2009-2010 in Model Orchard at College of Horticulture, Rajendranagar, Hyderabad to assess the influence of NAA 25 and 50 ppm, GA_3 50 and 100 ppm, Kinetin 15 and 50 ppm, Ethrel 250 and 500 ppm on yield and quality parameters of Phalsa (*Grewia sub-inaequalis* DC)". Among all the treatments, GA_3 100 ppm was most effective in improving yield per plant (3.05 kg), yield per hectare (7.63 t ha^{-1}) and hundred fruit weight (61.48g). Ethrel 500 ppm recorded maximum total soluble solids content (25.72 %). Maximum reducing sugar (18.91%), TSS to acid ratio (10.98), pulp weight (51.45g), pulp to stone ratio (5.85g) and minimum titratable acidity (2.26 %) and stone weight (8.83g) was recorded with GA_3 100 ppm. Kinetin 30 ppm recorded maximum shelf life (51.46 hrs) of the fruits.

Key words: Phalsa, plant growth regulators, yield, quality, shelf life

INTRODUCTION

Phalsa (*Grewia sub-inaequalis* DC) belongs to the family Tiliaceae is one of the hardy tropical and Subtropical fruit plant, withstand drought and grown under adverse climatic conditions. The ripe Phalsa fruits are consumed fresh, as desserts or processed into refreshing fruit and soft drinks enjoyed during summer months in India (Salunkhe and Desai, 1984). The fruits are excellent for processing into quality beverages, ready to serve, nectar, syrup and squash.

Application of growth substances viz. Auxins and Gibberellins has been effective in increasing fruitset and yield in several fruit crops including Phalsa (Randhawa *et al*. 1959). Application of GA_3 results in increased yield and better grade Phalsa fruits (Randhawa *et al*., 1967). Ethrel sprayed at full bloom stage found to be increasing TSS content of the Phalsa fruits (Rema and Sharma., 1991) and efficacy of kinetin in increasing shelf life by reducing the physiological loss of weight of fruit crops was shown by various workers (Dedolph *et al*., 1961: Randhawa *et al*., 1976).

MATERIALS AND METHODS

A field experiment was conducted in Model Orchard at College of Horticulture, Rajendranagar, Hyderabad on healthy Phalsa bushes during 2009-2010. The experiment was laid out in a Randomized Block Design with nine treatments and replicated thrice. The treatments consists of two levels each of Naphthalene acetic acid 25 and 50 ppm, Gibberellic acid 50 and 100 ppm, Kinetin 15 and 50 ppm, Ethrel 250 and 500 ppm and control. The growth regulators were applied twice *i.e*., first sprays at pre bloom and second sprays at post bloom stage.

Data was recorded on fruit weight and yield characters and chemical analysis was done to determine quality parameters of the fruit. The weight of the fruits was recorded in grams taking a random sample of 100 fruits from the harvest of each treated bush using a YAMATO balance.TSS was determined by hand refractometer and acidity was estimated as per the method of Ranganna (1986). Reducing sugars were estimated by Fehling's method using methylene blue as indicator and expressed in terms of percentage (A.O.A.C. 1980). Pulp weight and Stone weight based on 100 fruit weight and sixty per cent of fruits spoilage considered as the end of shelf life and the time (hrs) was recorded.

RESULTS AND DISCUSSION

Among the different growth regulators applied maximum 100 fruit weight (61.48g) sprayed with GA_3 at 100 ppm followed by GA_3 at 50 ppm (59.33g) shown in the table 1. Minimum was recorded with NAA at 50 ppm (55.12g) and control recorded a weight of (57.14g). Similarly, (Reddy, 1977) and (Prasad, 1990) reported that fruit weight was increased due to GA_3 sprays in Phalsa, (Al-Dujaili *et al*., 1987; Hallbrooks and Mortenson, 1988) in grapes. The beneficial effect of GA_3 in increasing fruit weight seems to be through enhanced mobilization of food reserves (Nanda and Purohit, 1965). The reduction in the fruit weight was maximum with NAA 50 ppm, which may be due to very high fruit set resulting in competition among the developing fruitlets for food. The results obtained in respect of NAA are also agreement with the findings of (Reddy 1977) who reported that Celemone sprays (NAA) did not increase fruit weight in Phalsa. Prasad (1990) reported reduction in fruit weight due to NAA application which is in conformity to the present findings.

Bushes treated with GA_3 at 100 ppm produced significantly more yield (3.05 kg/plant) and less yield was recorded with ethrel 500 ppm 0.645 kg/plant. Yield recorded with NAA 25 ppm (2.76 kg/plant) was on par with GA_3 at 50 ppm (2.88 kg/plant) but significantly superior over kinetin 15 ppm (2.34kg/plant) and Kinetin 30 ppm (2.38kg/plant) which in turn on par with each other. The increase in the yield due to GA_3 treatment due to increase in fruit set and fruit weight. The higher fruit yield might be due to GA_3 mediating process for faster translocation and mobilization of stored metabolites or photosynthates from source points (Singh *et al*., 2003). Increased yield due to GA_3 application of Phalsa was also reported by (Randhawa *et al*., 1959; Singh *et al*., 1966; Reddy 1977;Moti singh 1986).

Significantly higher content of TSS was obtained when the bushes were sprayed with ethrel 500 ppm (25.72 %) followed by ethrel 250 ppm (25.10%) and minimum was recorded in control (19.80%). Increased in the TSS by ethrel may be due to quick metabolic transformation of starch and pectin into soluble compounds and rapid translocation

of sugars from the leaves to the developing fruits (Tripathi and Sukhla, 2007). Similar finding was also reported by (Rema and Sharma, 1991) in Phalsa.

Highest GA_3 treatment at 100 ppm was more effective in reducing acidity (2.26%) when compared to control (2.55%). Lower acidity (2.34%) was recorded with ethrel 250 ppm which was on par with higher concentration ethrel 500 ppm (2.40%). The acidity of the fruit under the influence of growth regulators applied declined because it might have converted fastly into sugar and their derivatives ((Koruna *et al*., 2007) or due to faster degradation of organic acids (Dutta *et al*., 2008). (Prasad, 1990) also reported similar results with GA_3 in Phalsa, (Sharma and Dhillon, 1984) in litchi.

Maximum content of reducing sugars (18.91 %) was observed with GA_3 at 100 ppm followed by ethrel 500 ppm (18.79 %). The content of reducing sugars was higher with of GA_3. Increased in reducing sugar with higher concentration of GA_3 was reported by (Prasad, 1990) in Phalsa and (phaniprasasd, 1980) in guava, (Thilak, 1980) in Thompson Seedless grapes. Gibberellins have been shown to act through auxin synthesis hence the exogenous application of GA might have supplemented the endogenous auxin and causes greater influx of sugars in the fruits (Mohammed and Hulamani, 2001). Reducing sugar per cent age (18.67 %) was also improved with 500 ppm ethrel in Phalsa. Present finding was confirmed with the findings of (Rema *et al*., 1993) with ethrel in Phalsa.

Maximum TSS: acidity ratio (10.98 %) was observed with GA_3 100 ppm followed by ethrel 250 ppm (10.73%). This might be due to early and rapid degradation of acid and its conversion into sugars (Koruna *et al*., 2007). This conformity with the findings of (Thilak, 1980) Thompson seedless and (Mohammed and Hulamani, 2001) in Arkavati grapes.

Bushes treated with GA_3 at 100 ppm produced significantly higher pulp weight (51.45g/100 fruits) over other treatments followed by GA_3 at 50 ppm (50.78g100 fruits) but on par with each other. On the other hand, minimum pulp weight was recorded (40.25g/100 fruits) in control. The increase in the pulp weight may be due to the cell multiplication and cell enlargement or may be enhanced uptake of water and accumulation of sugar and other food reserves in greater amount as well as increased volume of intercellular spaces in the pulp of fruit due to GA_3. This finding substantiate the earlier reports on this aspects (khan *et al*., 1976; Singh and Lal,1980) in litchi, (Ruby Rani and Brahmachari,2004) in mango and (Prasad and Bajpai, 1963) who also observed similar responses of Phalsa with the fruits application of GA_3.

Bushes treated with GA_3 at 100 ppm produced significantly minimum stone weight (8.83g) and maximum stone weight was recorded (10.81g) in control. GA_3 were found effective in producing parthenocarpic fruits in multiseeded fruits but in single seeded Fruits they reduced the size and weight of the seed (Sharma and Dhillon, 1984).These results are in agreement with the findings of (Rao and Rao 1963) in Phalsa, (Islam and Siddique 1973) in guava, (Sharma and Dhillon 1984) in litchi.

Bushes treated with GA_3 at 100 ppm produced significantly higher pulp to stone ratio (5.85) followed by GA_3 at 50 ppm (5.41). On the other hand, less pulp to stone ratio was recorded (3.72) in control. The increased in the shelf life due to application may be attributed to efficacy of kinetin to increase endogenous kinins, stimulates protein synthesis as well as nucleic acid synthesis thereby delaying the senescence and reduce the physiological loss of weight during storage. Similar results were reported by earlier worker in grapes (Dedolph *et al*., 1961; Randhawa *et al*., 1976 and Dhillon., 1985), in apple (Mir *et al*., 1996). GA_3 treatments also improved the shelf life.

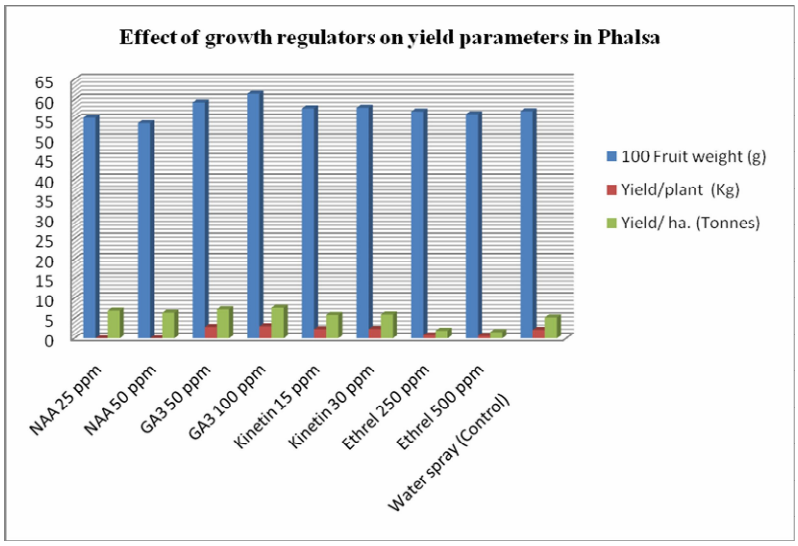

Fig. 1: Effect of growth regulators on yield parameters in Phalsa

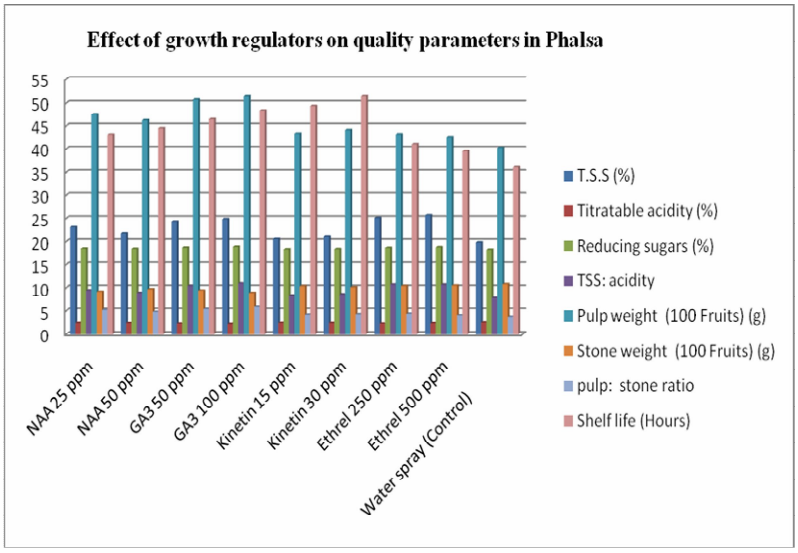

Fig.2: Effect of growth regulators on quality parameters in Phalsa

Table 1: Effect of growth regulators on yield and quality in Phalsa (*Grewia sub-inaequalis* DC)

Treatment	100 Fruit weight (g)	Yield/ plant (Kg)	Yield/ ha. (Tonnes)	T.S.S (%)	Titratable acidity (%)	Reducing sugars (%)	TSS: acidity	Pulp weight (100 Fruits) (g)	Stone weight (100 Fruits) (g)	pulp: stone ratio	Shelf life (Hours)
NAA25 ppm	55.51	2. 76	6.89	23.05	2.45	18.52	9.41	47.57	9.12	5.22	43.11
NAA 50 ppm	54.12	2. 56	6.40	21.68	2.44	18.46	8.87	46.26	9.65	4.79	44.50
$GA_3$50 ppm	59.33	2.88	7.25	24.31	2.33	18.71	10.43	50.78	9.38	5.41	46.52
$GA_3$100 ppm	61.48	3.05	7.63	24.83	2.26	18.91	10.98	51.45	8.83	5.85	48.40
Kinetin15 ppm	57.89	2.34	5.85	20.56	2.48	18.34	8.28	43.31	10.35	4.18	49.38
Kinetin30 ppm	58.03	2.38	5.95	21.04	2.46	18.40	8.56	44.10	10.10	4.26	51.46
Ethrel250 ppm	57.08	0.784	1.95	25.10	2.33	18.67	10.73	43.14	10.41	4.36	41.08
Ethrel500 ppm	56.40	0.645	1.60	25.72	2.40	18.79	10.72	42.52	10.52	4.04	39.53
Water spray (Control)	57.14	2.16	5.24	19.80	2.55	18.25	7.77	40.25	10.81	3.71	36.12
S. Em ±	0.20	0.05	0.11	0.10	0.02	0.01	0.11	0.39	0.03	0.05	0.43
C.D.at 5%	0.60	0.15	0.33	0.31	0.06	0.05	0.32	1.18	0.08	0.14	1.30

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RESEARCH ARTICLE

Anthropogenic Impacts on Hydrochemical Characteristics of River Gomti in Lucknow City, India

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ABSTRACT

The present investigation attempted to assess the extent of anthropogenic impacts on surface water quality of River Gomti in Lucknow city. Five surface water samples were collected from Gomti River in urban Lucknow during post-monsoon season (October to December, 2010). The physico-chemical characteristics, total coliforms, fecal coliforms and heavy metals were analyzed as per American Public Health Association Standard Methods. The physico-chemical analysis revealed some of the parameters such as phosphate, DO, COD, BOD, total coliform, fecal coliform are recorded well above the prescribed standard limit for drinking water. Iron (300 to 400 μg/l) and cadmium (1 to 4 μg/l) levels were recorded higher than the prescribed limit of world health organizations. Mixing of industrial effluents, municipal sewage and solid waste dumping are suspected sources of pollution in River Gomti at Lucknow.

Key words: River Gomti, surface water, physico-chemical characteristics, heavy metals, anthropogenic pollution.

INTRODUCTION

Urbanisation in India is taking place faster than the rest of the world. Rapid urbanisation, high economic activity and mushrooming of industries ultimately lead to the tremendous pressure on freshwater resources. Further, it alters land use changes from unaltered natural land to artificial land use (e.g. roads, residential areas, commercial areas). Apart from point and non-point sources of water pollution, land use changes also further aggregates the water

quality in rivers running through urban areas. Evidently, earlier studies also hinted that urban areas are the largest source of organic and inorganic pollutants in river water [1-2]. Rivers in urban areas have also been associated with water quality problems because of the practice of discharge of untreated domestic & industrial effluents and solid waste dumping into the water bodies leads to the deterioration of riverine ecosystem [3-7]. Some of the studies reported the positive association between the area of urbanisation and the concentration pollutants in river water [8-10].

In general, surface water contamination leads to considerable impact on human health, balance of aquatic ecosystems, socio-economic development and prosperity [11]. Among the pollutants, heavy metals are known of their toxicity, persistence, and non-degradability in the environment [12-14]. Some of the heavy elements such as lead, mercury, arsenic, and cadmium are highly toxic for the humans even at trace level of exposure [15]. Therefore, monitoring of heavy metals in freshwater system is highly essential to assess the safety of the environment and human health in particular.

River Gomti is a major tributary of the Ganga river system in India. The river flows a total distance of nearly 730 km before merging with the River Ganga. During its course of flow it receives massive pollution load both from the point and non-point sources. Because of high pollution load, River Gomti has been known as a one among the most polluted rivers in India [16]. River Gomti serves as a major source of domestic water supply of the Lucknow city, the State capital of Uttar Pradesh. Area of Lucknow is 337.5 Sq.Km with population of about 32 lakhs. In this context, the present study attempted to evaluate the impact of anthropogenic activities on surface water quality of Gomti River in Lucknow city.

MATERIALS AND METHODS

Surface water samples were collected in acid washed polythene bottles from Lucknow city in five stations (Kudia Ghar (S1), Saheed Smarak (S2), Gaughat (S3), Mid-Lucknow (S4), and Pipraghat (S5) during October to December, 2010. The parameters such as pH, electrical conductivity and total dissolved solids were measured in the field using portable water monitoring kit (make: Deep vision). Total alkalinity, total hardness, calcium, magnesium, chloride were estimated by titration method. Sodium and potassium levels were measured flame photometerically (make: Systronics). Sulphate, nitrate and phosphate were estimated spectrophotometrically. All the analysis was carried out as per Standard Methods of American Public Health Association [17]. Heavy metals were determined after digesting a known volume of water sample with nitric acid by Atomic Absorption Spectrophotometer (Varian Spectra- AA220). The bacteriological analysis such as total coliform count and faecal coliform were measured by Most Probable Number (MPN) and faecal coliform count (FCC), respectively. All the collected water samples were analyzed within 24 hr. The numbers of bacterial colonies were counted by colony counter. The results obtained were evaluated in accordance with the norms prescribed under Bureau of Indian Standards (BIS) [18] and World Health Organization (WHO).

RESULTS AND DISCUSSION

Descriptive statistics of physico-chemical characteristics and heavy metal concentration are given in Table 1. The pH of the water samples ranged between 8.0 and 8.4 indicates alkaline nature and it falls within the prescribed standard limit (6.50-8.50) of World Health Organisation. Mean level of electrical conductivity and total dissolved solids recorded as 1047 μS/cm and 1621 mg/l, respectively. Higher TDS in water system increases the chemical and biological oxygen demand and ultimately depletes the dissolved oxygen level in water. TDS in water originates from natural sources, sewage, urban runoff and industrial effluents. The total suspended solids were observed in the range between 24 and 57 mg/l.

The concentration of calcium and magnesium was observed in the range from 124 to 150 mg/l and 98 to 144 mg/l, respectively. The mean level of sodium and potassium content in water samples were recorded as 111 and 15 mg/l, respectively. Elevated level of sodium exposure may adversely affect the cardiac, renal and circulatory functions in human [19]. Concentration of chloride lies between 145 to 198 mg/l with a mean of 170 mg/l. Chloride concentrations of all the samples were found well within the permissible limit of 250 mg/l [20].

Dissolved oxygen content of samples ranged between 1.5 and 2.5 mg/l with a mean concentration of 2.03 mg/l. While comparing the dissolved oxygen content of Gomti River samples with World Health Organization standards, all the samples exceeded the permissible limit of 6 mg/l. Very low level of DO in surface water probably attributed to the mixing of organic rich municipal sewage into river. In Lucknow city, about 450 MLD the untreated domestic wastewater directly discharge into Gomti River [16]. Chemical oxygen demand of samples varied between 59 and 78 mg/l with a mean level of 68.3 mg/l. High COD values (78 mg/l) at Pipraghat are due to the discharge of huge amounts of the untreated urban and industrial wastewater/effluents. BOD of water samples varies from 2.9 to 8.2 mg/l. BOD values indicate the extent of organic pollutants in the aquatic systems, which adversely affect the water quality.

Phosphate concentration was observed in the range between 0.2 and 0.3 mg/l. The phosphate values exceeded the permissible limit (0.1 mg/l) of US Public Health Standards [21]. High concentration of phosphate is indicative of pollution and untreated sewage discharge, detergents, and fertilizer runoff are the major sources of anthropogenic phosphorus in freshwater system [22]. The concentration of Nitrate was observed in the range from 0.58 to 7.2 mg/l. Maximum level (7.2 mg/l) of nitrate was recorded at Mid-Lucknow. Nitrate concentration of 10 mg/l or greater is considered as an indication of contamination [23] and nitrate level of 8.5 mg/l are considered to be in the category of low level contamination [24]. High level of nitrate in drinking water may leads to potential health risks such as methmoglobinemia or 'blue-baby-syndrome' particularly in pregnant women and bottle-fed infants, respectively [25]. The concentration of sulphate was observed in the range between 16 and 23 mg/l. Sulphate values in all sampling stations were well below the prescribed by Bureau of Indian standards limit of 400 mg/l. Fluoride content in all water samples recorded (0.8 to 1.0 mg/l) within the optimum concentration of 1.5 mg/l, as recommended by World health organization. Coliform bacteria are known as a reliable indicator of organic pollution because they are unable to survive in clean water beyond a limited time [26]. The total coliform and faecal coliform were recorded in the range from 70000 to 270000 coliform/100 ml and 49000 to 80000 coliform/100 ml, respectively. These values are much higher than recommended values of 1 coliform/100 ml. Most of these coliforms were of a faecal type probably due to residents and animals faecal wastes discharge from adjacent villages and forest. High level of faecal contamination in rivers may be attributed to rather warmer zones and domestic discharges [16].

Heavy metals concentration in Gomti River samples were observed in the following sequence: Fe > Zn > Cr >Cd = Ni> Pb (Figure 1). Iron (Fe) concentration in Gomti River samples were ranged between 0.3 and 0.4 mg/l with mean level of 0.33 mg/l. High level of Fe (Gaughat, Mid-Lucknow, Pipraghat) could be attributable largely to effluents the river receives from many iron based industries located along its banks. Except Kudia Ghar, in all the sampling sites the Fe levels exceeded the standard limit of 300 µg/l [27].

Lead is second among the top 20 priority list of hazardous substances [28]. Apart from leaded gasoline, sewage sludge and agricultural runoff are the major sources of Pb contamination in the watersheds [29]. In the present study, Pb level was found range between 0.02 and 0.03 mg/l with a mean level of 0.02 mg/l. In the case of chromium, all the samples (0.03 to 0.05 mg/l) recorded within the standard limit of 0.05 mg/l of WHO. Singh and Steinnes [30] categorized various anthropogenic activities which are attributed to increase concentrations of Cr in natural waters are (i) run-off from agricultural and urban areas, (ii) discharges from mining, factories and municipal sewer systems, (iii) leaching from dumps and former industrial sites and (iv) atmospheric deposition. Concentration of nickel (Ni) and zinc (Zn) recorded in the range from 0.03 to 0.04 mg/l and 0.11 to 0.15 mg/l, respectively.

In all the sampling locations Zn concentration was observed within the permissible limit of 3 mg/l [27]. The maximum concentration (0.004 mg/l) of Cd observed in Pipraghat (S5) was probably due to coal combustion which is very frequent in industries and domestic purpose. The major source of Cd is the coal combustion, metal industry and waste incineration [31]. Comparatively, low level of heavy metals in surface water samples of River Gomti probably due to the neutral to alkaline nature of the river water, most of the heavy metals have precipitated and settled as carbonates, oxides, and hydroxide bearing sediments [32]. Analysis of variance not yielded statistically significant difference between sampling sites and sampling events in heavy metal concentration at 95% confidential level.

CONCLUSION

This study investigated the extent of human impact on physico-chemical characteristics and heavy metals concentration in the Gomti River. The physico-chemical characteristics and heavy metal content of surface water samples reveals that some of the parameters such as phosphate, DO, COD, BOD, total coliform, fecal coliform, iron, cadmium are well above the prescribed standard limit for drinking water. The contamination of surface water in all the sampling sites by faecal coliform indicates unregulated discharge of faecal wastes from residents and animals in the Lucknow city. Mixing of industrial effluents, municipal sewage and solid waste dumping are suspected sources of pollution in River Gomti at Lucknow. Suitable management and restoration plans should be implemented on priority basis for avoiding further detoriation of river water quality.

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Table. 1 Descriptive statistics for surface water characteristics and heavy metals concentration in River Gomti during 2010 (October – December).

Parameter	Unit	Minimum	Maximum	Mean	Standard deviation
Temperature	(°C)	22.0	24.0	23	1.00
Turbidity	NTU	10.0	14.0	12.33	2.08
pH	-	8.0	8.4	8.17	0.21
Conductivity	μS/cm	974.00	1110.00	1047.00	68.62
TDS	mg/l	1508.00	1714.00	1621.00	104.54
TSS	mg/l	24.00	57.00	39.00	16.70
DO	mg/l	1.50	2.50	2.03	0.50
BOD	mg/l	15.00	20.00	17.00	2.65
COD	mg/l	59.00	78.00	68.33	9.50
Na	mg/l	88.00	136.00	111.00	24.00
K	mg/l	13.00	17.00	15.00	2.00
Ca	mg/l	128.00	152.00	141.67	12.34
Mg	mg/l	98.00	144.00	118.67	23.35
Cl	mg/l	145.00	198.00	170.33	26.58
Alkalinity	mg/l	198.00	245.00	217.67	24.42
SO_4	mg/l	70.00	82.00	76.67	6.11
NO_3	mg/l	12.00	14.00	13.00	1.00
F	mg/l	0.80	1.00	0.90	0.10
Phosphate	mg/l	0.20	0.30	0.23	0.06
Cd	mg/l	0.001	0.004	0.002	0.002
Total Coliform	MPN/100ml	70000	270000	170000	100000
Faecal Coliform	MPN/100ml	49000	80000	63000	15716

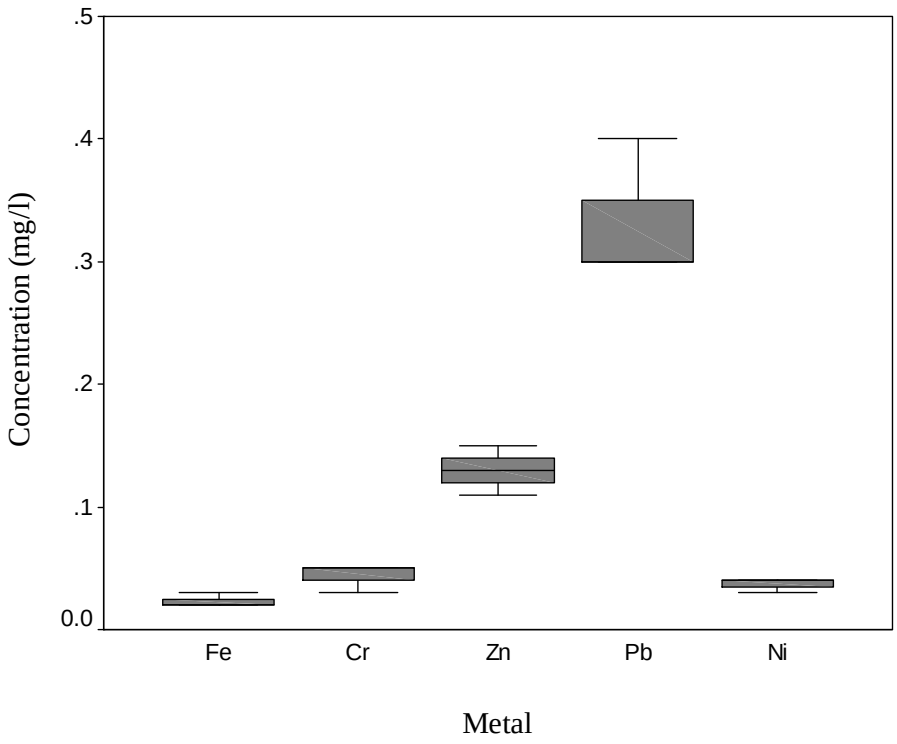

Figure. 1 Heavy metal concentration in surface water samples of Gomti River in Lucknow city. (The bottom boundary of each box indicates the 25th percentile, lines within the box mark the median and the top boundary of the box indicates the 75th percentile).

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RESEARCH ARTICLE

Biological Synthesis of Nanoparticles by using Crabshell (*Scylla serrata*) and Exploration of their Medicinal Properties

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ABSTRACT

The paper is regarding the ecofriendly, extracellular, biological synthesis of nanoparticles; by using some biological agents like crab shell (*Scylla serrata*). Crab shells are the biological agents which are very cheap and readily available everywhere. And also for exploration of medicinal properties of silver nanoparticles synthesized by crab shell extract. The investigation reveals that so produced silver nanoparticles possesses medicinal properties related to drug delivery system which was tested against many human pathogens like (*Staphylococcus aureus, Klebsiella pneumoniae, and E.coli).* Other than that particle size of nanoparticles was also very small, which is a useful characteristic for a various processes like preparation of bionanochips, Nanofibers.

Keywords: Nanoparticles, crab shell, antibiotic activity, drug delivery system.

INTRODUCTION

From the time of the first talk on nanoparticles given by physicist Richard Erymann on December29,1959, various researches going in the area of nanoparticles synthesis by various ways and exploration of properties of their different formulations, sizes and shapes. Because of tremendous applications of nanoparticles like use in integrate circuits, cell electrodes, Antimicrobial deodorant fiber, catalysis, chemical analysis(mouxing el.al) , drug delivery, solar energy absorption , optical receptors , biolabelling (Minaeian el.al). Initially, nanoparticles were prepared by chemical synthesis, but the process of that was very costly, environment polluting & also skill demanding. So, to overcome from these disadvantages of chemical synthesis, researches are going on biological synthesis of nanoparticles by using some biological agents like plant extracts and micro-organisms such as fungus and bacteria. Instead of nanoparticles prepared by chemical synthesis, the particle prepared by biological agents seems to be having potent antimicrobial abilities. The present investigation is based on potent silver nanoparticles synthesis

property of crab shell(*Scylla serrata*)and antimicrobial activity of the same, against human pathogens (*Staphylococcus aureus, Klebsiella pneumoniae,& E.coli)*.We, authors, proudly say that this is the first paper regarding study of nanoparticles forming & antimicrobial properties of crab shell.

MATERIALS AND METHODS

Silver nitrate solution was purchased from HiMedia laboratories Pvt. Limited, Mumbai, India. The crab shells were collected from fish market, Chennai, India.

Preparation of silver nitrate solution

3mM silver nitrate solution was prepared in 100 ml deionized water. All the glasswares were sterilized to avoid any disturbance in reaction.

Preparation of extract

The crab shell was washed several times with deionized water. 25g of the crab shell was grinned for 30 min. in 100ml water, with the help of mortar and pestle and filtered. Then, the filtrate was centrifuged for 30 min at 5000 rpm and supernatant was collected. The supernatant was cooled to room temperature and used as reducing agent and stabilizer.

Synthesis of silver nanoparticles:

10 ml of the grinned crab shell extract was added to 100ml of 3mM $AgNO_3$ solution, and kept below room temperature. Bioreduction of silver ions in the solution was monitored by using UV-Vis spectra of the solution at periodic intervals. The nanoparticles synthesis was confirmed by UV-Vis spectra plasma curve. The solution was centrifuged and the particles characterized.

Particles characterizations

Nanoparticles were characterized by UV-vis, XRD, and FTIR & SEM-EDS.

UV-vis analysis

The bio reduction of Ag^+ in aqueous solution was monitored by periodic sampling (0.2ml) of the suspension, then diluting the samples with 2ml deionized water and subsequently measuring UV-Vis spectra, UV-Vis spectra were recorded at 1hr, 2hr, 3hr, & 4hr time interval, by using UV-Vis spectrophotometer (ELICO-SL 159).

SEM analysis

SEM analysis was done using LEO-1530 (LEO) at 20 kV. Thin film of sample was prepared on carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed by using a blotting paper and then the film coated on the grid was allowed to dry for analysis.

XRD analysis

The air dried nanoparticles were coated onto XRD grid and analyzed for the formation of Ag nanoparticles by Philips X-Ray Diffractometer with Philips PW 1830 X-Ray Generator operated at a voltage of 40kV and a current of 30mA with Cu Kal radiation. The diffracted intensities were recorded from 10' to 80' of 20angles.

FTIR analysis

The dried Ag nanoparticles were subjected to FTIR analysis by KBr pellet (FTIR grade) method in 1: 100 ratios and spectrum was recorded in Nicolet Impact 400 FT-IR Spectrophotometer using diffuse reflectance mode operating at a resolution

Assessment of Antibiotic property of silver nanoparticles

Five different Human pathogens were used for assessment of antibiotic property, they are *Staphylococcus aureus(*MTCC *96), Klebsiella pneumoniae (MTCC 109) , & E.coli (MTCC 118).* Disc diffusion method was used for analysis. Antibiotic Tetracycline was used for comparison of antibiotic properties against silver nanoparticles and also for drug delivery. Concentration & quantity of antibiotic & nanoparticles used for investigation was as stated in Table:1

Table:1 Concentration of the test samples.

No.	Name of the test sample	Concentration of sample	Amount transferred to the disc
1	(C)CONTROL(DISTILLED WATER)	----------	10μl
2	(NP) NANOPARTICLES	10mg/ml	10μl
3	(AB) ANTIBIOTIC (TETRACYCLINE)	10mg/ml	10μl
4	(AB+NP) ANTIBIOTIC+NANOPARTICLES	5mg/ml + 5mg/ml	10μl

RESULTS AND DISCUSSION

Preparation of silver nanoparticles by above described method is a fast method, in around 1hr the nanoparticles started to form.(Fig.1) UV-vis spectrum of solution showed peak at 450 nm, and O.D.

SEM Analysis

SEM analysis of silver nanoparticles shows that the average size of particle is 22nm and particle size was between 18-30. And also the particles were mostly spherical, average particle size has been estimated by using Debye-Scherrer formula (Fig:2).

XRD Analysis

XRD analysis showed three distinct diffraction peaks at 26.459^{o}, 32.555^{o}, 46.705^{o} and can be indexed the angle values of (110), (111), (211) crystalline planes of cubic Ag. This analysis revealed that nanoparticles are in orthorhombic crystals. The high peaks in the analysis indicate the active silver composition with the indexing (Fig:3).

FTIR Analysis

FTIR spectral analysis showed array of absorbance bands in 500 cm^{-1} – 2000 cm^{-1}. The spectral band peaks are along the range of between 614cm^{-1}- 3270cm^{-1} with prominent peaks at 614cm^{-1}, 1525cm-^{1}, 1636cm^{-1}, and 2921cm^{-1} which were interpreted for the identification of the functional moieties in the air dried silver nanoparticles(Fig :4).

Antibacterial Activity of Silver Nanoparticles against Human Pathogenic Bacteria

Disc diffusion method

Zone of Inhibition in the plate showed that silver nanoparticles synthesized using filtrate of crab shell is having the antibacterial activity against test pathogens namely *Staphylococcus aureus, Klebsiella pneumoniae and E.coli.* The results stated here revealed the fact that nanoparticles and antibiotics alone were not working at all. But, when we coated the nanoparticles by antibiotic, they started to work on all the microbes, used for investigation. Means, we can say that silver nanoparticles produced by crab shell extract may be used for drug delivery system, but this work is preliminary stage regarding to potent drug delivery system, further work is required in this area.

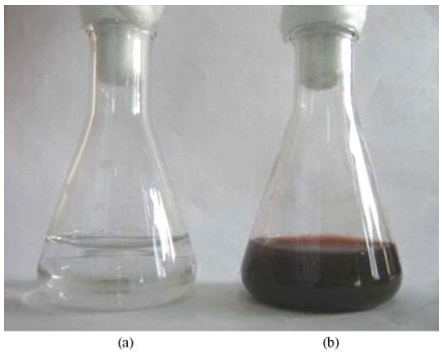

Fig.1 Colour change of solution

(a) Before adding crab shell extract

b) After adding crab shell extract & incubation of 1hr.

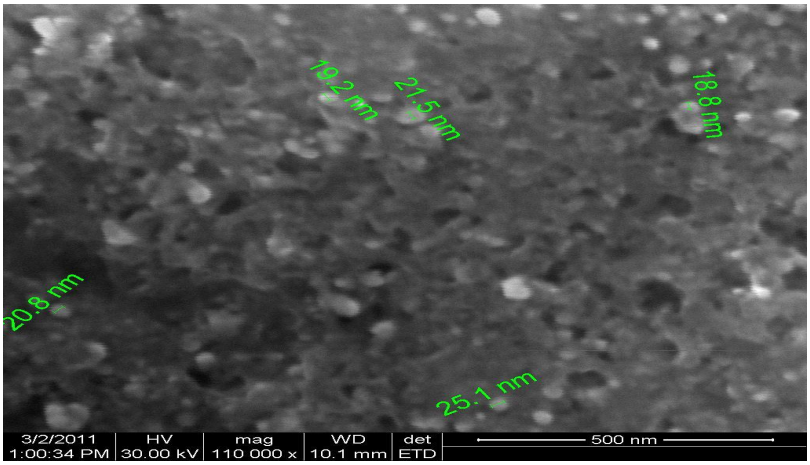

Fig.2 SEM analysis of Nanoparticles.

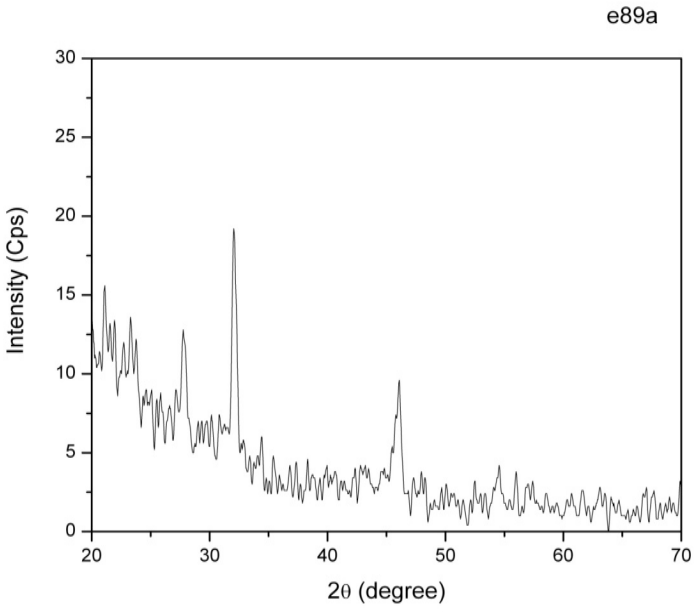

Fig:3 The XRD pattern of the silver nanoparticles

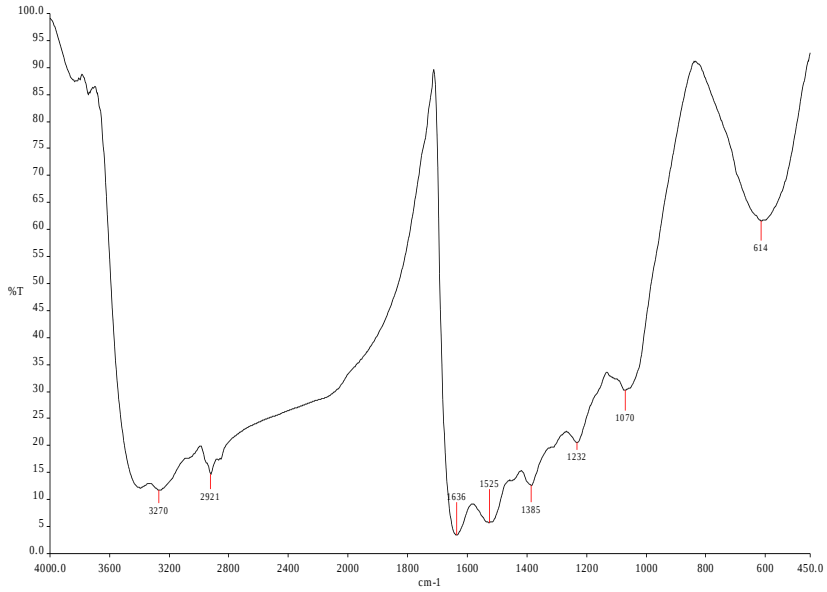

Fig: 4. FTIR spectrum of the silver nanoparticles.

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RESEARCH ARTICLE

Removal of Mercury from Synthetic Effluent using Activated Rice Husk Carbon and Activated Carbon as an Adsorbents

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ABSTRACT

Mercury is a poisonous and harmful metal and creates a major problem in environmental pollution. Mercury can be removed from effluent by various methods and some of the methods are sodium sulphide treatment, ferrous sulphate treatment, ion exchange resin method and adsorption method. The adsorption method was taken up as it is a cheapest technique for the treatment of various types of effluents and the various experiments were conducted for the removal of mercury from synthetic effluents using rice husk ash and activated rice husk carbon. The parameters studied were concentration of adsorbents and time.

Key words: Adsorbents, synthetic effluent, rice husk and activated carbon

INTRODUCTION

Mercury is a toxic material and it causes damage not only to plants but also to human beings. The maximum limit for the presence of mercury in industrial effluents as per Indian standards is 0.01 mg/L. whenever the concentration of mercury crosses this permissible limit value it causes serious concern since it also causes some disease. It has received environmental concern only after the 'MINAMATA' episode in Japan during 1952-53. There, fishermen families and house hold cats became stricken with a mysterious disease that weakened their muscles, impaired their vision, led to

mental retardation and sometimes resulted in paralysis and death. A large portion of mercury found in the environment is derived from industrially produced mercury. Industries responsible for production of mercury are the burning of fossil fuels, composites, incinerators, mining and extraction of mercury from cinnabar, the chloro alkali industries, paper and pulp, paints, fungicides electrical equipments. Nearly all chlorine is manufactured by electrolysis of brine, using mercury cells in which the cathode is a flowing sheet of elemental mercury. Also all of the hydrogen gas produced during the preparation of chlorine by the mercury cell process contains mercury. In paper and pulp industries organic mercury compounds are often used extensively. It has been found that some 5-20% of the mercury is discharged into waterways, with the rest remaining in the product and finally being released into the environment.

The main objective is to remove the harmful mercury from effluents by the adsorption process using adsorbents like activated carbon, activated rice husk carbon and rice husk ash. To achieve these parameters such as optimum time of adsorption, optimum dosage of adsorbents were investigated.

MATERIALS AND METHODS

Determination of optimum dosage

0.15 gm of $HgCl_2$ was dissolved in 100ml distilled water in 6 beakers. To each, a known amount of adsorbent was added and the mixture was stirred for half an hour. This mixture was filtered and the amount of mercury in filtrate was determined gravimetrically. To estimate mercury from the filtrate obtained the following procedure is to be followed.

Twenty ml of dilute HCl was added to the filtrate, to this two spoons of fused sodium sulphide flakes were added to saturate the solution with H_2S gas. This results in the precipitation of Hg as mercuric sulphide. The precipitate was filtered, dried and weighed. The amount of Hg in it was calculated stoichiometrically.

Determination of contact time

From the above mentioned procedure, optimum dosage of adsorbent was taken in a solution (0.15 grams $HgCL_2$ in 100 ml distilled water) in five different beakers. The mixture was stirred continuously for 1, 1.5, 2, 2.5, and 3 hrs. Then the adsorbent was filtered off and the amount of mercury in filtrate was determined gravimetrically. The difference in the initial amount of mercury in solution and amount of mercury in filtrate gave the amount of mercury adsorbed.

RESULTS AND DISCUSSION

Experiments were conducted to remove the mercury from effluent by the procedure described above. The effect of the following parameters on the percentage removal of mercury were studied using adsorbents like activated carbon, activated rice husk and rice husk ash. The results of activated carbon, activated rice husk carbon with different dosages and different contact time intervals are mentioned in the tables 3.1, 3.2, 3.3 and 3.4 respectively. The data revealed that the increases the amount of adsorbents added increase in the amount of mercury adsorbed. The increase in the amount of mercury adsorbed with an increase in the dosage of adsorbents is due to enhanced total surface area of the adsorbents.

The mechanism and rate of adsorbent are functions of n and k respectively. For a good adsorbent, n value should be greater than one. A smaller value of n-1 indicates better adsorption and formation of relatively strong bond between the adsorbate and adsorbent. The value of n given in the above table indicates that bond formation between mercury and activated rice husk is stronger than rice husk ash and activated carbon.

Determination of optimum dosages

The parameters are time 0.5 hour and concentration being varied from 0.2 to 0.7 grams. The result of the experiment to determine the optimum dosage of activated carbon, activated rice husk, rice husk ash is given below.

Table 3.1: Determination of optimum dosages

Dosage of activated carbon (grams)	Residual mercury concentration (ppm)	Mercury removed (grams)	Percentage of mercury removed
0.2	0.077	0.247×10^{-2}	3.10
0.3	0.072	0.747×10^{-2}	9.39
0.4	0.039	4.047×10^{-2}	50.92
0.5	0.018	6.147×10^{-2}	77.37
0.6	0.017	6.23×10^{-2}	78.34
0.7	0.013	6.59×10^{-2}	82.92

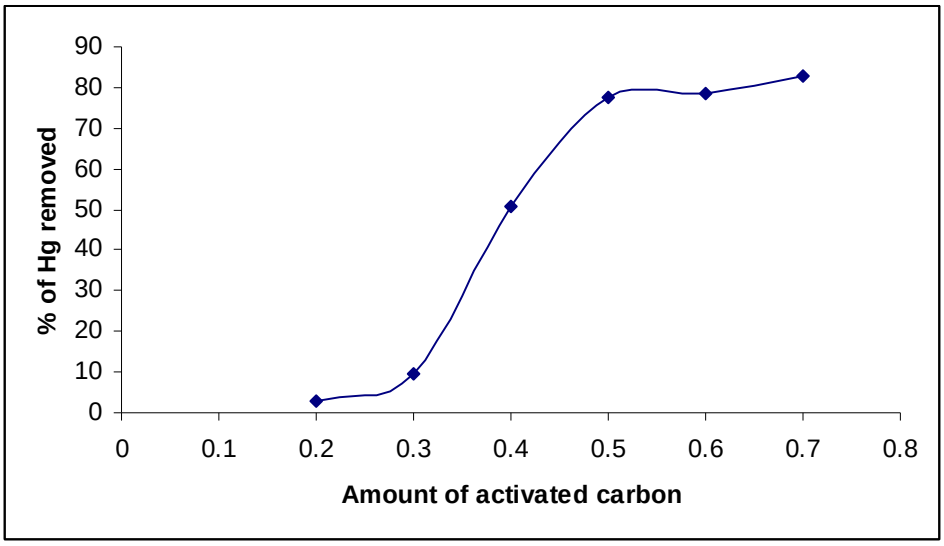

Fig.3.1: Optimum dosage for activated carbon

Effect of contact time

The parameters of time was varied from1 to 3 hours of interval and concentration of 0.5gms of activated carbon is kept constant for each half hour interval of time.

Table 3.2: Effect of contact time

Time of contact (hr)	Residual mercury concentration (ppm)	Mercury removed (grams)	Percentage of mercury removed
1.0	0.01547	6.45×10^{-2}	81.16
1.5	0.01167	6.78×10^{-2}	85.31
2.0	0.00747	7.2×10^{-2}	90.60
2.5	0.00597	7.35×10^{-2}	92.48
3.0	0.00467	7.48×10^{-2}	94.12

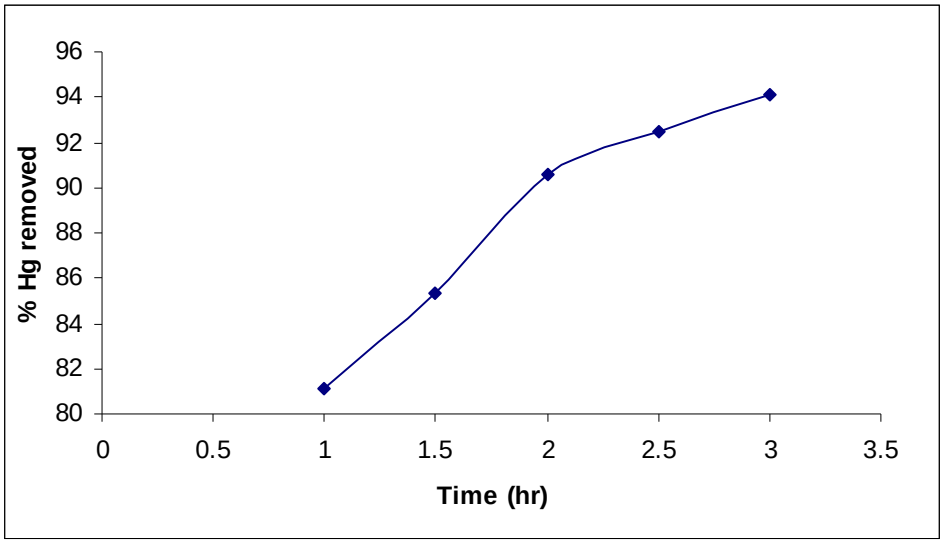

Fig.3.2: Effect of contact time

Activated rice husk carbon

In this case the parameter such as time 0.5 hour is kept constant and concentration be varied from 0.2 to 0.8 grams of activated rice husk carbon.

Table 3.3: Determination of optimum dosages

Dosage of activated carbon (gm)	Residual mercury concentration (ppm)	Mercury removed (grams)	Percentage of mercury removed
0.2	0.078	0.001	1.25
0.3	0.077	0.001	2.03
0.4	0.036	0.043	54.1
0.5	0.031	0.048	60.4
0.6	0.030	0.049	61.09
0.7	0.029	0.05	62.9
0.8	0.030	0.049	61.65

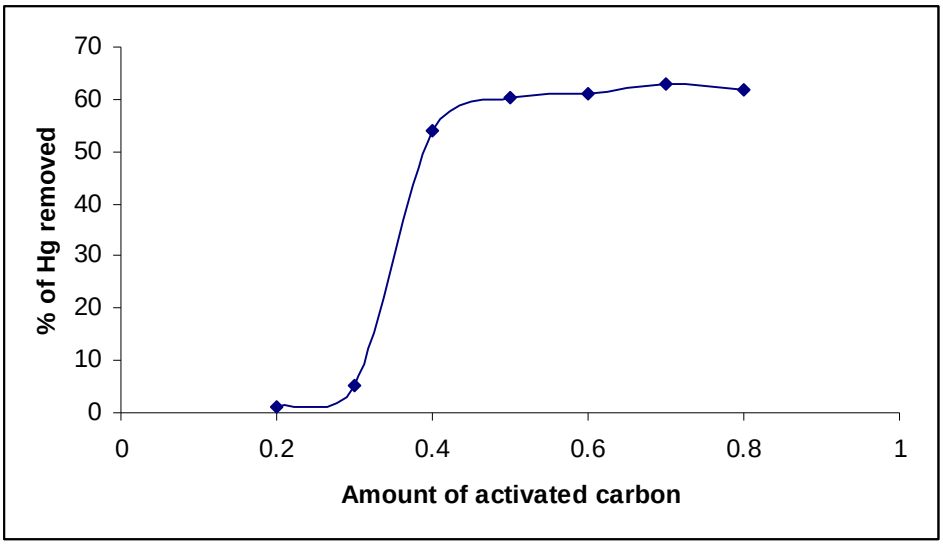

Fig.3.3: Optimum dosage for activated carbon

Effect of contact time

To verify the effect of contact time, the time was varied from 1 to 3 hours of interval and concentration of 0.6 grams of activated rice husk carbon is kept constant for all durations of time.

Table 3.4: Effect of contact time

Time of contact (hr)	Residual mercury concentration (ppm)	Mercury removed (gm)	Percentage of mercury removed
0.5	0.003	0.049	61.6
1.0	0.028	0.050	63.54
1.5	0.027	0.052	65.43
2.0	0.025	0.053	67.32
2.5	0.021	0.057	72.32
3.0	0.016	0.062	79.16

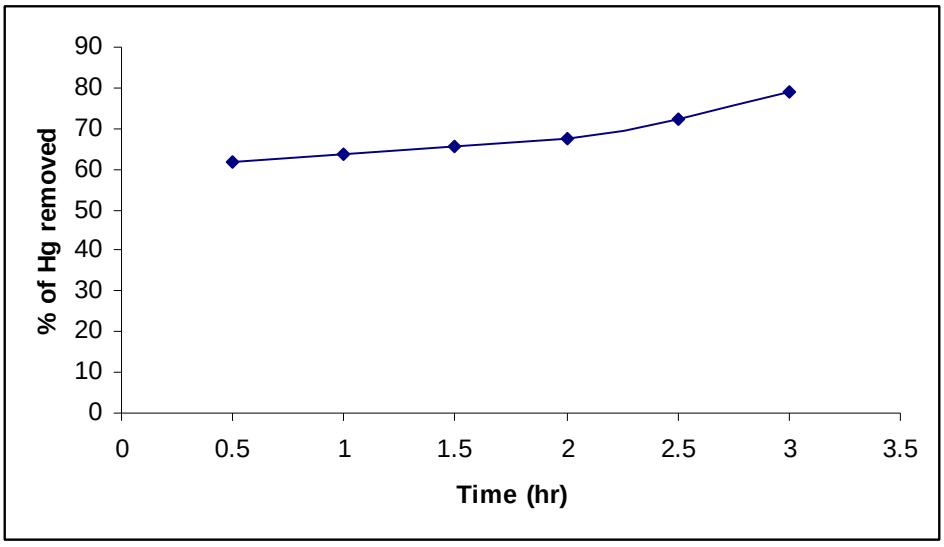

Fig.3.4: Effect of contact time

CONCLUSION

The following are the conclusions drawn from the present work. Activated carbon and activated rice husk carbon are found to be good for removing Hg by adsorption from synthetic solution. The percentage removal is also good for both the adsorbents and nearly about 75% Hg removal is observed in both the cases in just 0.5 hours.The dosage of activated rice husk carbon required is slightly greater than the dosage of activated carbon to achieve a particular percentage removal of Hg. This is due to the large surface area of activated carbon. The dosage of activated rice husk carbon required is little larger than the activated carbon.

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RESEARCH ARTICLE

An Investigation on Heavy Metals Accumulation in Water, Sediment and Small Marine Food Chain (Plankton and Fish) from Coromandel Coast, Southeast Coast of India

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ABSTRACT

The accumulation level of heavy metals viz., Co, Zn, Ni, Cd and Pb were determined in sediment, water, plankton, and fish (*Mugil cephalus*) samples collected from four different sampling areas *viz.,* Pulicate lagoon, Muttukadu Backwater, Cuddalore and Nagapattinam in the Bay of Bengal, southeast coast of India. The trend of heavy metals accumulation was found to reported as follows Co > Zn > Ni > Cd> Pb in sea surface water, Co > Zn > Ni > Pb > Cd in sediments; Zn > Co > Ni > Pb > Cd in plankton and Zn > Ni > Co > Pb > Cd in fish. From the present study, it is clearly understood that the zinc was observed as maximum in plankton sample at Pulicate lagoon followed by fish which collected from Muttukadu Backwater. Similarly, copper was reported as maximum in the surface waters of Nagapattinam and sediment samples in Muttukadu Backwater. The level of copper and zinc metals in water, plankton, and fish flesh were exceeded the acceptable levels for a food source for human consumption. The results of this study indicated that the metals accumulated in the Coromandel Coast of India were taken up by fish through water and food and regardless of their biological needs showed high metal concentrations.

Keywords: Coromandel Coast, heavy metals, sediment, water, plankton, fish.

INTRODUCTION

Heavy metals play an important role in human society due to their special properties including malleability, ductility, resistance to corrosion, and high electric and thermal conductivity, etc. In aquatic systems, heavy metals have received considerable attention due to their toxicity and accumulation in biota [1]. Metals generally enter the aquatic environment through atmospheric deposition, erosion of the geological matrix, or due to anthropogenic activities caused by industrial effluents, domestic sewage, and mining wastes [2,3]. Some of these metals, such as Cd and Pb, are toxic to living organisms even at quite low concentrations, whereas others, such as Zn and Cu, are

biologically essential and natural constituents of aquatic ecosystems, and generally only become toxic at very high concentrations. Together with increasing use of heavy metals, the level of heavy metal pollution has been increased dramatically over the years. Anthropogenic sources of heavy metals in coastal environments include industrial and municipal waste products, urban and agricultural runoff, fine-grained sediments eroded from polluted catchment areas, atmospheric deposition, antifouling paints from ships, and acid mine drainage. Human activities such as dredging and reclamation in coastal environments can remobilize the heavy metals from marine sediments to seawater column [4].

Pollution by heavy metals in coastal environment has become a global phenomenon because of its toxicity, persistence for several decades in the environment, bioaccumulation and biomagnifications in the food chain. Metals transferred through aquatic food webs to fish, humans, and other piscivorouss animals are of environmental and human health concern. High levels of Hg in fish from apparently pristine lakes have resulted in the adoption of conservative fish consumption advisories in many states [5,6]. In Asia, investigations on the measurement, distribution and fate of heavy metals in the marine environment have been reported for a number of countries including India, Thailand, Malaysia, Japan, Korea and China [7-11]. In recent years metal concentrations were found to be increased in coastal and marine eco-systems. As a result, aquatic organisms are exposed to elevated levels of heavy metals [12,13]. The distribution and behavior of heavy metals in the marine environment, as well as their impact upon marine organisms and human health, are of great concern due to their persistent, non-biodegradable and toxic properties. In this context, the present study was aimed to study the heavy metals accumulation in seawater, sediment, plankton and planktivorous fish of selected coastal areas of Coramandel coast of Tamil Nadu, Southeast coast of India. For the present investigation the heavily industrialized coastal area such as Pulicate Lagoon, Muttukadu Backwater, Cuddalore and Nagapattinam in the eastern zone of the Bay of Bengal was selected to study the the heavy metal levels in the seawater and sediments and determine the heavy metal accumulation levels in the plankton and common edible fish, *Mugil cephalus*.

MATERIALS AND METHODS

Coromandel Coast is located on the east coast of India. In that we selected four sampling areas *viz.* Pulicate Lagoon (Lat 13°43'N Long. 80°18'E), Muttukadu Backwater (Lat 12°49'N Long. 80°15'E), Cuddalore (Lat 11°43'N Long. 79°49'E) and Nagapattinam (Lat.10°45'N Long. 79°51'E) in the Bay of Bengal, Southeast coast of India (Figure.1). The selected areas represents great interest because it is a highly industrial belt consisting of many major industries involved in the production of chemicals, plastics, agricultural chemicals and human activities in and around the four areas have altered ecosystem prominently.

For heavy metal analysis water sample was collected from the sampling stations in clean polyethylene bottles. The filtered water samples were pre-concentrated with APDC-MIBK extraction procedure as described AAS [14] and aspirated to a Flame Atomic Absorption Spectrophotometer. Sediment samples were collected using a clean plastic spoon and samples were transferred to clean polyethylene bags. The sediment sub-samples were dried at 150^{o}C for 5-hr, ground to powder in glass mortar, and stored in pre-cleaned polythene bags. Sediment samples were determined by aspirating the solution to a standard Flame Atomic Absorption Spectrophotometer [15].

Plankton samples were collected from four different stations using standard plankton net (48μm) and collected plankton samples were stored in ice box with an ice and were stored frozen. Heavy metals from plankton were extracted followed the method described by [16]. Fish sample was collected in sampling area. Samples were immediately kept in pre cleaned polythene bags, which were sealed and kept in deep freezer (-20°C) until further analysis. Fish was cleaned and washed with filtered water to remove dirt and slick. The soft tissue was removed and dried at 60ºC at oven and dried tissue was reduced into fine powder in a pestle and mortar and the resulting powder

was sieved using a plastic sieve with 0.2 mm opening size and was stored in desiccators for further heavy metal analysis [17].

RESULTS AND DISCUSSION

Pulicate lagoon

At Pulicate lagoon, the maximum level of copper was recorded as 110.125 ppm in water whereas the minimum value of 2.05 ppm was observed in sediment samples. No copper was reported in fish collected from the Pulicate lagoon. The zinc concentration was varied from 8.4 to 11.925 ppm. The maximum zinc (11.925 ppm) was observed in water and minimum (8.4 ppm) in sediment samples. The cadmium concentration was found to vary from 0.175 to 0.25 ppm with the maximum level (0.25 ppm) observed in fish whereas the minimum noticed in water samples. No cadmium was observed in sediment collected from the Pulicate lagoon. The recorded nickel concentration of Pulicate lagoon was ranged between 1.95 and 7.85ppm. The maximum nickel accumulation was obtained in plankton while the minimum in sediment. No nickel was noticed in water sample collected from the Pulicate lagoon while the lead concentration of Pulicate was ranged between 0.225 and 2.1 ppm with maximum (2.1 ppm) in fish and minimum (0.225 ppm) in sediment.

Muttukadu Backwater

The recorded copper concentration of Muttukadu Backwater was found to range between 0.525 and 143.572ppm. The maximum (143.572 ppm) was obtained in water whereas the minimum (0.525 ppm) found in fish. The zinc content was varied from 7.2 to 120.3ppm with highest in plankton whereas the lowest in sediment .The recorded range of cadmium was reported between 0.1 and 0.575ppm with the maximum (0.575ppm) in water and minimum (0.1 ppm) in plankton. The reported level of nickel was found between 1.15 and 5.025 ppm with highest accumulation in plankton whereas the lowest level in sediment. Similarly, the lead was recorded in the range between 0.6 and 1.325 ppm. The maximum value (1.325 ppm) was observed in fish and minimum value (0.6 ppm) in water.

Cuddalore

The recorded range of copper in Cuddalore waters was 0.55-98.625 ppm. Of these, the maximum copper was observed in water whereas the minimum reported in fish. The zinc level was reported in the range between 2.85 and 118.25 ppm. Of these, the plankton was found to report with maximum zinc content of 118.25 ppm whereas the minimum (2.85 ppm) was noticed in sediment. The cadmium was noticed in the range between 0.1 and 0.275 ppm. The maximum cadmium was obtained in plankton and minimum in water. No cadmium was noticed in sediment and fish samples collected from the Cuddalore water. Here, the nickel level was showed in the range between 1.775 and 6.875 ppm with maximum value in water while the minimum in sediment. The lead concentration was recorded in the range between 0.1 and 1.6 ppm. The maximum (1.6) lead was observed in plankton while the minimum (0.1) in water.

Nagapattinam

The recorded copper range at Nagapattinam was varied from 1.35-152.975 ppm. The surface water was accumulated with maximum copper while minimum in sediment. The zinc recorded in the range between 1.0 and 119.9 ppm. Of these the plankton recorded with highest zinc whereas the low zinc was observed in water. The cadmium content of

Nagapattinam was varied from 0.075-0.35. The highest (0.35 ppm) cadmium was observed in fish whereas the lowest (0.075 ppm) in water. The recorded nickel content was varied from 3.5 to 14.75 ppm with peak concentration in plankton whereas the least level in sediment. The lead was recorded in the range between 0.175 and 1.9 ppm. Of these, the maximum content was observed in fish and minimum in sediment.

Among the five heavy metals analyzed, the seawater showed the maximum copper level it might be due to land runoff and the mechanical and chemical weathering of rocks, the components also washed from the catchment areas through runoff and windblown dust [15]. Similarly copper content was found to obtain more in plankton which second to water it might be due to accumulation or transferring of metals from water [18]. The present study clearly indicated that zinc was accumulated more in plankton followed by fish compare to water and sediment. The rich cadmium accumulation in plankton might be due to the rich accumulation capabability of plankton. This is supported by earlier workers who obtained similar results [19-21]. Similarly, cadmium, nickel and lead also observed more in plankton than water, sediment and fish. The metals such as cadmium, copper, nickel, zinc and lead can capable of binding with phytoplankton cell surface and further it can transfer to zooplankton which feeds on them. The present study was strongly agreed by earlier authors [22,21].

The metal concentrations are ranked as follows: Co > Zn > Ni > Pb > Cd. The highest concentration of copper was occurred in the water sample of Nagapattinam (152.97 ppm) coastal area followed by Muttukadu Backwater (143.57 ppm). The copper contents in the water samples were much higher than the FAO-permitted level of 30µg/g [23] and Chinese food standards (10µg/g) [24]. Excessive intake of copper may lead to liver cirrhosis, dermatitis and neurological disorders [25]. Copper toxicity in fish is taken up directly from the water via gills, the present study showed the similar accumulation of copper in the muscles [26]. Effects of high concentrations of copper on fish are not well established, however, there is evidence that high concentrations in fish can experience toxicity [27]. Copper can combine with other contaminants such as ammonia, mercury and zinc to produce an additive toxic effect on fish [28, 29].

In the present study, the highest concentration of zinc was occurred in plankton which was collected from the Pulicate lagoon (120.55ppm). The presently obtained result was comparatively higher than Chinese food standards (50µg/g) [24] but less than Hungarian standards (150µg/g) [30]. The present study indicated that the present values are lower than the permitted level of Hungary and above permitted level of China. Therefore, we can conclude that these metals have posed no threat for consumption of mullet, *Mugil cephalus* fish collected from the present study areas. Toxicity due to excessive intake of zinc has been reported to cause electrolyte imbalance, nausea, anemia and lethargy [31].

In this study, we found that the highest concentration of lead was occurred in plankton collected from the Nagapattinam (1.32ppm). The fact that toxic metals are present at low concentrations in plankton is of particular importance in relation to the FAO/WHO [32] standards for Pb. The maximum permissible doses for an adult are 3 mg Pb per week, but the recommended doses are only one-fifth of those quantities [32]. Chinese food standards [24] are 1µg/g. Turkish acceptable limits and EU limits are 0.4µg/g. The highest concentration of cadmium was occurred in plankton, collected from Cuddalore (0.27ppm). Cadmium concentrations are lower than other metals from those reported earlier by [33] in fish from Turkish Lakes. These values were found to be lower than the acceptable limit proposed by the EU [33] and TFC [34]. For muscle tissue they found a significant increase in heavy metal concentrations with increasing fish age. Higher concentrations of metals were found in younger fish and this generally reflects the short residence time of these metals within the fish, combined with the higher rate of metabolism compared to older organisms [35]. Similar increase of metals levels in tissues of some invertebrate and fish species were observed during summer months that were related to the increased metabolism due to high temperature [36, 37].

The values of organic matter in the sediments were high during hot seasons which, might be attributed to the flourishing of phyto- and zooplankton, leading to high organic productivity during this period especially spring [38]. The bioaccumulation factor of Ni in the Antarctic fishes was about one order of magnitude lower than those found in fishes from other ocean waters [39], which is due to a low concentration of blood cell in the Antarctic fishes for adapting themselves to the cold water condition.

CONCLUSION

It is clearly understood that the planktonic organisms found in the Coromandal coastal water are able to accumulate much of zinc than other metals owing to discharges from power plants, aquaculture, agriculture, port and ship wastes. The present study concluded that the planktonic organisms are capable of accumulating more heavy metals and it might be transferring it to planktivorous fishes. Further, long term investigations are needed on heavy metals accumulations in plankton species in precise to have a clear picture on biomagnification rate of heavy metals to higher trophic levels.

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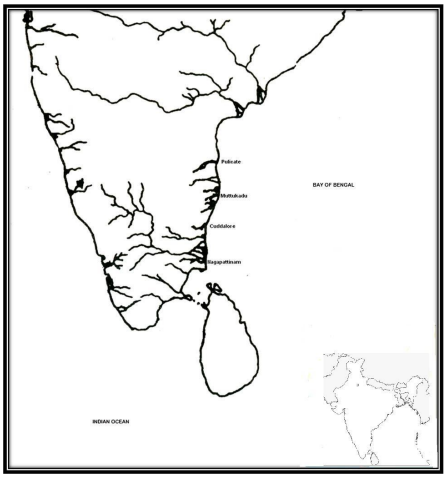

Figure-1. Location of the sampling area

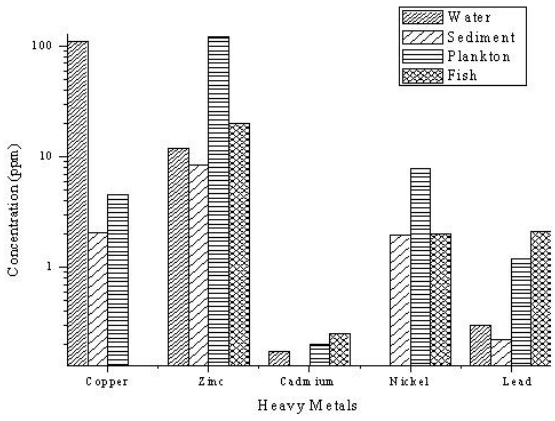

Figure – 2. Heavy Metals level in marine food chain at Pulicate lagoon

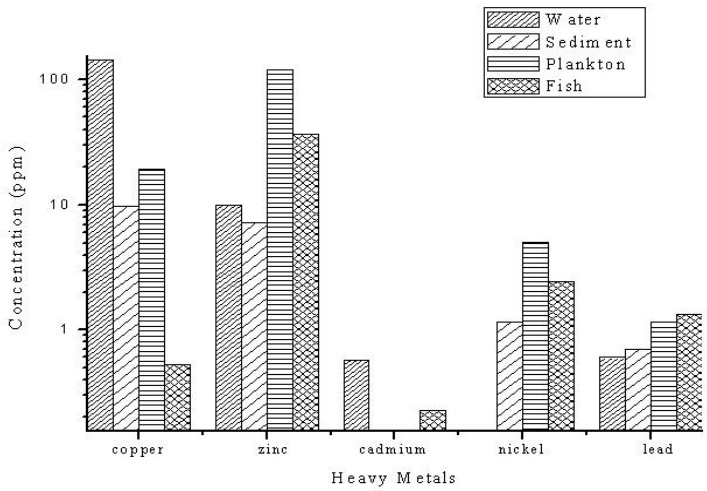

Figure – 3. Heavy Metals level in marine food chain at Muttukadu Backwater

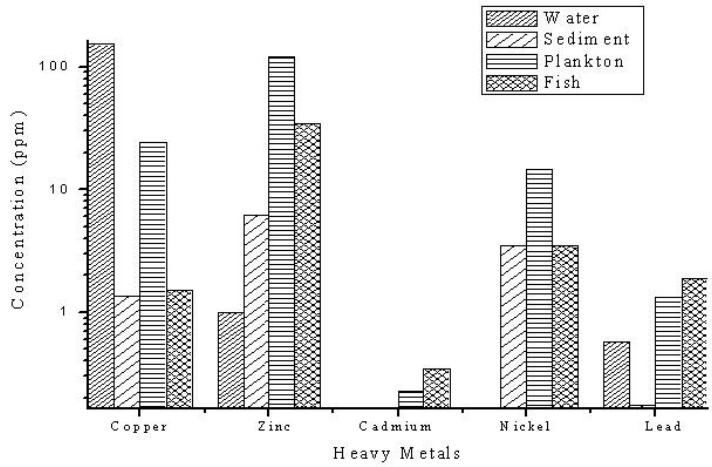

Figure – 4. Heavy Metals level in marine food chain at Nagappattinam water

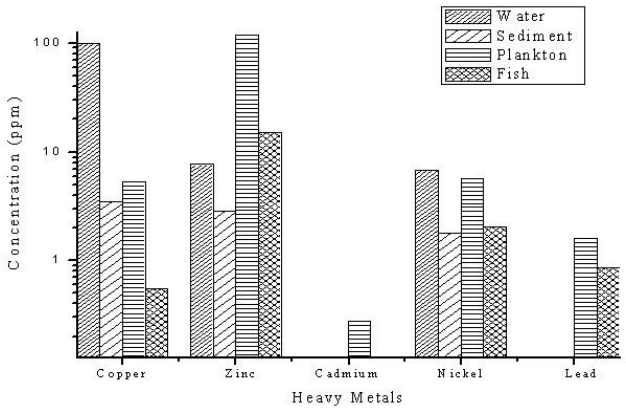

Figure – 5. Heavy Metals level in marine food chain at Cuddalore water

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RESEARCH ARTICLE

Design and Development of Soil Moisture Deficit Based Drip Automation System

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ABSTRACT

Automated drip irrigation and soil moisture sensors developed were based on soil moisture and soil electrical resistance. Sensors evaluated were for monitoring the soil moisture content based on electrical resistance variation with moisture content. The controllers and sensors were calibrate with red soil and black soil and shows satisfactory performance for black and red soil based on calibration curve developed from moisture of soil and electrical resistance of soil. From the regression analysis, R^2 values of 0.98 and 0.88 observed for black and red soil.

Keywords: drip irrigation, soil moisture sensors, controllers, regression analysis

INTRODUCTION

The primary source of water in agricultural production in most parts of the world is rainfall. The main factors that characterized of rainfall amount, frequency and intensity; the value of which vary spatially and temporally. When the weather does not provide enough rainfall to feed agricultural needs, farmers should supplement water available through surface and groundwater by some type of irrigation to manage soil moisture and nutrient concentration to create the optimum growing environment.

The recent irrigation techniques introduce automated irrigation using sophisticated equipments to supply water to the plant as soon as they need it. Automated irrigation systems can increase crop yields, save water usage, energy and labour costs as compared with manual systems (Mulas, 1986). Automated irrigation has a number of advantages including greater precision, efficient use of water and reduction in human error (Castanon, 1992). It is very useful, particularly in humid areas where unpredictable and unevenly distributed summer rainfall disrupts fixed irrigation schedules. Automated irrigation system also facilitates high frequency and low volume irrigation.

Microprocessors are used in irrigation control units to operate electric motors and solenoid valves. Thomson et al. (1982) reported on the use of microprocessor control units for center pivot irrigation. Pogue (1990) describes how the Watermark soil moisture sensors were used to override landscape irrigation controllers and Phene et al. (1992) used electronic measurement of evaporation pan losses to control a high-frequency subsurface drip system with a microprocessor. Finally, McCann et al. (1997) documented how microprocessors are being used to move control from the irrigation system level to individual sprinkler level to provide a variable rate water application on continuous move sprinkler systems.

Automatic irrigation systems presently available are costly were not adopted by most of the Indian farmers. Therefore, an appropriate low cost technology has to be developed to facilitate high water use efficiency. Therefore a study was conducted to design low cost sensor and with locally available material was designed.

MATERIALS AND METHODS

Design and fabrication of soil moisture sensors was dine with the technical help of Handson technology Ltd and labrotary experiments on soil moisture content were conducted at Precision Farming Development Centre (PFDC) of TNAU Coimbatore.

Design details

Controller

The system designed was to be simple, lightweight and easy to handle. The front panel has four keys to accept user inputs. A LCD displays the response and status of the system. at the rear side 2×6 sensor interface helps easy connectivity for the sensor modules. 2 × 8power interfaces provide 3 phase 230 v AC. Supply input and out put for motor control. Two D9 connectors used were to interface with computer. Program interface is for loading for firmware updates and not for use by end users.COM1 interface is for RS-232 serial communication with computers. A PIC16F877A controller used for data acquisition and for activation irrigation pumps the heavy-duty 3-pole relay is used; three- phase or single-phase connection can be made. Figure 1 shows the schematic layout of circuit for controller.

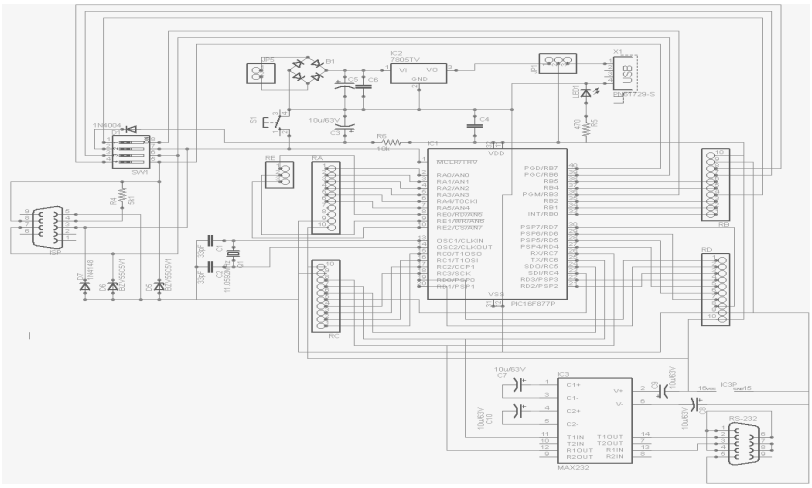


Fig 1. Schematics and Layouts of the boards used in the system.

Whole circuit simulated with MPLAB software. The simulation software MPLAB is that program package that makes writing and developing a program easier.

Sensor

The soil moisture sensors designed were to measuring Electrical resistance of soil that is indicator of soil moisture. The material used for fabrication of sensor is stainless steel to avoid oxidation with soil shown in Fig.2

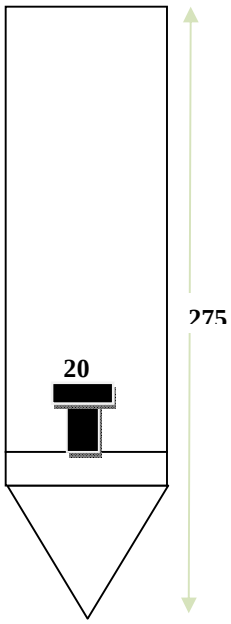


Fig.2 Soil Moisture Sensor

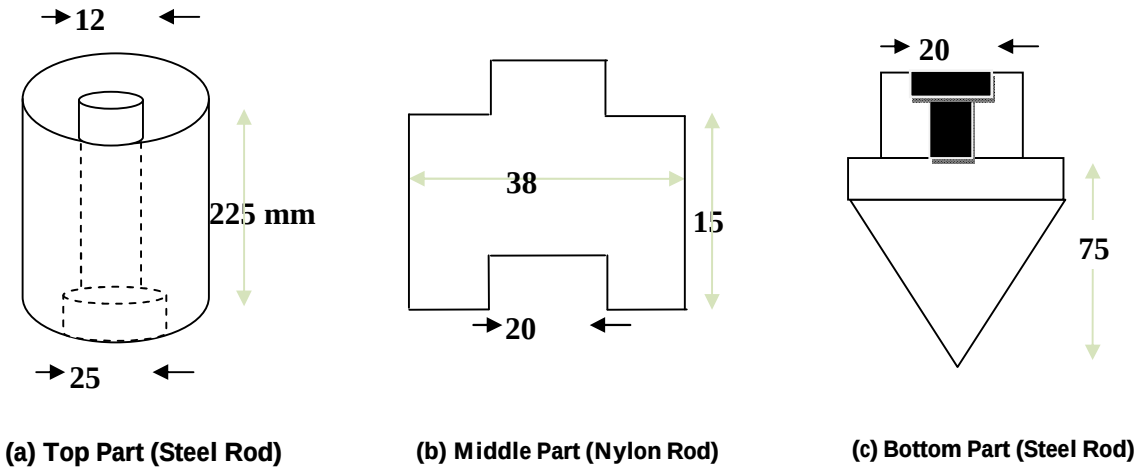


Fig.3. Schematic Diagram of Soil Moisture Sensor

The soil moisture sensor used for the study consisted of two cylindrical electrodes with a nonconductor medium in between. The electrode is round having 20 mm outer diameter with 2 mm thickness and at the tip of sensor made as conical shape for proper penetration into the soil. The complete length of the sensor was 300 mm and weight 1700g (Fig.3).

The soil moisture content was sensed by measuring the resistance between the electrodes, which is a function of soil moisture content. The sensors based on electrical resistance were evaluated for two different soils (black and red). A simple linear regression analysis was done to evaluate the performance of soil moisture sensors. The experimental setup is shown in Fig.4.

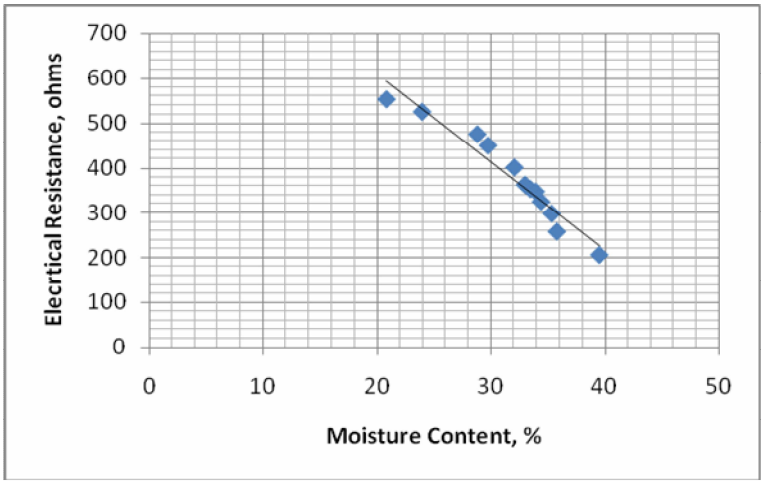
Fig.4. Experimental setup

RESULTS AND DISCUSSION

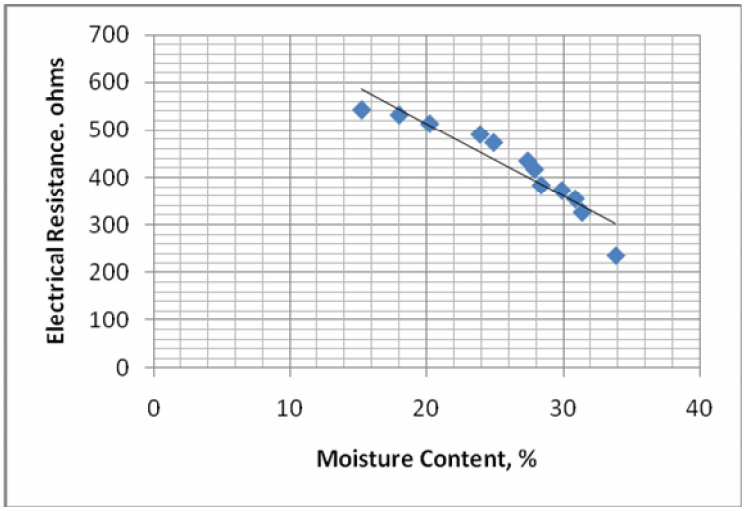
The measurement of electrical resistance for two different soils namely blac and red soil showed that when the polarity across the electrode changed, the resistance reading had considerable variation... The performance curves of sensor for two different soils are shown in Fig.3 to Fig.4.

From the linear regression statistical analysis, it was observed that R2 value for black and red soil as 0.93 and 0.88 respectively. It was observed that nearly a constant trend in the linear relation between electrical resistance and soil moisture content. It was observed that the maximum electrical resistance of 537 ohms at 15 .97 %, moisture for red soil and 461 ohms fro black soil at 20.15 % moisture content. The minimum of 225 ohms at 33.32 % for red soil and 205 ohms at 39.50 % moisture content for black soil were observed, because it is due to soil moisture content and it will conduct the electric current maximum electrical resistance and minimum indicates the soil is under dry and wet condition respectively. The different soils have different physical properties like bulk density, water holding capacity and textural arrangement so it important to calibrate the sensor in each different type major soils where soil moisture will used.

Experience with the on-time loggers has led to some recommendations concerning their improvement. For standardization of the sensor, there is a need to calibrate under different soils with different saline irrigation water both under laboratory and field conditions. There is a future scope for further optimization of eleoctrode geometry to improve the shape of the sensor and the cost of unit. Also system can be further modified as solar power based and wireless control system. The capacity to store the data can modify in order to store large number of reading.



Graph.1 Relation between mean value Electrical resistance and Moisture content of Black Soil during third trail



Graph 2. Relation between mean value Electrical resistance and Moisture content of red Soil during third trail

CONCLUSION

Successfully soil moisture based drip automation system and soil moisture sensor was developed based on electrical conductivity of soil. Different calibration curve were obtained for black and red soil, which is useful for scheduling irrigation, and system was easy to operate and install in the filed. The helps to provide quick information made light in weight, and easy to handle.

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RESEARCH ARTICLE

Selection of the Best Enzyme for the Production of Detergents

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ABSTRACT

The varieties of enzymes such as amylase enzyme, protease enzyme, lipase enzyme etc were most widely employed by the detergent industry. In the present study all these three enzymes and their mixture, pine oil detergents and a normal detergent was tested and analysed separately. The various experiments were conducted to find out the moisture content, pH value and foamability present in enzymes and their mixture. The good results were found in the detergent which is obtained from mixture of enzymes.

Keywords: Enzyme, detergents, moisture content, pH value, foamability.

INTRODUCTION

The study deals with an aggregate planning for a small scale production unit using different enzymes to meet the changing demand of the society. Soaps are the earliest form of detergents, though at present, the term detergent is used for synthetic detergents derived from petroleum products. Due to tremendous strides in petrochemical industries, propylene became available which was polymerized to propylene tetramer that became the major feedstock for the manufacture of synthetic surfactant known as Linear Alkyl Benzene Sulphonate (LABS).

To improve the detergency of cake, certain other components known as builders, fillers , brighteners and enzymes, etc. are also added. The surfactants have molecular structure that have hydrophilic groups on one end and hydrophobic groups on the other end which imparts the special characteristics of soil removal from the surface of the clothes[2,3]. Synthetic detergents are not only used as cleaning materials but also have industrial applications in textiles, pesticide industry as carriers, etc. A detergent is a material used for cleansing agents which have all the properties of soaps, but which actually does not contain any soap. These can be used both in soft and hard water as they give foam even in hard water some of the detergents give foam even in ice cold water[4]. The success of any

cleansing is to supply compounds with hydrophobic and Hydrophilic groups which will also appreciably decrease the surface tension and increase wet ability.

An interesting and unique feature of detergent industry in India is the existence of non-power operated units which do not use any electrical power for the production of detergent powder. But the production technology of detergents have been changed from slower batch processes to quicker continuous processes involving costly equipments, high technique in process control, more skilled personnel and requiring large input. These emphases practical aspects of detergent production with latest development and other special products based on synthetic surfactants manually. This is an attempt to fill the need of those of starting detergent industries in small scale sector and necessarily contains analytical methods for testing and evaluation of raw as well as final products. Our detergent cake gives sparkling whiteness on clothes. These are very efficient in removing dirt, stains and marks created by sweat. Apart from this white fastness quality, our detergent cakes have a very nice aroma, which brings a pleasant feeling among the users and are very gentle on the skin. They contain surfactants, detergent boosters, brighteners and artificial colors and fragrances. Cakes are not for use in your washing machine, but meant for hand-washing.

They are highly concentrated cleaning agents without the toxicity and damaging effects of harsh bleach or other chemicals. The oxygen bleaches are often used for both stain and odor treatment. Other ingredients include citric acid and enzymes that are produced from natural food cultures and actually eat away at the stains/odors with protein basis.

MATERIALS AND METHODS

Washing Soda

Sodium carbonate (also known as washing soda or soda ash), Na_2CO_3 is a sodium salt of carbonic acid. It most commonly occurs as a crystalline heptahydrate, which readily effloresces to form a white cake, the monohydrate.

Acid Slurry

Acid slurry or alkyl benzene sulphonate is prepared by sulphonation of Linear Alkyl Benzene.

China Clay

Calcite is also known as chalk powder or natural calcium carbonate. It comes in bright white colour and it has nodular particle size with strong crystal structure.

Sodium Silicates

Among the different forms of carbonates a available, sodium carbonate (soda ash) has been found to be the most suitable for adding in detergents. Soda ash (Na2 Co3) provides high alkalinity and softens water by precipitation of calcium and Magnesium carbonates, provided that the PH of the solution is over 9 and remains so after the precipitation has occurred

Silicates play a very important role in the formulation of washing materials and particularly those based on synthetic detergents. Sodium silicates are usually made by the fusion of sand, containing high proportions of silica with soda ash in on electric furnace according to the equation.

$Na_2Co_3 + Sio_2 \rightarrow Na_2Sio_3 + Co_2$

Sodium Tripolyphosphate

Builders boost detergent powder, and complex phosphates, such as sodium tripoly phosphates, have been extensively used. These are more than water softeners which sequester water hardening calcium and magnesium-ions. They prevent redeposition on fabrics of soil from the wash water. Among all the builders used in detergent the most popular and important one is the sodium tri poly phosphate.

Carboxymethyl Cellulose

CMC (Carboxy methyl cellulose) is used as an anti- redisposition agent in detergent. When cellulose, which is insoluble in glucose units of cellulose there are three-OH groups each of which can be displaced by CH2COOH group, but this does not happen. Therefore, the number of carboxy methyl cellulose groups added per glucose unit is called the degree of substitution.

Citric Acid

Citric acids are produced by culturing a citric acids-accumulating and hydrocarbon-assimilating strain of yeast of the genus Candida in an aqueous medium containing, as main carbon source, at least one normal paraffin with from 9 to 20 carbon atoms, inclusive, in the molecule, at a specific pH, and recovering accumulated citric acids from the culture broth.

Bleaching Powder

Bleaching agents eliminate stubborn stains and ensure hygiene by killing bacteria through a chemical oxidation performed by a per oxygen generator, usually sodium perborate. The latter is usually active only above 60°C and so, for lower washing temperatures, an activator is added: e.g. tetra acetylethylenediamine (TAED).

Whitening Agent

The present invention relates to compositions comprising specific amphoteric fluorescent whitening agents (FWA) and dye fixing, dye transfer inhibition and/or fabric softening agents, formulations for the treatment of textiles comprising such compositions, and the use of such compositions and/or formulations.

These additives are often use to enhance the appearance of color of fabric and paper, causing a "whitening" effect, making materials look less yellow by increasing the overall amount of blue light reflected.

Pine Oil

It is an Anti-microbial agent for detergent and it kills or inhibits growth of micro-organisms. Hand soap, dishwashing liquid,, kitchen sponges, toothbrushes, toothpaste, mattresses, cutting boards, window cleaner, socks, cycling shorts, chop sticks, pencils, and now facial tissues are all being marketed for their ability to kill germs.

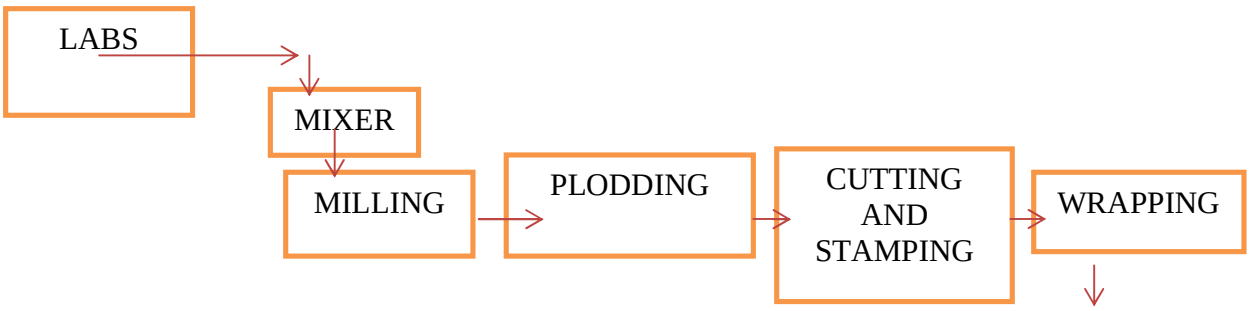
Role of Enzymes

In particular: proteases, lipases and amylases are the enzymes used in case of detergents. Catalyse the degradation of some stains and thus facilitate their elimination.Enzymes have effectively assisted the development and improvement of modern household and industrial detergents. The major classes of detergent enzymes—proteases, lipases, amylases—each provide specific benefits for application in detergent cake laundry and automatic dishwashing. Historically, proteases were first to be used extensively in laundry detergents. In addition to raising the level of cleaning, they have also provided environmental benefits by reducing energy consumption through shorter washing times, lower washing temperatures, and reduced water consumption. Today proteases are joined by lipases and amylases in improving detergent efficacy especially for household laundering at lower temperatures and, in industrial cleaning operations, at lower pH levels. Celluloses contribute to overall fabric care by rejuvenating or maintaining the new appearance of washed garments. Enzymes are produced by fermentation technologies that utilize renewable resources.

Determination of Active Matter Content

When equivalent amounts of cationic and anionic detergents are present in a two- phase mixture of water and chloroform, methylene blue will color the two phases to the same degree. Sodium alkyl benzene sulphonate and sodium lauryl sulphate or any other detergent bromide.

Flow Sheet for the Manufacture of Detergent Cakes



In the process flow sheet, the major raw materials are washing soda; china clay and linear alkyl benzene sulphonates (LABS) which is a acid slurry were in India are fed to the mixer. Later, including surfactants, builders, enzymes are added where each enzyme perform their significant role in for the production of detergent cake. The final process is to the add colour and perfumes and particular or desired shapes are given to detergent cakes.

Procedure for Normal Detergent Cake

In this process, the major raw materials are washing soda (100 grams), china clay (87.5 grams) and LABS (25 grams) which is a acid slurry fed into the mixer. In normal detergent sodium silicate is used were it is added to a mixer. Sodium silicates are corrosion inhibitors for domestic purpose but it hardens the detergent cake fast. The 2.5 grams of colour and 10 ml of perfumes are added due to which particular desired shapes are given to detergent cakes.

Procedure for Detergent Cake Using Pine Oil

The major raw materials are washing soda (100 grams), china clay (87.5 grams) , LABS (25 grams) which is a acid slurry and 5 grams of pine oil are fed to the mixer. Further the 25 grams of sodium silicate, 4 grams of sodium polyphosphate, 4 grams of CMC and 4 grams of citric acid, were added to it. In the final process 2.5 grams of colour and 10 ml of perfume are added to give particular or desired shapes to the detergent cakes.

Procedure for Protease Hydrolyz E Enzymes

In this process the 5 grams of protease hydrolyze enzyme, washing soda (100 grams), china clay (87.5 grams), LABS (25 grams) which is a acid slurry are fed to the mixer. The enzymes are called protease hydrolyzed peptide bonds in proteins. These all raw materials are mixed uniformly. Further the 25 grams of sodium silicate, 4 grams of sodium polyphosphate, 4 grams of CMC, 4 grams of citric acid, 6 grams of pine oil and 3.75 grams of whitening agent a fluorescent were added to it. In the final process 2.5 grams of colour and 10 ml of perfume are added to give particular or desired shapes to the detergent cakes.

Procedure for Lipase Hydrolyze Enzymes

To produce detergent by this enzyme, the 5 grams of lipase hydrolyze enzyme, washing soda (100 grams), china clay (87.5 grams), LABS (25 grams) which is acid slurry are fed to the mixer. The remaining same procedure is followed as mentioned above. These enzymes removes gravy lipsticks, stains etc.

Procedure for Amylase Hydrolyze Enzymes

In this process 5 grams of amylase hydrolyze enzymes were used instead of lipase hydrolyze enzymes and the same procedure is followed as discussed in the procedure of protease hydrolyze enzymes. The enzymes called amylase hydrolyze starch to clean food stains such as chocolates, pasta and potatoes.

Procedures for Mixture of All Types of Enzymes

In this process, the mixture of all variety of enzymes mentioned above of 5 grams are taken and rest procedure is similar. This mixture is the best and beneficial cake in households, commercial purposes etc.

Reagents Preparation

Cationic solution (solution A)

It can be titrated with standard solution of cethyletrimethyle ammonium. Weight 1.5 ± 0.001gm of cethyletrimethyle ammonium bromide into a 250 ml beaker. Add 100 ml of distilled water and stir until dissolved. Transfer quantitatively to a 1 liter volumetric flask and make to volume. Mix thoroughly and standardize against solution B.

Anionic solution (solution B)

Weigh accurate amount of standard alkyl sulphate of known combined SO_3 into a 250 ml beaker. Dissolve in 100-200 gm of warm water. Transfer quantitatively to a 1 liter volumetric flask and make to volume with water at room temperature and mix thoroughly. This is the primary standardization against which solution A is standardized. Solution B is 0.004N

Methylene Blue indicator

Dissolve 0.1 gm of methylene blue in 100 ml of water. Transfer 30 ml of this solution to a 1 liter flask. Add 500 ml of water, 608 ml of concentrated sulphuric acid, and 50gm of sodium phosphate ($NaH_2PO_4H_2O$) and shake until solution is complete, dilute to make Chloroform-Analytic reagent grade.

Procedure

A. weigh accurately a sample of sufficient size to give approximate 0.320 of combined SO_3 into 250 ml of sample size is essential. Use 700 to 800ml of water to transfer quantitatively to a 1 liter volume flask. Warm on stream-bath and shake gently until the sample is dissolved and solution is clean. Cool, dilute to the mark and mix thoroughly.
Note: - titration value v should be as near to 10ml as possible, say, between 8 and 12 ml never outside 5 and 15 ml.

B. Pipette 10 ml of sample solution into a 100 ml glass stopped cylinder (25×300 mm). Add 25±0.5 ml methylene blue solution and 10±0.5ml chloroform (see note). Titrate with solution A to the correct end point, shaking the cylinder carefully after each addition (to avoid emulsion) and maintaining temperature within limits of 20-30°C by emulsion in water bath, if necessary. As the end point is approached, the rate of transfer of color increases and solution shaking after each addition. If the approximate titration volume of A is known 80% of the required titrating solution should be added before shaking since this avoids emulsion formation.Application of vacuum to the titration cylinder may help to break some emulsion, if formed. The end point is reached when both layers have some colour intensity.The end point is very sharp and 0.05ml will cause a distinct change in colour distribution at or near the equivalent point.
Note: The volume of methylene blue solution and chloroform may be changed if found advantageous provided the same volumes are used in standardizing solution A and B.
After the completion of above procedure the sample was tested for percentage moisture, pH and foamability so as to know the quality of the detergent.

RESULTS AND DISCUSSION

The following table shows the values of the moisture content, pH and amount of foam obtained.

Enzymes/ Other items	Weight of sample Taken	Moisture Content	pH value obtained	Foamability Test
Normal Detergent	--	11%	10	90 ml foam
Pine Oil Detergent	5 grams	7.69	9.8	80 ml foam
Protease Enzyme Detergent	5 grams	7.29	9.5	90 ml foam
Lipase Enzyme Detergent	5 grams	7	8.3	70 ml foam
Amylase Enzyme Detergent	5 grams	8.3	7.5	75 ml foam
Mixture of Enzymes Detergent	5 grams	7	7.2	110 ml foam

Results of moisture content

In the above table the results shows that the moisture content is content is high for the detergents produce normally. As there is more moisture in the normal type detergents which results in quick loss of detergent cake after applying. But in case of detergent manufactured by mixture of enzymes the moisture content is low in such cases the loss of the detergent cake after may be ignored.

Results of pH value

The higher value of pH indicates the presence of more OH ions and this type of alkaline detergents causes incrustations due to the formation of trihxlomethanes which are responsible for causing cancer in human beings. This high value of pH was found in normal, pine oil and protease enzyme based detergents shown in the above table. The mixture of enzyme detergent was shown normal pH value which is the better selection for the production of detergents.

Results of foamability test

The results for foamability obtained by Rosemair instrument were shown in the table above. Again the higher amount of foam was found in the detergent which is obtained by mixture of enzymes. Because of the low density in the foams than in oil and grease foam can enter deeper and deeper in to the cloth to remove excessive amount of the dirty contaminants.

CONCLUSION

In production of detergent cake enzymes play a very important role. Each enzyme performs its own functions like removal of soils, salads, and lipsticks, chocolate and so on. Another major important function is removal of oily, greasy substances from the enzymes. The manufacturing of detergent cake that is "MIXTURE OF ALL ENZYMES CAKE" is a better cleaning agent because its pH value, foamability test, Moisture content test and active matter test shows a good result. But the drawback is economic cost is high of enzymes.

Now a day in production of detergent enzymes are not used due to high cost. Subsequently customers will not purchase it and loss of production takes place to respective industries. Implementation is going on enzymes to reduce the cost as well as the acid slurry dodecyl benzene is more costly than linear alkyl benzene sulphonate and not available in INDIA.

These products have good demand in domestic as well as in International market. So there is a very good scope for new entrepreneurs to venture into this field. This project is very useful for entrepreneurs, technocrats and for those who want to diversify in to this field.

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RESEARCH ARTICLE

Solid Waste Management in Tiruchirappalli, TamilNadu,India

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ABSTRACT

This study is made to ascertain "Solid Waste Management in Tiruchirappalli, Tamil Nadu". This study is purely based on secondary data. There are 10 Municipal Corporations in Tamil Nadu among these Tiruchirappalli Municipal Corporation has been selected as the study area. Data regarding Municipal solid waste collection, segregation, transportation and disposal has been collected from the Tiruchirapplli Corporation. This study has three objectives. The second objective is to quantify the solid waste generated. Tiruchirappalli Municipal Corporation area generates 381 MT of solid waste per day. The provisional population of Tiruchirappalli City is 746,173 as per census 2001.The second objective is to randomly characterize the waste and method of disposal. Result revealed that in the study areas the highest percentage of Bio-degradable material is about 75%. The third objective is to analysis the current status and problems of Solid Waste Management (SWM) in the study areas. Shortage of skilled manpower for collection of waste, lack of awareness on implication of solid waste collection among people of the study area and inadequacy of vehicles for waste collection. This study reveals that, there is no segregation of waste at source in study areas.

Keywords: Urban local Bodies (ULB), Municipal Solid Waste (MSW), Solid waste management (SWM), Waste generation.

INTRODUCTION

The waste generated in solid state as a result of various human activities and normally discarded as useless or unwanted is termed as solid waste. Solid waste consists of highly heterogeneous mass of discarded materials from residential, commercial, industrial, agriculture and mining activities. It is unique because it can move from land to water or air [1]. Municipal Solid Waste (MSW) Management is the responsibility of Urban Local Bodies (ULBs) in

India. It is the responsibility of the ULBs to collect, transport and process/dispose the collected waste in an environmentally responsible manner [2]. Improper solid waste management leads to substantial negative environmental impacts (for example, pollution of air, soil and water, and generation of greenhouse gases from landfills), and health and safety problems (such as diseases spread by insects and rodents attracted by garbage heaps, and diseases associated with different forms of pollution). Municipal (or local) authorities charged with responsibility of providing municipal solid waste management services (together with other municipal services) have found it increasingly difficult to play this role. The difficulty has been aggravated by lack of effective legislation, inadequate funds and services, and inability of municipal authorities to provide the services cost-efficiently.

The public sector in many countries is unable to deliver services effectively, regulation of the private sector is limited and illegal dumping of domestic and industrial waste is a common practice. In general, solid waste management is given a very low priority in these countries.The population is the main problem to increasing the quantity of waste generation. The waste quantities are estimated to increase from 46 million in 2001 to 65 million in 2010 [3]. Urbanization and civilization also plays a vital role in solid waste generation. The increase in population is directly proportional to the quantity of waste generated. Reduction of solid waste generation per capita is intricate in the present urban scenario. But proper management practices and awareness root level can reduce the quantity of solid waste that has to be disposed off. Solid waste disposal has been an environmental threat to both urban and rural areas. It is seen that there is increased awareness in urban areas towards SWM than in rural areas, but a lot more activities with regard to Reduce, Reuse and Recycle have put into day to day practice.

India has a mixed demographic profile with about 307 million (about 30%) of the total population living in urban area and by 2011 it will be 395 million (RMC, 2004). The present system of SWM in most of the urban areas are highly problem due to limited finance, inadequate services and coupled with public apathy towards the same [4]. Indian cities generate on an average of 300-400 gms/capita/day solid waste and of which only 60-80% of the waste is collected on daily basis and rest of the waste is left to decay on the street, roads, drains etc, which affects vector transmitting diseases [5].

Generally solid waste contains a small percentage of recyclable material and the remaining is made up of compostable and inert materials like ash and road dust. These materials will be collected through trucks from various zones and transported to the disposal land filling sites. Rag pickers play a vital role in SWM. They collect recyclable waste from the streets, bins and disposal sites like plastic, metal, glass and rubber etc., which they sell to recycle vendors for their livelihood. From last few years, the solid waste collection service is extended to the societies, apartments and private bungalows but not extended fully to slums and chaws on a regular basis

MATERIALS AND METHODS

The necessary data has been collected from Tiruchirappalli Corporation. The collected data has been classified and tabulated. The diagrammatical and graphical representation is also used to illustrate the studied phenomena. This study mainly deals with the present solid waste management status in Tiruchirappalli city.

Physical Features

Tiruchirappalli is one of the most famous temple town of Tamilnadu, also called Tiruchi, this is the fourth largest town of Tamilnadu. Tiruchirappalli falls under Cauvery river basin. The topology of Trichy is flat. It lies at an altitude of 78 m above sea level. The area of the city is 146.90 sq.kms while the urban agglomeration is spread over an area of 180 sq.kms. There is also a plan to increase the area of the corporation to 223 sq.kms which would result in an increase in population of 0.9 million (2001). The river Kaveri (also called Cauvery) and the river Coleroon (also called

Kollidam) flows through Trichy, the latter forms the northern boundary of the city. The river Cauvery flows along WNW-SSE direction through the city. The Cauvry River is the most important river in the district and the tributaries of Cauvery, i.e. Coleroon River, Koraiyar River, Ariyar, Malattur channel, Uyyakondan channel also drain in Triuchirappalli. The variation of temperature throughout the year exhibits hot and dry climate with high temperature and low degree of humidity [6].
Summer temperature : 41.10°C (maximum) 36.40°C (minimum)
Winter temperature : 21.31°C (maximum) 18.60°C (minimum) [6]

City Management and Governance

Tiruchirappalli Municipality was constituted on 08.07.1866. The Municipality was upgraded as City Municipal Corporation with effect from 01.06.1994 by adding the adjacent Municipalities, Town Panchayats and Village Panchayats. [6]

The administration of the City Municipal Corporation is carried out according to the Tiruchirappalli City Municipal Corporation Act 1994. The City is subdivided into 4 Administrative Zones and 60 Wards for effective administration. The same has been given in the Table 1. he Tiruchirappalli City Municipal Corporation Council was constituted with a Mayor and 60 Ward Councilors (inclusive of Mayor) representing each ward. The Worshipful Mayor conducts council meetings, at least once in a month.[6]

The departments that facilitate and provide urban basic services in the City Corporation are as follow:

- Administrative Department
- Engineering Department
- Public Health Department and
- Town Planning Department

Demography and Density

The administrative jurisdiction of the TCC is spread over 146.90 sq.km. The provisional population of Tiruchirappalli City is 746,173 as per census 2001. The latest census breakup for 2001 covering the erstwhile Municipalities, Town Panchayat and Village Panchayats is not readily available; therefore the overall census figure for the city alone is indicated. Accordingly, the population has increased from 669,452 in 1991 to 746,137 in 2001 with a growth rate of 11.45% between 1991 and 2001. The population of City increased from 323,693 in 1951 to 746,137 between 1951-1961 and 27.81% during 1961-71; due to the accelerated industrial growth that took place in and around the Tiruchirappalli area. After the formation of the City Corporation the rate of population growth has declined from 20.99% during 1971-81 to 15.67% during 1981-91. Subsequently, rate of growth has declined to 11.45% during 1991-2001. This shows that the natural growth is prominent than migration factors, as migration appears restricted.

Waste Generation from Tiruchirappalli Corporation (Tire 1)

Rapid urbanization, increasing commercial and industrial activities and changing life styles in Tiruchirappalli leads to a steady increase in the generation of solid waste. Tiruchirappalli City Corporation (TCC) is responsible for the collection, transportation and disposal of all solid waste generated in the city, except for untreated bio-medical waste and hazardous industrial waste, which is taken care of by the respective generators. TCC has been divided into 4 zones with 15 wards in each zone. Following are the list of zones and population break has been given in the table 2:

- K.Abishekapuram
- Ariyamangalam
- Golden Rock

TCC generates approximately 381 M.T of waste per day. TCC reportedly collects and transports approximately 360 M.T (94% of total waste generated). Table 3 provides the zone wise details on waste generated in a day. The primary sources of solid waste in Tiruchirappalli city are local households, markets, commercial establishments, hotels, restaurants and hospitals. The major source of waste generation has been provided in the table 4 and break-up of the same refer table 5 & Figure 1. Apart from wastes generated from these areas, wastes are also collected from drains in the form of wet silts.

It is observed from table 6 and Figure 2 that one third of wastes generated in TCC are from residential areas and it is followed by markets and commercial establishments with each sector accounting for approximately 22% of the total waste generated in a day. Similarly as per the table 7 and Figure 3 the per-capita waste generation in Ariyamangalam zone is also high with 593 g/capita/day when compared with the other zones and it is also more than the average per capita generation of the city. Waste Characterization and Composition of solid waste in Tiruchirappalli city is given in the Table 8. Analysis of the physical characteristics of municipal solid waste as per information provided by ULB is furnished in Table 9.

Waste Collection

Existing solid waste collection system mainly comprises of collection from the door step by means of hand-carts/cycle-rickshaw and collection through community bins/containers. Collection of solid waste is now being performed in an unorganized way just to keep the garbage away from the city area. At present Door-to-Door collection is introduced in some wards. But source segregation is not performed in all the zones. Moreover, the Primary Collection of Garbage at the doorsteps in some of the streets in selected wards in Tillai Nagar is being done with the help of NGOs.

Presently, the collection of waste in the city is managed by 1,679 sanitary workers supervised by 54 sanitary supervisors. In addition 17 Sanitary Inspectors are also involved in monitoring the collection and disposal of solid wastes. Sweepers and sanitary workers have been engaged for sweeping the solid wastes from the streets. They accumulate the collected waste into small heaps and subsequently the same is either loaded manually or mechanically on to the solid waste transportation vehicles for onward transportation to the disposal site. Apart from sanitary workers Self Help Groups (SHGs) are also involved in the street sweeping and toilet maintenance. The present collection and transportation system involves multiple handling of solid waste.

The basic mode of Waste Collection is through community storage/collection points. Generally in any major City Corporation 3 types of Waste Collection Points exist, namely,

- Major Collection Points
- Minor Collection Points and
- Sub-centers

Major collection points:

In Major collection points large quantity of wastes arrives mainly from the following;

- Markets (Vegetable Market, Fruit Market etc.,)
- Tourist Spots (Temple, Bus Stand etc.,)
- Commercial Establishments & Domestic Sector (Hotels, Residential Area, Street etc.,)

Tiruchirappalli City Corporation has 4 major collection points where the waste arrival varies from as low as 5 tpd to as high as 50 tpd depending on the types of wards the collection points encompass. Waste uploading at Gandhi Market shown in the Figure 4. Details of major collection points are given in Table 10.

Minor Collection Points

In Minor Collection Point, Wastes from roadside bins are stored temporarily prior to transportation to the disposal site. Push Carts and bins made of Concrete/Masonry/Steel are used for collection of waste in Minor Collection Points. Wastes collected are then transported through Dumper Placer, Auto-Rickshaws and Lorries to the Dump yard via Sub-centers. Type & no. of bins used for waste collection in TCC are shown in Table 11.

Sub – Center

It is a Temporary Transfer Station and acts sometimes as a sub area for waste storage to prevent overflow of wastes in Major Collection Points. TCC has given in the Table 12.Wastes are collected from these Sub-centers only on weekends (once-a-week) and dumped at Ariyamangalam Dump Yard. Tipper Lorries are employed to transfer wastes from the Sub-center to Dump Yard.

Waste Collection Mechanism

The Waste Collection Mechanism employed by the TCC is detailed below: the chart showing the waste collection mechanism has given in Figure 5.

- Waste is collected twice a day in all wards during the time 6:30 – 10:30 hrs and 14:30 – 17:30 hrs.
- In narrow streets and densely populated localities where waste dumping in a bin is difficult, bullock carts and auto rickshaws are employed for waste collection on door-to-door basis. Refer the Figure 6 showing the door-to-door collection.

Waste Transportation

Transportation of Wastes collected forms the heart of the Waste Management Programme. In India no single mode of transportation has proved effective and economical due to various constraints encountered like narrow street, dense population, unorganized dumping, poor quality waste segregation etc.

Hence it is quite essential that various types of vehicles be used for effective waste transportation. The type of vehicles used is TCC range from primitive hand carts to most modern mechanized dumper placer and compactors. Table 13 shows types of vehicles used in TCC for secondary transportation of MSW. Smaller capacity vehicles are utilized for waste transportation from collection points to Sub centers. Bigger vehicles (Tractor, Tipper Lorry etc.) transport the wastes from Sub-centers to Ariyamanga+lam Dumping Yard (Figure 7: Tipper carrying the waste to dumping yard).

Privatization

Pay and use toilets and bathrooms have been privatized in Central Bus stand, Chatiram Bus stand & Gandhi Market and have been leased to a NGO called "AWAKES". The same NGO is engaged in the task of sweeping and collection of garbage in the above locations and surrounding roads.

"KALAI EXNORA" the NGO (otherwise called kalki chartable trust) also involving the sweeping and collection of refuse in the ward no. 17, 18 & 31. This NGO collecting 10 Rs from each house hold and shops.

Trichy District "Exnora" Door to door collection of garbage in residential areas through various Civic EXNORAs functioning in the City/Municipalities/Panchayats.

Waste Processing and Disposal

Tiruchirappalli City Corporation owns 47.70 acres of land in **Ariyamangalam** village situated along Trichy – Thanjavur Main road at a distance of 10-km away from the city centre and these lands are used for dumping the solid waste. This site is delimited with a compound wall all around. TCC constructed a water tank inside the Compost Yard for firefighting during the summer season. The image of the existing disposal sites is furnished in Figure 8. The process of collection and transportation system is coordinated through wireless communication devices. The TCC office maintains the records on amount of waste collected by each transportation unit on daily basis.

Waste to Bio-manure

Vegetable waste from the Ghandhi Market is dumped in the left corner of the compost yard. This vegetable waste is used for the purpose of producing the Bio-manure with the permission of TCC Commissioner.

Proposed Landfill Site

Tiruchirappalli City Corporation also owns another 570 acres at **Panjapur** village (including STP) and this site is proposed to be used as a Compost Yard and landfill site in the future. The proposed Landfill site is about 8 km away from the town.

CONCLUSION

Conclusion can be made that the solid waste generation depends upon the area density and population. In practice the control of waste generation is very difficult. Reduction of solid waste generation per capita is intricate in the present urban scenario. But proper management practices and awareness root level can reduce the quantity of solid waste that has to be disposed off. The TCC generates about 381 MT waste per day and collects 94% of it. But before the disposal mechanism, the 3R technology (Reduse, Reuse and Recycle) has to be implemented.Simple practices like source segregation, quantification, delineating proper collection centers and coordinated transportation will make SWM a successful endeavor. In order to achieve this public awareness in both urban and rural areas is very essential.

Table 1: Administrative Zones of Tiruchirappalli City

Zone	Area		Population	
	km²	%	Lakhs	%
K Abishekapuram	63.67	43.3	1.9716	23.94
Ariyamangalam	12.79	8.7	1.9800	23.95
Golden Rock	43.41	29.6	1.8593	22.50
Srirangam	27.03	18.4	2.4497	29.61
Total	**146.90**	**100**	**8.2606**	**100.00**

Table 2: Zone Details

Administrative Zone	Representative wards
Srirangam	1,2,3,4,5,6,8,9,10,11,12,13, 16,17,18
Ariyamangalam	7,14,15,19,20,21,22,23,24,25,26,27,28,29,33
Golden Rock	30,31,32,34,35,36,37,38,39,42,43,44,46,47,48
K. Abhishekapuram	40,41,45,49,50,51,52,53,54,55,56,57,58,59,60

Table 3: Waste Generation Details

Parameter	Abishekapuram	Ariyamangalam	Golden Rock	Srirangam	Total
No of Wards	15	15	15	15	**60**
Waste Generation (tpd)	99.4	117.36	79.43	85.13	**381.62**
% to total	26.10	30.80	20.80	22.30	**100.00**
Per-capitaWaste Generation (gm / d)	502	593	427	348	**461 (Avg.)**

Source: ULB

Table 4: Commercial Establishments

Commercial Establishment	No.
Hotels	123
Markets	9
Marriage Halls	162
Parks	24
Theatres	34

TABLE 5: WASTE GENERATION SOURCES - BREAK UP

Sources	Abishekapuram		Ariyamangalam		Golden Rock		Srirangam		Total	
	Tpd	%	Tpd	%	Tpd	%	Tpd	%	Tpd	%
Bus Stands & Temples	4.97	5	11.74	10	19.86	25	21.28	25	57.85	15.06
Commercial Establishments	14.91	15	17.6	15	31.77	40	17.03	20	81.31	21.80
Markets	19.68	20	46.94	40	7.94	10	12.77	15	87.53	22.80
Residential Area	59.84	60	41.08	35	19.86	25	34.03	40	154.93	40.34
Total	**99.4**	**100**	**117.36**	**100**	**79.43**	**100**	**85.11**	**100**	**381.62**	**100**

Source: Tiruchirappalli City Corporation

Table 6: Source-wise Reconciliation

No	Waste Origin	Tpd
1	Bus Stands & Temples	59.32
2	Commercial Establishments	81.26
3	Markets	88.24
4	Residential	152.50
Total		**381.32**

Table 7: Ward wise Per-capita Waste Generation

Ward No	Population	Average Waste Generation tons / d	Per Capita Waste Generation (gm/ d)	*Ward No*	Population	Average Waste Generation tons/ d	Per-Capita Waste Generation (gm/ d)
1	15 080	5.75	381	***32***	21 710	**Railway Contract Area**	
2	14 530	5.0	344	***33***	15 640	4.18	267
3	13 490	7.25	537	***34***	15 100	6.45	427
4	14 940	6.75	452	***35***	15 540	2.95	189
5	14 940	10.75	720	***36***	11 490	4.00	348
6	15 300	4.375	286	***37***	13 040	1.50	115
7	12 520	4.07	325	***38***	15 600	8.00	512
8	14 430	4.0	277	***39***	14 620	2.75	188
9	25 380	1.375	54	***40***	12 850	3.25	253
10	27 620	8.375	303	***41***	12 020	5.25	437
11	22 210	4.75	214	***42***	12 580	2.50	198
12	13 490	4.563	338	***43***	9 370	4.75	506
13	13 940	4.875	350	***44***	14 360	20.65	1438
14	13 870	7.51	541	***45***	15 100	10.9	720
15	13 460	10.05	747	***46***	12 510	9.00	719
16	12 470	6.188	496	***47***	15 050	6.50	431
17	12 090	8.063	667	***48***	13 860	5.38	387
18	14 880	3.063	206	***49***	14 730	5.45	369
19	11 780	7.87	668	***50***	15 910	9.6	617
20	11 400	7.71	676	***51***	15 546	4.5	290
21	12 280	4.41	359	***52***	13 960	8.3	595
22	13 660	7.08	518	***53***	6 900	3	434
23	12 520	2.47	197	***54***	11 410	5.25	460
24	12 240	3.77	308	***55***	14 660	2.65	179
25	14 250	4.06	284	***56***	12 700	16.65	1199
26	12 520	5.98	477	***57***	13 860	5.5	397
27	15 600	4.30	276	***58***	13 340	6.5	487
28	13 110	3.59	274	***59***	11 980	5.6	467
29	13 150	2.78	211	***60***	12 950	7	541
30	11 600	2.50	215	***Gandhi Market***		37.53	-
31	11 210	2.50	223	***Total***	**826636**	**381.32**	**461 (Avg)**

TABLE 8: EXISTING SOLID WASTE MANAGEMENT SYSTEM – TIRUCHIRAPPALLI CITY CORPORATION

Particulars	Quantity
1. Estimated quantity of waste generated / day	381 MT
2. Composition of solid waste (MT per day)	
i. Domestic waste	280.50 MT
ii Commercial waste	65.00 MT
iii Industrial waste	4.50 MT
iv Market waste	25.00 MT
v Bio-medical waste	Dedicated System
vi Street sweepings	5.00 MT
vii Drain cleanings	1.00 MT
viii Construction material / debris	20.00 MT

Table 9: Physical Characteristics of Solid Waste in Tiruchirappalli

No	Components	Weight (kg)	% by Weight
	Organic		
1	Banana Leaves & Stem	108,673	28.57
2	Food & Vegetable Waste	73,070	19.21
3	Organic Silt	51,016	13.41
4	Leaves and Branches & Wood	49,109	12.91
5	Coconut Leaves Waste	4,564	1.2
6	Papers	5,886	1.55
7	Flower Waste	1,145	0.3
8	Coconut Husk	1,498	0.39
9	Beedi Leaves Waste	3,504	0.92
10	Gunny Bags	2,185	0.57
11	Oil Cake	186	0.05
12	Bagasse	283	0.07
13	Cow Dung	389	0.1
14	Mat Waste	254	0.06
15	Coconut Shell	424	0.11
16	Paddy Straw	113	0.03
17	Beef Waste	1,040	0.27
18	Coir Waste	45	0.01
Sub Total		**303,384**	**79.73**
	Inorganic		
19	Inorganic Silt	60,321	15.86
20	Plastics	10,949	2.88
21	Textile Waste	2,392	0.63
22	Rubbers	1,975	0.53
23	Rexins	774	0.2
24	Metals	157	0.04
25	Glass	289	0.08
26	Thermocole	196	0.05
Sub Total		**77,053**	**20.27**
Grand Total		**380,437**	**100**

TABLE 10: MAJOR WASTE COLLECTION POINTS AND WASTE ARRIVAL QUANTITY

No	Ward no	Location	Waste arrival tons / d
1	-	Gandhi Market	30 – 40
2	12	Marakkadai	10 –15
3	44	Central Bus Stand	5 – 15
4	9	Chathram Bus Stand	5 – 7
Total			**50 – 77**

Table 11: Type & No of Bins Used

Type	Number	%
Dumper Bins	215	7.07
Concrete	1,436	47.25
Masonry	228	7.50
Push Carts	1,160	38.17
Total	3,039	100.00

TABLE 12: SUB-CENTERS' DETAILS

SL No	Name of Place	Location		Extent (sq. m.)
		Ward	Zone	
1	Anna Nagar	50	K-Abishekapuram Zone	4,125.52
2	Kasivilingi	58	K-Abishekapuram Zone	6,173.36
3	Ambedkar Nagar	4	Srirangam Zone	6,825.00
4	Moolaithoppu	1	Srirangam Zone	7,365.00
5	Ponmalai patti	36	Golden Rock Zone	--

SOURCE: TIRUCHIRAPPALLI CITY CORPORATION

TABLE 13: VEHICLES USED FOR SECONDARY TRANSPORTATION OF MSW

S. NO	TYPE OF THE VEHICLE	NOS.	WASTE CARRYING CAPACITY (TONS)	NO OF TRIPS / DAY	TOTAL VEHICLE CAPACITY (MT)	ACTUAL CARRYING CAPACITY (MT)
1	Lorry	6	5.0	3	90	45
2	Tractor(2) Trailer (4)	2	2.0	3	12	6
3	Mini-Lorry	8	2.5	3	60	30
4	Dumper Placer	10	2.0	10	60	30
5	Tipper	22	4.0	3	264	132
6	Auto	16	0.5	5	40	20
7	Compactor	1	7.0	3	21	10.5
8	Tractor Dozer Cum Backhoe Loader	1	-	-	-	-
9	Bulldozer	1	-	-	-	-
10	Excavator Cum Loader	1	-	-	-	-
Total		**68**	**-**	**-**	**547**	**273.5**

Source: Tiruchirappalli City Corporation

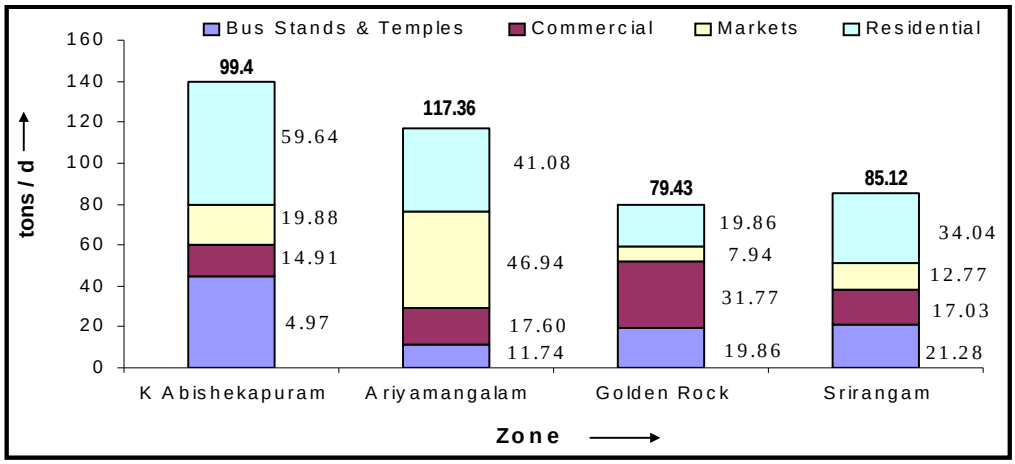

Fig 1: Waste Generation Sources – Break up

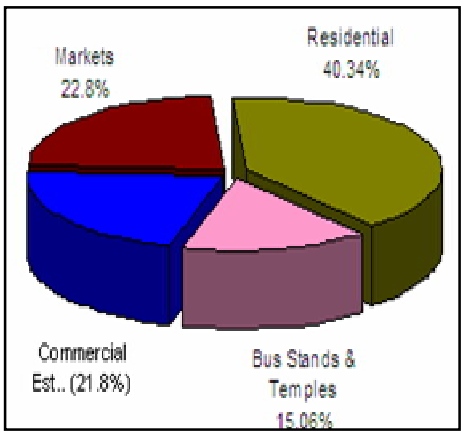

Fig 2: Per-Capita Waste Generation

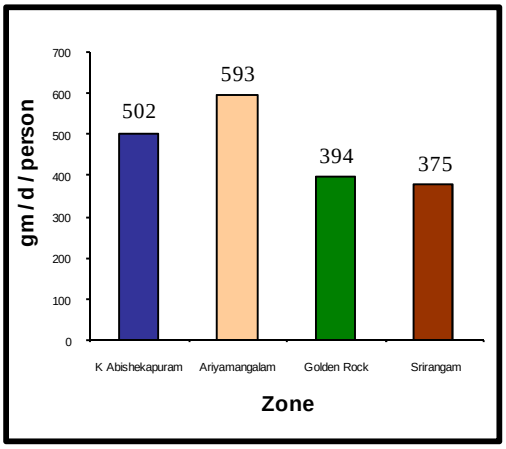

Fig 3: Source wise Break-up

Figure 4: Waste uploading at Gandhi Market (Major collection point)

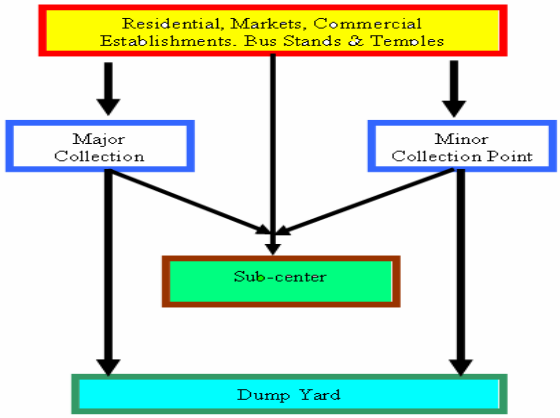

Figure 5: Waste collection Mechanism

Figure 6: Door- to -door waste collection Sevasangam (Ponmalai Zone Ward no: 44) TCC

Figure 7: Waste transportation to dumping yard at (near by Paalpannai) TCC

Figure 8: Dumping yard, Ariyamangalam (TCC)

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RESEARCH ARTICLE

Species Diversity of Avenue Trees in the Coimbatore City, TamilNadu, South India

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ABSTRACT

The purposes of avenue trees in the cities are mainly because of ornamental, shade, aesthetic and medicinally valuable. Therefore species diversity and their proportion availability in a given area are highly needed for the better management of urban greening. The study was carried out in Coimbatore City with the following objectives, to quantity the occurrence, relative abundance and regeneration and recruitment classes of various plant species. Tree species occurrence, frequency was collected by foot survey. Tailoring tape was used to measure GBH and BA of each tree species. Tree species and their occurrence map were developed by using map info 6.0 Software Program. A total of 24 tree species comprising of 456 individuals were recorded from the study area. The family Leguminaceae attributed more number of individuals followed by Meliaceae and Bignoniaceae. The result of habitat preference of the tree species revealed that the majority of them were exotic (55.92%). Three dominant tree species recorded in the study area were *Peltophorum inermae* (n=97), *Poinciana regia* (n=94) and *Enterolobium saman* (n=69). *Thespesia populnea* attributed highest percentage in recruitment class (33.33%). In the regeneration class *Enterolobium saman* and *Bauhinia purpurea* (15%) scored more numbers in total standing trees. The result of Girth at Breast Height at various class interval of trees revealed that the GBH of 51-100cm trees (n=102) were more represented in the over all vegetation cover. The GBH of 400-600 cm trees were represented comparatively very low numbers. Seedlings were represented only in the middle part of the city. The encounter rate revealed that the tree species such as *Peltophorum inermae* (4.8 trees/km) scored highest value followed by *Poinciana regia* (4.7 trees/km) and *Enterolobium saman* (3.4 trees/km).

Key words: Urban greening, Species diversity, Dominant tree species, Avenue trees
Abbreviations : GBH - Girth at Breast Height, BA-Basal Area

INTRODUCTION

India is one of the world's twelve-mega diversity nation has almost all climatic conditions (George and Varghese, 1993). India has a typical woodland climate which is favorable to the growth of vegetation. The texture of soil, availability of water resources, the amount of rainfall, riverbanks and the temperature play an important role in the growth of variety of trees in an area (Randhawa, 1965). The total geographical area in the country is around 3287263 Sq. Km. In which the forest covered 678333 Sq. Km and the area of cultivable non forest is 2188688 Sq. Km (http://www.fsi org.net/ fsi 2003/ states/index.asp.)

The vastness of the plant genetic resource available in India may be gauged from the fact that 238 families comprising of 16,000 species, nearly 16 percent of flowering plants of 425 families are known to exist in India. Overall the plant wealth of the country was assessed about 45,000 species (Joshi, 1993).The geographical status of the state of Tamil Nadu consisting of 130057 Sq.Km of which 22699 Sq.Km was under the forests, which accumulated for 17.45% of the total geographical area of the state. The cultivable non forest area (CNFA) was 98851 Sq.Km and the total tree cover was 4991 sq km (http://www.fsi org.net/fsi 2003/states/index .asp.).

Vegetation plays an aesthetic link between man and environment. Plants are enhancing the quality of environment by influencing the life supporting systems (Shukla and Chandel, 1972). Simpson and Pherson (1996) stated that the trees are considered as nature's air conditioners and they reduce the annual energy use for cooling by 10% to 50 % and electricity use by 23% in California. The reason behind for planting avenue trees in cities and along the roadsides because we believe that no road or street is dressed or furnished until it has been planted to furnish shade, frame vistas of outlying beauty and prevent natural calamities. Plants are the ameliorators of summer air temperature through evaporation. Vegetation playing an effective role in the urban environment, supporting many fundamentals like hydrological cycles, nutrient cycles, and gas balance. So the above all play an essential role in function as a whole (Ramakrishna and George, 1994). In recent days most of the vegetation covers were severely affected by various human induced activities. This resulted increase of CO_2 accumulation in the atmosphere that ultimately increasing global warming across the world. This kind of changes severely altered many plant species distribution, composition, genetic structure and even extinction of many useful plants (Chandra and Joshi, 2002). Dattraja (1992) also stated that the species diversity is an important criterion for any vegetation study.

The purposes of avenue trees in the cities are mainly because of ornamental, shade, aesthetic and medicinally valuable. Homogenous stand of the plants never fulfill the above mentioned functions in a given area. Species diverseness not only render the above needs as well as to withstand for long run. Therefore species diversity and their proportion availability in a given area are highly needed for the better management of urban greening. Species diversity is the number and variety of species found in a given area in a region (Sharma, 1975). Patwardhan (2001) studied the avenue tree species diversity with special reference to their habitat, nomenclature, locality and benefits of the each tree species. Similar type of study was attempted by Sudha and Ravindharnath (2000) at Bangalore and Randhawa (1956) in Chandigar.

Although vast bio-geographical areas are effectively preserved by various levels of policies and intensive research studies, the micro environments such as avenue trees, social forestry were less attempted for research. But these micro environments role in high carbon polluted cities are remarkable. Therefore, considering the lacunae this present study was attempted to exhibit the need of establishing avenue trees in the South India's Industrial City where actually the avenue trees are highly warranted to absorb the pollution.

Objectives

- To quantity the occurrence of various plant species
- To find out the relative abundance of tree species
- To know the regeneration and recruitment classes
- To suggest management recommendations for urban greening.

Study Area

Coimbatore is an inland district of the southern part of Indian Peninsula, elongates from the north to south between 76° 39' and 77°56' of east, latitude 10°12' and 11°57' of the north latitude. The extent of the area of the city is around 6,024sq.miles. The sample area taken for the study was in Rathna Sabapathy puram (R.S. Puram) of Coimbatore City, which is one of the developed and the developing areas of the city. The area has many shopping complexes, residential areas, hospitals etc. The soil of the district is chiefly red sand and gravel with moderate area of red loam and black loam or sometimes black clay.During the study period the temperature was maximum (30.7°C) in the month of September and minimum (17.2°C) in the month of December. The rainfall was recorded maximum in the month of October (235.1mm).

METHODS

The short term study was undertaken in R.S.puram, which is a small portion of the Coimbatore City selected as a sample area. The starting point as well as the and point of the streets were marked using the GPS (Global Position System) in order to derive the length of the streets .Like wise the geo-coordinates position of each tree was marked.The botanical name of the tree species, the family and the local name was recorded by referring Mathew (1956) and Gamble (1957).

Total number of individuals of each tree species was noted. Of which three dominant tree species were recorded based on their occurrence and frequency. Habitat preference, status, trend of each tree species were categorized and tabulated.

The total 456 species were classified into 5 types. Namely.Forest, Plantations, Habitations, Exotic, Forest/Exotic

The following simple arithmetic calculations were attempted to derive Relative proportion, percentage of regeneration and Recruitment and class and encounter rate of species occurrence in the study area.

$$\text{Relative proportion} = \frac{\text{Total number of individuals of each species}}{\text{Total number of trees in the study area}} \times 100$$

Similarly the occurrence and total number of regeneration and recruitment types of trees were noted.

Regeneration - Saplings less then 1m height

Recruitment - Saplings less than 10 cm GBH

The regeneration and recruitment percentage was calculated as follows

$$\text{Regeneration percentage} = \frac{\text{The frequency of each species}}{\text{Total number of regenerated trees}} \times 100$$

$$\text{Recruitment percentage} = \frac{\text{The frequency of each species}}{\text{Total number of recruited trees}} \times 100$$

For each trees their Girth at Breast Height (GBH) (Dattaraja, 1992) and the Basal Area (BA) were measured by using a tape (Agni *et al.,* 2000). The total numbers of trees were arranged in the ascending order of GBH and BA classes. And the total number of individuals occurred in each GBH class and BA classes were taken and their percentage occurrence for each class was calculated from the total numbers.

$$\text{Class percentage} = \frac{\text{Total number of trees in each class}}{\text{Total number of trees in the study area}} \times 100$$

A graph was constructed by using the tree species on the x-axis and encounter rate/km on the y-axis and also GBH map was developed to exhibit the occurrence of big trees in the study area.

Encounter rate of urban tree species per kilometer was estimated as follows

$$\text{Encounter rate/km} = \frac{\text{Number of individuals}}{\text{Total length Surveyed (Km)}}$$

RESULTS

A total of 24 tree species comprising of 456 individuals were recorded from the study area over a period of three months. The family Leguminosae attributed more number of individuals followed by Meliaceae and Bignoniaceae. Among the tree species *Peltophorum inermae* (n=97), *Poinciana regia* (n=94) and *Enterolobium saman* (n=69), ranked first three positions. On the other hand, species such as *Chorisia speciosa* (n=1), *Eugenia jambolana* (n=10), *Plumeria alba* (n=1*) Santalam album* (n=1*) Pisonia morindifolia* (n=2) and *Tectona grandis (*n=3) positioned last ranks with their availability being lowest. Species such as *Azadiracta indica* (n=35) C*assia fistula* (n=25) and *Millingtonia hortensis* (n=22) have scored average ranks in the overall vegetation cover (Table 1).

The result of habitat preference of the tree species in the study area revealed that the majority of them were exotic (n=255) which were introduced from both western and eastern countries. One fourth of them were being cultivated near human habitations and a few of them were plantation species (Table 2).

Exotic tree species contributed relatively high proportion (55.92%) out of the overall composition of the trees followed by Forest/Exotic (15.79%), human habitation species (14.04%) and forest species (13.16%). On the contrary, plantation species (1.10%) showed the lowest proportion availability in the study area (Table 2).

Availability of the regeneration and recruitment classes of the various tree species out of the total numbers were varied among the species. *Thespesia populnea* attributed highest percentage in recruitment class (33.33%) in the regeneration class *Enterolobium saman* and *Bauhinia purpurea* scored equally (15%). out of the total standing trees. Recruitment numbers were same in the species such as *Pongamia pinnata,Cassia fistula*, *Terminalia catapa*, *Poinciana regia* and *Dombeya sp.* (8.33%) . Similarly regeneration class was also same in some of the species of *Cassia fistula, Azadiracta indica* and T*erminalia catapa* (2.5%) in the total trees category (Table 3)

In total 456 tree individuals Girth at Breast height (GBH) were classified into 50cm interval to know the availability of different age groups of trees in the study area. The result revealed that the GBH of 51-100 cm trees (n=102) represented the most in the over all vegetation cover, followed by 151-200 cm (n=90) and 201-250 cm (n=63). It is interesting to note that, the GBH between 400 and 600 trees represented comparatively very low numbers in the over all vegetation cover in the study area (Table 4)

Basal Area (BA) of the various tree species in the study area was not much varied in the classes between 50 cm and 400 cm (Table 5). The numbers of individuals were very low in the basal area class between 450 cm and 600 cm. Basal area of 51-100 cm was proportionately high (21.49%) in the overall vegetation cover. On the contrast, it was very low (0.22%) in the 501 –550 cm class.

Encounter Rate

The encounter rate revealed that the *Peltophorum inermae* (4.8 trees/km) scored highest value followed by *Poinciana regia* (4.7 trees/km) and *Enterolobium saman* (3.4 trees/km) in the study area. Other tree species such as *Azadiracta indica* (1.8 trees/km) and *cassia fistula* (1.2 trees/km) and *Milingtonia hortensis* (1.1 trees/km) were recorded in considerable numbers.

DISCUSSION

A short term study on tree diversity of the R.S.Puram urban area was carried out from October to December. A total of 456 trees were recorded comprising of 24 species. Past studies such as Patwardhan (2001) quoted 380 tree species till date have been recorded, Ghate (1990) recorded 33 species but he could not record 57 species recorded by Vartak (1964) in the Pune urban area. Among the families Leguminosae attributed more numbers than other families in the overall standing trees. Among the tree species *Peltophorum inermae, Pionciana regia* and *Enterolobium saman* ranked the first three positions. Habitat preference of the tree species in the study area showed that the majority of the tree species are exotic species. Patwardhan (2001) have also recorded majority of the tree species of Eucalyptus or Palms in Pune urban area. Generally district administration prefers exotic tree species for urban greening. This is mainly due to its survival capacity against any climatic conditions as well as ornamental values of the most of the tree species in the urban area were planted for ornamental purpose and to provide good shade to the public.

Forest and some human habitation tree species were also considerably recorded in the study area. This could be to maintain our endemic species as gene pool in the urban area. Santapau (1958) also found in habit ever green forests species in moister areas, nearest locality From Pune city from 70 km away. It was interesting to note that the seedlings of the urban tree species (Regeneration and Recruitment class) were mainly of endemic species such as *Thespesia populnea, Bauhinia purpurea, Pongamia Pinnata, Cassia fistula*, and *Terminalia catapa*. The seedlings and their growth status of this urban area is another endeavor in this direction. This seemed to be an encouraging trend which would definitely pave the way to the public. The needs and values of our own tree species used by our ancient people, also this will reduce the invasion of exotic species into our natural ecosystem. Because many of the exotic species are noxious in nature and also they bring adverse effect to the natural system. Patwardhan (2001) has pointed out that the increase in population of exotic trees will lead to the declining of native species. His finding has supported to my finding.

The Girth at Breast Height (GBH) of majority of the trees belongs to those between 51 cm and 250 cm among all the standing trees. This is an encouraging trend for having low GBH class trees in the urban area. Because this standing trees population definitely support in future as green mass in the study area. The low representation of trees in the GBH class between 400 and 600 cm trees are need to be replaced by planting new seedlings.

The total Basal area of the urban trees growing in the study area is 88.9 sq.m. Among the tree species, *Peltophorum inermae* covered more place in the overall sampled area (21.908 sq.m) Followed by *Poinciana regia* and *Enterolobium saman*

Management Recommendations

1. Only tree species were found in more numbers in overall vegetation. The other species may be planted in future.
2. The centre part of the study area alone attributed more seedlings of the tree species. This indicate that future plantation is needed in the peripheral area.
3. Since most of the trees are matured trees. Plantation may be undertaken by the concern authorities for the future wealth of green cover.

ACKNOWLEDGEMENTS

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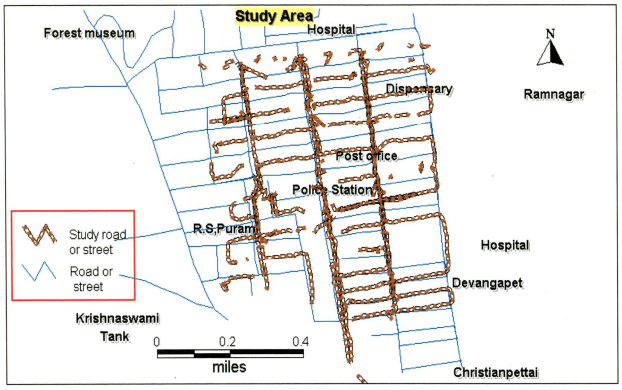


Map:1 Study Area

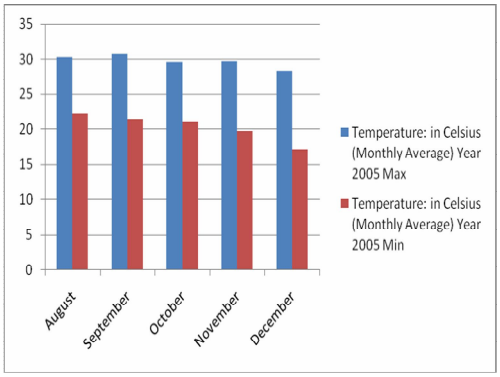


Fig:1 Temperature Ratio of Study Area

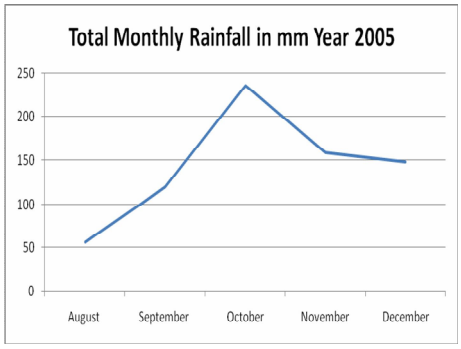
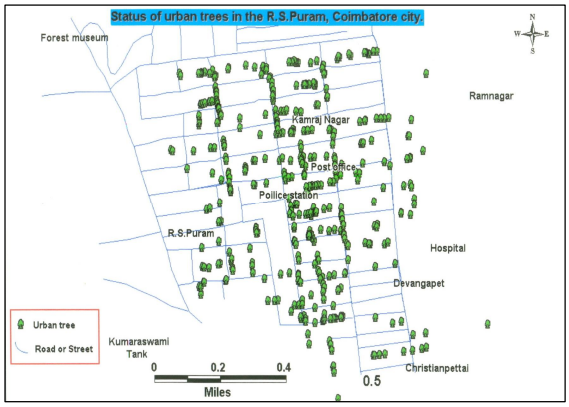
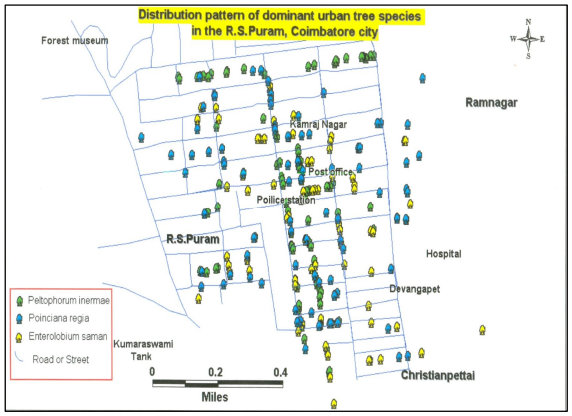


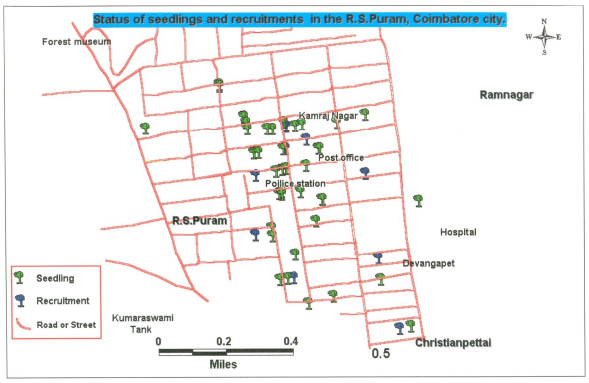
Fig:2 Rainfall of Study Area



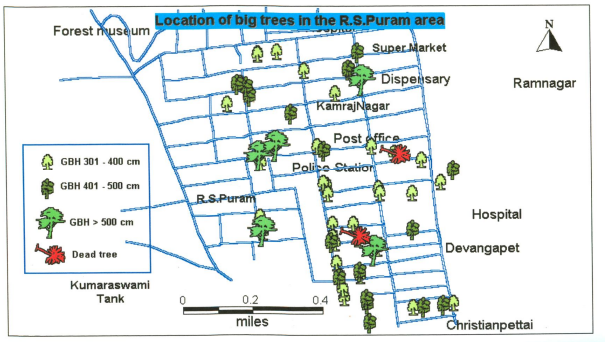
Map 2. Occurrence of various tree species



Map 3. Three dominant tree species recorded in the study area



Map 4. Status of seedlings and recruitments in the study area



Map 5. Occurrence of big trees

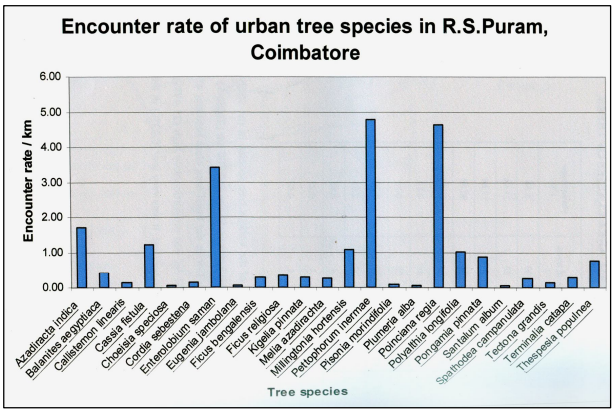


Fig:3 Encounter rate of urban tree species in RS puram

Table 1. Status of various tree species in the study area

Local Name	Family	Name of the Tree Species	Total No. of Individuals	HP	Status	Trend	Benefit
Vepan	Meliaceae	*Azadiracta indica*	35	H	O	D	S/M
Vilvam	Simarubaceae	*Balanites aegyptiaca*	9	E	R	D	O
Bottle brush	Myrtaceae	*Callistemon linearis*	3	P	R	D	O
Konnai	Leguminosae	*Cassia fistula*	25	F	O	D	O/M
Paruthi	Bombaceae	*Chorisia speciosa St. Hill.*	1	F	R	D	F
Aechinaruvihli	Boraginaceae	*Cordia sebestena*	3	F/E	R	D	O
Amaivagai	Leguminosae	*Enterolobium saman*	69	F/E	C	I	O/S
Naval	Myrtaceae	*Eugenia jambolana*	1	F	R	D	F/S
Aala maram	Moraceae	*Ficus bengalensis*	6	H	R	D	S
Arasa maram	Moraceae	*Ficus religiosa*	7	H	R	D	S
Jhas phanoos	Bignoniaceae	*Kigelia pinnata*	6	E	R	D	S/F
Mallay vembu	Meliaceae	*Melia azedarach*	5	F	R	D	S/M
Mara malli	Bignoniaceae	*Millingtonia hortensis*	22	E	R	D	O
Perungondrai	Leguminosae	*Peltophorum inermae*	97	E	A	I	O/S
	Nyctaginaceae	*Pisonia morindifolia*	2	P	R	D	S/O
Perungalli	Apocynaceae	*Plumeria alba*	1	E	R	D	O
Mayirkondrai	Leguminosae	*Poinciana regia*	94	E	A	I	O/S
Ashoke	annonaceae	*Polyalthia longifolia*	21	E	R	D	O/S
Pongam	Leguminosae	*Pongamia pinnata*	18	F	R	D	S/O
Sandanam	Santalaceae	*Santalum album*	1	F	R	D	S
Patadi	Bignoniaceae	*Spathodea campanulata*	5	E	R	D	O/S
Thekku	Verbanaceae	*Tectona grandis*	3	F	R	D	S
Badam	Combretaceae	*Terminalia catapa*	6	F	R	D	F/S
Kallal	Malvaceae	*Thespesia populnea*	16	H	R	D	O/S

HP-Habitat preference: F-Forest, **P**-Plantations, **H**-Habitatious, **E**-Exotic **Status: A**-Abundant, **C**-Common, **O**-Occasional, **R**-Rare **Trend: I-**Increase, **D-**Decrease; **Benefit: O-**Ornamental, **F-**Fruit bearing, **S-**Shade, **M-**Medicinal

Table 2. Tree species richness against habitat types.

Sl. No.	Habitat Types	Total No. of Individuals	Relative Proportion(%)
1	Forest	60	13.16
2	Plantations	5	1.10
3	Habitatious	64	14.04
4	Exotic	255	55.92
5	Forest / Exotic	72	15.79

Table 3 Regeneration and Recruitment Classes of Trees

Sl.No.	Name of the Species	Regeneration		Recruitment	
		Frequency	Percentage	Frequency	Percentage
1	*Mangifera indica*	1	2.5	0	0
2	*Pongamia pinnata*	4	10	1	8.33
3	*Enterolobium saman*	6	15	3	25
4	*Plumeria alba*	2	5	0	0
5	*Cassia fistula*	3	7.5	1	8.33
6	*Thespesia populnea*	4	10	4	33.33
7	*Auracarea*	1	2.5	0	0
8	*Cordia sebestena*	1	2.5	0	0
9	*Spathodea campanulata*	1	2.5	0	0
10	*Azadiracta indica*	3	7.5	0	0
11	*Terminalia catapa*	3	7.5	1	8.33

12	*Bauhinia purpurea*	6	15	0	0
13	*Poinciana regia*	2	5	1	8.33
14	*Melia azedarach*	1	2.5	0	0
15	*Santalum album*	2	5	0	0
16	*Dombeya sp.*	0	0	1	8.33

Table 4. Various GBH classes of trees.

Sl. No.	GBH Class (cm)	No. of trees	Percentage (%)
1	0-50	39	8.55
2	51-100	102	22.37
3	101-150	52	11.40
4	151-200	90	19.74
5	201-250	63	13.82
6	251-300	45	9.87
7	301-350	21	4.61
8	351-400	21	4.61
9	401-450	13	2.85
10	451-500	5	1.10
11	501-550	2	0.44
12	551-600	3	0.66

Table 5. Various basal area classes of trees.

Sl. No.	BA Class (cm)	No. of trees	Percentage (%)
1	0-50	30	6.58
2	51-100	98	21.49
3	101-150	54	11.84

4	151-200	95	20.83
5	201-250	59	12.94
6	251-300	44	9.65
7	301-350	26	5.70
8	351-400	29	6.36
9	401-450	12	2.63
10	451-500	4	0.88
11	501-550	1	0.22
12	551-600	4	0.88

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RESEARCH ARTICLE

Influence of Drip Irrigation Methods on Growth and Yield of Onion at Raichur Region

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ABSTRACT

An experiment was conducted to study the effect of drip and surface irrigation methods on growth and yield of onion. Five different irrigation treatments were evaluated like 60, 80, 100 and 120 per cent ET using drip irrigation and surface irrigation. The highest yield was recorded in 80 per cent ET (28.76 t/ha) using drip irrigation than other treatments. The lowest yield was recorded in surface irrigation (14 t/ha). The plant height, leaf width, number of leaves, average bulb weight and bulb diameter was higher in drip irrigation method.

Key words: Onion, irrigation, drip and yield

INTRODUCTION

Onion is an important commercial vegetable crop. The productivity of onion in our country is lower than many other onion-producing countries (Pandey *et al.*, 2004). The low productivity of onion could be attributed to low inheritance potential of short day onion varieties predominately grown in our country, higher disease incidence, shortage of timely inputs particularly water, *etc.*. Irrigation is one of the most crucial inputs for onion. The shortage of irrigation

at bulb development, which usually coincides with summer season, affects the yield drastically. In last few decades, emphasis has been given in enhancing the productivity of irrigation water. Onion is mostly grown as irrigated crop in our country and surface irrigation is commonly used. The productivity of water in surface irrigation is low due to higher percolation, distribution and evaporation losses. The modern systems of irrigation such as drip, sprinkler ensures higher water use efficiency. Several research workers reported that through micro-irrigation, higher crop yields can be obtained along with considerable saving in irrigation water (Sezen *et al.*, 2006; Kumar *et al.*, 2007). The results of micro-irrigation are though rewarding in fruit crops as also effective in widely spaced vegetable crops. There has always been apprehension about suitability of drip for closely-spaced vegetables, while sprinklers are used for variety of crops. Thus, a study was conducted to study the efficacy of drip and irrigation over surface irrigation.

MATERIALS AND METHODS

The experiment was conducted during September 2010 to December 2010 at Main Agricultural Research Station, UAS Raichur 16°15' N latitude and 77°20' E longitude and is at an elevation of 389 m above mean sea level (MSL). The climate is semi-arid and average annual rainfall is 722 mm. The soil was clay loam in texture and had pH of 7.33. There were five irrigation treatments *i.e.* 60, 80, 100 and 120 percent ET in drip irrigation and surface irrigation, taken for the studies which were laid out in randomized block design with four replications. Seedlings of onion (Nasik red) were transplanted at spacing of 15 cm X 7.5 cm The seedlings were transplanted in 16 beds of 10 m x 1.5 m (drip) while 4 beds for furrow irrigation. Three laterals of 12 mm diameter were used each bed with a inline dripper at 40 cm distance and discharge of 2 l h^{-1}. Irrigation was provided daily after calculating water requirement based on past 24 hours of pan evaporation while in furrow irrigation it was scheduled ones in seven days.

The recommended doses of fertilizer, *i.e.* 125 kg nitrogen, 50 kg phosphorus and 125 kg potassium per hectare were applied (19% N, 19% P, 19% K), urea (46 % N) and murate of potash (60 % K). The half dose of nitrogen and full doses of phosphorus and potash were applied as basal and remaining 50 % of nitrogen (75 kg/ha) was divided in 7 equal doses and applied at weekly interval through fertigation or broadcasting starting from two weeks after transplanting. The recommended plant protection measures were taken as and when required. The irrigation was stopped at 15 days before harvesting. The observations on plant morphological characters, yield and yield contributing characters were recorded and quantity of water applied was also worked out.

RESULTS AND DISCUSSION

The results revealed that there was significant difference in growth and yield of onion under different irrigation methods. The data on the influence of methods and different levels of drip irrigation on plant height at 30, 60, 75 and 90 days after transplanting are presented in Table 1. The results indicated that at 30 days after transplanting, plants receiving water at 80 per cent ET recorded significantly maximum height (42.80 cm) followed by the treatment that received water at 100 per cent ET (38.10 cm) and 120 per cent ET (36.23 cm). The minimum values were noticed in control treatment (30.61 cm) which was significantly with 60 per cent ET treatment (32.66 cm). Similarly, at 60 days after transplanting, plants receiving water at 80 per cent ET recorded significantly maximum height (58.04 cm) followed by the treatment that received water at 100 per cent ET (50.13). The lowest value was found in control treatment (35.11 cm). Similar trends were noticed at 75 days after transplanting. The plants receiving water at 80 per cent ET recorded (63.26 cm) which was on par with 100 per cent ET (57.20). The minimum height was produced by control treatment (38.78 cm) and 60 per cent ET treatment (45.78 cm). Finally at 90 days after transplanting, plants receiving water at 80 per cent ET recorded the height (68.16 cm) which was on par with 100 per cent ET (61.28). The minimum height was found at control treatment (41.89 cm).

Table 1. Effect of irrigation methods and irrigation levels on plant height (cm) at different intervals

Treatment	**30 DAT**	**60 DAT**	**75 DAT**	**90 DAT**
T_1 (60% ET)	32.66	40.28	45.78	51.48
T_2(80% ET)	42.80	58.04	63.26	68.16
T_3(100% ET)	38.10	50.13	57.20	61.28
T_4(120% ET)	36.23	48.17	51.61	55.56
T_5(Surface irrigation)	30.61	35.11	38.78	41.89
$CD_{0.05}$	4.60	7.06	7.54	7.96

The data pertaining to number of branches 30, 60, 75 and 90 days after transplanting are presented in Table 2. The results indicated that at 30 days after transplanting, plants receiving water at 80 per cent ET recorded the maximum number of leaves (7.03) which was on par with the 100 per cent (6.28) where as lowest values were found in control treatment (4.13). Similarly maximum number of leaves recorded at 60, 75 and 90 days after transplanting in 80 per cent ET.

The data pertaining to leaf width 30, 60, 75 and 90 days after transplanting are presented in Table 3. The results indicated that at 30 days after transplanting, plants receiving water at 80 per cent ET recorded maximum leaf width (1.29 cm) which was on par with 100 per cent ET(1.18 cm) where as lowest leaf width were found in control treatment (0.93 cm). Similarly a highest leaf width was recorded at 60, 75 and 90 days after transplanting in 80 per cent ET. The effect of irrigation methods and irrigation levels on leaf width at different intervals is depicted graphically in Fig. 1

Table 2 Effect of irrigation methods and irrigation levels on number of leaves per plant at different intervals

Treatment	30 DAT	60 DAT	75 DAT	90 DAT
T_1 (60% ET)	5.43	8.60	9.20	7.98
T_2(80% ET)	7.03	11.05	13.93	13.40
T_3(100% ET)	6.28	10.60	12.70	12.70
T_4(120% ET)	5.70	9.88	12.10	11.73
T_5(Surface irrigation)	4.13	6.23	6.78	5.93
$CD_{0.05}$	1.04	1.48	1.81	1.39

Table 3. Effect of irrigation methods and irrigation levels on leaf width (cm) at different intervals

Treatment	30 DAT	60 DAT	75 DAT	90DAT
T_1 (60% ET)	1.08	1.18	1.37	1.51
T_2(80% ET)	1.29	1.39	1.68	1.72
T_3(100% ET)	1.18	1.28	1.51	1.57
T_4(120% ET)	1.14	1.19	1.45	1.55
T_5(Surface irrigation)	0.93	0.99	1.04	1.10
$CD_{0.05}$	0.16	0.18	0.23	0.24

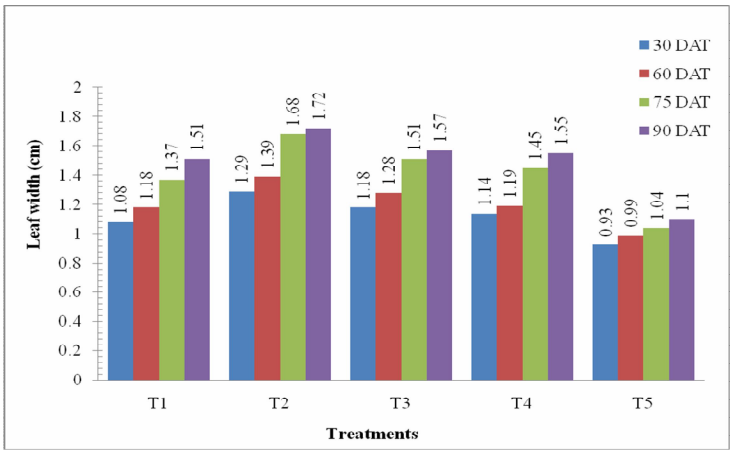

Fig. 1. Effect of irrigation methods and irrigation levels on leaf width (cm) at different intervals

The plant height, number of leaves and leaf width are the most important growth parameters which determine the canopy of plant. The canopy is directly related to productivity of crops. The moisture stress condition lead to poor cell elongation, low rate of photosynthesis and low carbohydrate assimilation resulting in poor plant growth. The onion plant height was more in 80 per cent ET treatment as compared to furrow irrigation. We can go for 80 per cent crop ET treatment because of saving of water in the latter treatment. Thus in drip irrigation treatments, the treatment which received water at 80 per cent ET level exhibited better plant growth in terms of plant height number of leaves due to favorable moisture conditions. These results are in agreement with the findings of Muthuchamy *et. al* (1993)

Bulb diameter

Data in respect of bulb diameter are presented in Table 4. The bulb diameter ranges from 34.53 to 68.57 mm in treatments under study. The maximum bulb diameter of 68.57 mm was found in case of 80 per cent ET, which was significantly higher than the control treatment (34.53 mm), 120 per cent ET (56.31 mm) and 60 per cent ET treatment (51.26 mm). But it was on par with 100 per cent ET (62.59 mm). According to Borivoji Pejic (2006), the maximum bulb diameter of 50.8 mm was found in case of 70 per cent ET.

Average bulb weight

The results of average bulb weight for different irrigation treatments are presented in Table 4. The maximum average bulb weight of 110.75 gm was found in case of 80 per cent ET, which was significantly higher than the control treatment (41.05 gm), 120 per cent ET treatment (89.53 gm) and 60 per cent ET treatment (66.88 gm).but it was on par with the treatment of 100 per cent ET (101.75 gm). According to Borivoji Pejic (2006), the highest average bulb weight of 76.8 g was found in case of 70 per cent ET.

The yield parameters like bulb weight and bulb diameter was produced highest in 80 per cent ET level followed by 100 per cent level. The overall trend for all the growth parameters was found to be superior in case of 80 per cent ET level. This may be attributed to the frequent and consistent application of water in the vicinity of the roots which provided better soil moisture regime in the crop root zone throughout the crop growth period. This 80 and 100 per

cent drip irrigation treatments has contributed to higher yield. Similar results were observed in the works of Prabakar (2000).

Total soluble solids (TSS)

Data pertaining to TSS of Onion crop are presented Table 4. It is seen from table that the treatment of 120 per cent ET recorded the highest TSS (13.50 brix), which was significantly higher than that 60 per cent ET treatment (12.25), 80 per cent ET (12.75) and control treatment (13.25) but it was on par with the treatment of 100 per cent ET (13.25).

Yield per hectare

The total marketable yield per hectare as influenced by irrigation methods and levels of drip irrigation are presented in Table 4. The total yield was significantly higher in 80 % ET (28.76 t ha^{-1}) using drip irrigation than other treatments. The lowest was found to be in furrow irrigation (14 t ha^{-1}). The effect of irrigation methods and irrigation levels on yield of onion at different irrigation levels is shown graphically in Fig. 2

The Onion crop performed well in terms of yield and yield contributing factors under drip irrigation as compared to furrow irrigation. The better performance of plant in terms of bulb diameter, average bulb weight and yield (Table 4) may be attributed to the frequent and consistent application of water vicinity of the roots which provides a good soil moisture regime in the crop root zone throughout the life period of crop. . These results are in agreement with findings of Sivanappan (1996)

The total water applied in different irrigation methods was lowest in 60 per cent ET (948.48 m^3/ha) fallowed by 80 per cent ET (1264.65 m^3/ha), 100 per cent ET (1580.81 m^3/ha) and 120 per cent ET (1896.97 m^3/ha). While it was highest in surface irrigation (2000 m^3/ha). The highest water saving over furrow was carried out in 60 per cent ET (65.73 %) fallowed by 80 per cent ET (55.18 %), 100 per cent ET (44.64 %) and 120 per cent ET (34.10 %) in Table 5. The higher water saving, water productivity of water in drip irrigation system is due to the reduction of various types of water losses during irrigation. . These results are in agreement with the earlier findings of Bafna *et al.* (1993).

Table 4. Effect of irrigation methods and irrigation level on yield traits, TSS and yield Onion

Treatments	Bulb diameter (mm)	Average bulb weight (gm)	TSS (brix)	Yield t/ha
T_1	51.26	66.88	12.25	21.17
T_2	68.57	110.75	12.75	28.76
T_3	62.59	101.75	13.25	26.72
T_4	56.31	89.53	13.50	25.02
T_5	34.53	41.05	13.25	14.00
CD (0.05)	7.60	17.75	0.84	2.89

Table 5. Effect of irrigation methods on water applied and water saving in onion

Treatment	Water applied in (m^3/ha)	% of water saving over furrow
T_1 (60% ET)	948.48	65.73
T_2 (80% ET)	1264.65	55.18
T_3 (100% ET)	1580.81	44.64
T_4 (120% ET)	1896.97	34.10
T_5 (Surface irrigation)	2000.00	

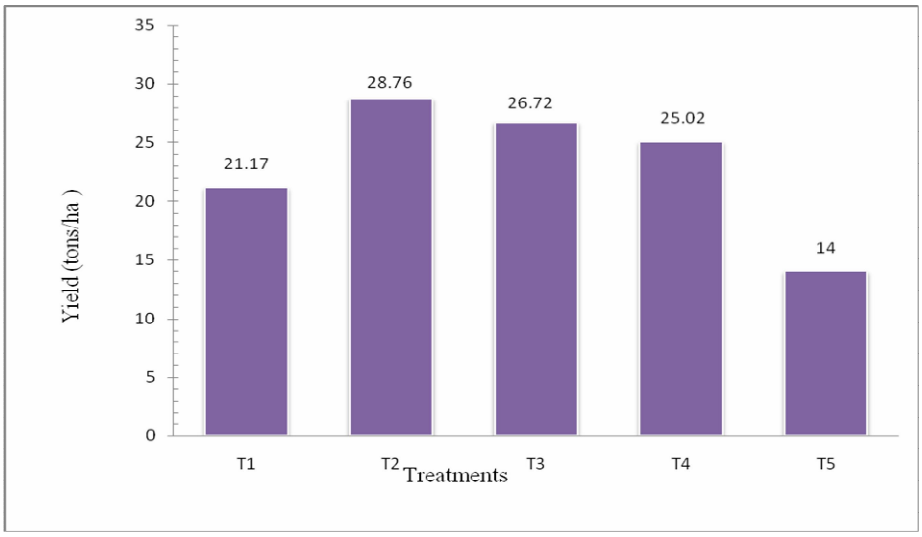

Fig. 2. Effect of irrigation methods and irrigation levels on yield (t/ha) of Onion

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RESEARCH ARTICLE

Screening of Antimicrobial Activity of Selected Medicinal Plants *Cassia surattensis* Burm.f and *Rhinacanthus nasutus* (*L*) Kure

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ABSTRACT

In vitro screening of antimicrobial activities of ethanol, chloroform, ethyl acetate and ethyl acetate:ethanol (1:1) extracts of selected medical plants *Cassia surattensis* Burm.f, and *Rhinacanthus nasutus* (L) Kure on selected pathogenic bacteria *S. aureus, B. subtilis, E. coli, P. aeruginosa, Klebsiella pneumonia* and selected fungal pathogens *Candida albicans* and *A. niger*. The two plant's leaf extract contains phytosterols, tannins and phenolic compounds, free amino acids and flavonoids. Ethanolic extract of *Cassia surattensis* inhibits all the test bacterial strains in the higher concentrations, but highest zone of inhibition is recorded in *B. subtilis*. The ethyl acetate extract of the same plant showed good effect on *S. aureus* and the ethanol:ethyl acetate extract is very good inhibitory effect on *E. coil*, *S. aureus* and *P. aeruginosa*. The extract had no effect on *C. albicans*, but good effect on *A. niger*. Ethyl acetate extract inhibits the growth of both *C. albicans* and *A. niger* only in highest concentration of the extract. Both ethyl acetate and ethanol mixture extract inhibits the growth of *A. niger* and *C. albicans* only in the highest concentration. Ethanolic extract of leaf of *Rhinacanthus nasutus* is also inhibits all the test bacterial strains in the higher concentrations like the ethanolic extract of *C. surattensis* and the highest zone of inhibition was recorded in *S. aureus, K. pneumonia* and *P. aeruginosa.* The ethyl acetate extract of the same plant had shown good effect on *E. coil* and *P. aeruginosa* at the highest concentration. *B. subtilis* and *K. pneumoniae* are resistant to all the concentrations of this extract, but *S. aureus* is mostly resistant to lower concentrations. The ethanol and ethyl acetate mixture extract had highly inhibited the growth of *S. aureus* and *E. coli,* the remaining bacterial strains are resistant to this extract. In the case of antifungal activity, the ethanolic and the ethyl acetate extracts highly inhibits both *C. albicans* and *A. niger* in the highest concentrations. But the ethanol:

ethyl acetate (1:1) extract had shown no inhibitory activity on both test fungal species even in the higher concentrations.

Key words: antimicrobial, *In vitro,* inhibitory activity, fungal pathogens, phytosterols. *Cassia surattensis, Rhinacanthus nasutus, S.aureus, Bacillus subtils, P. aeruginosa, E. coli* and *K. pneumoniae*.

INTRODUCTION

Plants have been an essential part of human society since the start of civilization. The earliest mention of medicinal use of plants in Hindu culture is found in "Rigveda", which is said to have been written between 4500- 1600 B.C (1). Medicinal plants contain numerous biologically active compounds, many of which have been shown to have antimicrobial properties (2). India having very diverse agroclimatic conditions is gifted with rich natural resources. Not only in India, has nearly fifty-six percent (56%) of low-income population in the world used herbal medicine and their supplements for their primary health care (3). Following the advent of modern medicine, herbal medicine suffered a setback, but during last two or three decades advances in phytochemistry and in identification of plant compounds effective against certain diseases have renewed the interest in herbal medicines (4).

Respiratory infections, gastro-intestinal infections and blood borne infections are the common health problems in rural communities in tropical developing countries. Numerous tropical medicinal plants are used traditionally which are remedial against these diseases (5, 6). Recent years, there are numerous synthetic antimicrobial drugs are available for medical practices to treat various microbial infections. Regular uses of these synthetic drugs cause adverse side effects to the patients like hypersensitivity, immunosuppression, damage the liver and kidney.

Nowadays multiple drug resistant microorganisms have developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. This situation, scientist are forced to search new antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents. The medicinal and aromatic plant's essences are rich in antibacterial and antifungal compounds could be an alternate way to combat against bacterial and fungal diseases (7). Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants (8). Hence the aim of this study is to determine the phytochemical constituents and investigate the antimicrobial properties so as to ascertain their uses in traditional medicines.

MATERIALS AND METHODS

Collection of plant materials

Cassia surattensis was collected from Pachaimalai and *Rhinacanthus nasutus* were collected from Srirangam, Tiruchirappalli district, Tamil Nadu state, India. The healthy plant samples were collected with a sterilized knife in clean polythene bag and brought to the laboratory. All the test plant's leaves were collected from the whole plants; air dried in the shadow and kept in the appropriately labelled sterile polythene bags.

Continuous hot extraction

Soxhlet apparatus was used to execute continuous hot extraction. The two test plant leaves were fine powdered by using marter and pestel and kept separately in a labelled polythene bags. The *Cassia surattensis* leaf powder was placed in the wide tube of the extractor a thimble made of filter paper is inserted in to this tube. The solvent ethanol was taken in the flask and heated, the vapours arise from the solvent, get in to the condenser through a side tube and the liquid condensed from the vapours drips in to the thimble. The solvent liquid level slowly arises and during this period, the dried materials get extracted of its soluble constituents. When the level of the liquid reaches the top of the siphon, it gets siphoned into the flask. The suction effect of the siphoning assists permeation of the solvent through

the drug. Again, a portion of the solvent from the solution vaporized and leaving the constituents in the flask itself and the process mentioned above is repeated. The same process was repeated again and again until the solutes extracted, this kind of continuous hot percolation was undertaken when the active constituents not readily soluble in the cold and the thermolabile. Like this, the other solvent ethyl acetate and mixture of ethanol and ethyl acetate (1:1) were used for extraction purpose. All the three plant's leaves were extracted and stored in a appropriately labelled containers for further studies.

Phytochemical Analysis

After the completion each extracts, the extract was filtered and the solvents were removed by distillation under reduced pressure, a coloured residue was obtained for phytochemical analysis.

Test for alkaloids

A small portion of Ethanol extract were separately stirred with a few drops of hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloid reagents

i) Mayer's reagent which gives yellow precipitate to detect the presence of alkaloids.
ii) Dragendroff's reagent shows the presence of orange brown precipitate to detect the presence of alkaloids.
iii) Hagar's reagent shows the presence of alkaloids due to the formation of yellow precipitate.
iv) Wgner's reagent shows the presence of alkaloid due to the formation of Reddish brown precipitate.

Test for Carbohydrates & Glycosides

I)**Molisch's Test**: A small quantity of extract was dissolved separatelyin 5 ml of distilled water and filtered. The filtrate was subjected to Molisch's test to detect the presence of carbohydrates.

ii) **Fehling's Test**: The filtrate was treated with 1 ml of Fehling's solution and heated. Appearance of reddish orange precipitate shows the presence of reducing sugars. The extract was hydrolysed with hydrochloric acid (HCl) for few hours on a water bath and the hydrolysate was subjected to legal's and Borntager's tests to detect the presence of different glycosides.

a) **Legal's Test**: 1.0 ml of pyridine and a few drops of sodium nitroprusside solution were added in the hydrolysed extract and sodium hydroxide solution was added. Appearance of pink to red colour confirm the presence of glycosides.
b) **Borntrager's test**: The hydrolysate was treated with chloroform and the layer was separated. Equal quantity of dilute ammonia solution was added with that supernatant of the chloroform treated extract layer. Ammonia layer acquires a pink colour and confirm the presence of glycosides.

Test for Phytosterols

Libermannan Burchard Test: 1.0 gm of extract was dissolved in a few drops of dry acetic acid, 3 ml of acetic anhydride was added followed by few drops of concentrated sulphuric acid. Appearance of bluish green colour shows the presence of phytosterols.

Test for fixed oils and fats

i) A small quantity of alcoholic extract was separately pressed between two filter papers and appearance of oil stain in the filter paper indicates the presence of fixed oil.

ii) Few drops of 0.5 N alcoholic potassium hydroxide was added to small quantity of alcoholic extract along with a drop of phenolphthalein. The mixture was heated on water bath for 1-2 hours. Formation of soap or partial neutralization of alkali indicates the presence of fixed oil and fats.

Test for Saponins: The extract was diluted with 20 ml of distilled water and it was agitated on a graduated cylinder for 15 minutes. The formation of 1 cm layer of foam shows the presence of saponins.

Test for tannins and phenolic compounds: Small quantity of alcoholic extract was taken separately along with water and tested for the presence of phenolic compounds and tannins.

i) When treated with dilute ferric chloride solution (5%) gives violet colour.
ii) 1.0% solution of gelatin containing 10 % NaCl gives white precipitate.
iii) 10% lead acetate solution gives white precipitate.

Test for Proteins and Free Amino Acids: Small quantity of extract was dissolved in few ml of water and subjected for the following tests.

i) **Million's reagent** – appearance of red colour shows the presence of proteins and amino acids.
ii) **Ninhydrin reagent** – appearance of purple colour shows the presence of proteins and free amino acids.
iii) **Biuret test_-** Equal volumes of 5% sodium hydroxide solution and 1% copper sulphate solution were added. Appearance of pink or purple colour shows the presence of proteins and free amino acids.

Test for flavonoids: The plant extract was mixed with

i) With aqueous sodium hydroxide solution. Blue-violet colour (anthocyanins) yellow colour (flavones) yellow to orange (flavonones).
ii) With concentrated sulphuric acid yellowish orange colour (anthocyanins) yellow to orange colour (flavones) orange to crimson (flavonones).
iii) Shinoda's test- the extract was dissolved in alcohol, to that piece of magnesium followed by concentrated hydrochloric acid drop wise are added and heated. Appearance of magenta colour shows the presence of flavonoids.

Test for lignin: With alcoholic solution of phlorogulucinol and hydrochloric acid are added, appearance of red colour shows the presence of Lignin.

Antimicrobial Activity- Disc Diffusion Method (9)

The two plant's leaves extracts were tested for antimicrobial activity in the disc diffusion assay using five bacterial pathogens *S. aureus*, *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The test bacterial strains were maintained in separate nutrient broth culture. 0.1 ml of each test bacterial cultures (18 hrs) was spread over appropriately labelled sterile Muller Hinton agar plates.

Disc preparations – 6.0 mm in diameter of whatman No.1 filter paper discs were sterilized and ethanolic extract of *Cassia surattensis* was added in that discs. Different concentrations of that extract in the discs such as 1.0mg/disc, 2.0mg/disc, 3.0mg/disc, 4.0mg/disc & 5.0mg/disc were prepared and the discs were allowed to dry. Like this, the remaining ehthyl acetate extract, chloroform extract and ethanol:ethyl acetate extract of *Cassia surattensis* impregnated discs were prepared with different concentration like the above mentioned concentration, the discs were dried and tested their antibacterial activity with five bacterial pathogens appropriately inoculated in Muller Hinton agar plates.

RESULTS

Phytochemical Screening

The phytochemical studies of the two plant's leaf extracts were identified and those are phytosterols, tannins and phenolic compounds, free amino acids and flavonoids. The phytochemicals alkaloids, carbohydrates, glycosides, saponins, fixed oils and fats are not detected in the materials used (Table.1). Hence, we can conclude that the antibacterial and antifungal activity of the ethanol and extract may be due to the above phytochemical components.

Antimicrobial screening

The present study has screened the antimicrobial activity of selected organic solvents (ethanol, ethyl acetate and ethanol : ethyl acetate) used extracts of leaf of *Cassia surattensis* Burm.f and *Rhinacanthus nasutus* (L) Kure on selected pathogenic bacteria *S. aureus, B. subtilis, E. coli, P. aeruginosa, Klebsiella pneumonia* and selected fungal pathogens *Candida albicans* and *A. niger*.

Antibacterial activity of *Cassia surattensis*

The ethanolic extract of leaves of *Cassia surattensis* was found highly effective against all the test bacterial strains at the concentration of 50%. The zone of inhibition of each test bacterial species were measured and compared with each concentration of ethanolic extract and the highest zone of inhibition was identified in the higher concentration. Each organism had good effect at concentration of 6.25mg/disc showed 6mm, 8.75mg and 10mg showed 7 - 9mm, 11.25mg showed 9 – 10mm but in 12.5mg the zone of inhibition is 17 mm as the highest, against *B. Subtilis*. The disc contains only ethanol (negative control) had no effect on all the test microorganisms (Table.2).

Ethyl acetate extract

Ethyl acetate extract of leaves had shown good effect on *S .aureus* (12mm) at concentration of 12.5mg/disc and also the concentration 6.25mg and 8.75mg showed 6 – 7 mm in *S. aureus, E. coli* and *P. aeroginosa,* the 10mg and 11.25mg of the extract showed 7 – 9 mm. For interest, the two solvent extracts of leaves of *Cassia surattensis* were mixed or combined together and tested against five microorganisms at varying concentrations. The mixed solvents ethanol and ethyl acetate (1:1) extract of leaves of *Cassia surattensis* had good effect on both *E. coli* and *S. aureus.* But they exhibited less activity (5 mm) against *P. Aeruginosa* (Table.3).

The selected pathogenic bacterial growth was also screened for inhibition zone measurement against two selected broad spectrum antibiotics – Gentamycin and Ciprofloxacin. Each antibiotic disc was used as a single concentration in 5μg / disc. All the bacterial strains were susceptible to Ciprofloxacin and their inhibition zones ranges from 7 mm – 30 mm. The Clotrimazole is active against only two organisms and its inhibition activity is 10 mm and 20 mm was measured to *C. albicans* and *A. niger*. (Table.13)

Antifungal activity of *Cassia Surattensis*

The antifungal activity observed by inhibitory zone formation. The ethalonic extract showed no inhibitory zone on *C. albicans*. But in *A. niger* the inhibition zone was ranging from 5 – 13mm (Table No.5). The ethyl acetate extract showed higher inhibition activity on *C. albicans* was ranging from 5 – 10 mm and in *A. niger,* the inhibitory zone was 5 –7 mm (Table.6). The ethanol : ethyl acetate (1:1) mixture extract showed 6 –12 mm of inhibition zone on *A. niger* at a concentrations between 6.25mg and 8.75mg (Table.7). But for *C. albicans* higher inhibition zone is 15 mm in the concentration of 12.5mg/disc.

The fungal growth characters were observed from 1st, 3rd and 5th day of incubation. All the growth characters including sporangium formation are appeared within three days in the control plate. The test plate cultured fungal colonies had shown very less expression of mycelium growth and belated sporangium formation during the 3rd day of observation in the *C. surattensis*.

Antibacterial activity of *Rhinacanthus nasutus*

The ethanolic extract of leaves of *Rhinacanthus nasutus* was found highly effective against all the test bacterial strains at the concentration of 11.25mg. The zone of inhibition of each test bacterial species were measured and compared with each concentration of ethanolic extract and the highest zone of inhibition was identified in the higher concentration 12.5mg. Each organism had good effect at concentration of 6.25mg showed 6mm – 7mm, 8.75mg showed 7 - 8mm, 10mg showed 9mm, 11.25mg showed11mm, in 12.5mg concentration showed 9 mm. The highest zone of inhibition is found as 25 mm on *Staphylococcus aureus* and 30 mm on *Klebsiella pneumonia*. The negative control disc had no effect on both gram positive and gram negative organisms (Table.8)

Ethyl acetate extract of *Rhinacanthus nasutus* had shown good effect on *Escherichia coli* (15mm) and on *P. Aeroginosa* (12mm) at the concentration of 12.5mg. The remaining concentrations 6.25mg, 8.75mg, 10mg and 11.25mg showed the zone of inhibitions are 5mm, 7mm, 7mm and 8 mm on *P. Aeruginosa* and 6mm, 7mm, 9mm and 7mm on *E. coli*. Like this, 4mm, 7mm, 6mm, 7mm of 8.75mg, 10mg, 11.25mg and 12.5mg of extract on *S. aureus* and 6.25mg concentration of the extract had no effect on *S. aureus*. The bacteria *B. subtilis* and *Klebsiella pneumoniae* are resistant to

all the concentration of ethylacetate extract of *Rhinacanthus nasutus* and the negative control of had shown no activity to the test bacterial strains (Table.9).

Ethanol and ethyl acetate (1:1) mixture extract of *Rhinacanthus nasutus* had shown good effect on *S. aureus, E. coli*, the zone of inhibition is 9mm and 10 mm in 12.5mg concentration of extract. 6.25mg and 8.75mg concentration of extracts had no effect on all the test bacterial strains, but 10mg and 11.25mg concentration of extracts showed 5mm and 4mm in diameter of zone of inhibition on *S. aureus* and 2mm and 5mm in diameter of zone on *E. coli*. Gentamycin and Ciprofloxacin (5mg/disc) were used as positive control to test their inhibition activity on the selected pathogenic bacteria (Table.10).

Antifungal activity of *Rhinacanthus nasutus*

The ethalonic extract showed 15mm of zone of inhibition on *C. albicans* and 12mm on *A. niger* (Table No.11). The ethyl acetate extract inhibit the *C. albicans* and *A. niger*, the zone of inhibition is 18mm and 25mm at the concentration of 12.5mg (Table.12) The ethanol : ethyl acetate mixture extract showed no inhibitory activity on both *A. niger* and *C. albicans*.

DISCUSSION

Plants such as *Cassia surattensis* and *Rhinacanthus nasutus* and are well known herbs used ayuverdic traditional medicine for their effectiveness against wide range of diseases including skin infections due to the advantage of the diversity of secondary metabolites responsible for their antibacterial activity. Moreover, HIV-positive patients have developed resistance to treatment with existing antibiotics (10). Despite the existence of potent antibacterial agents, the appearance of resistant or multi-resistant strains imposes the need for a permanent search and development of new drugs (11). *Cassia surattensis* and *Rhinacanthus nasutus* has been widely used as tincture for healing wounds and treating burns in homeopathy extensively (12, 13). The aqueous decoctions of these plants are reported to be antibacterial agents in traditional system of medicine (14).

Herbal drugs contain unique constituents which differs from one herb to another, hence the type and extent of their medicinal property also differs. (15, 16) Solubility of each constituent in an herb is very specific to different solvents used in the extraction process. The Ethanolic extract of both plant's leaves contain phytosterols, tannins and phenolic compounds, free amino acids and flavonoids. The preliminary phytochemical analysis of the extract revealed the presence of tannin, flavonoid and phytosterols. These compounds have been reported to inhibit bacterial growth [17].

The ethanolic extract of *Cassia surattensis* inhibits all the test bacterial strains in the higher concentrations of the extract, but the highest zone of inhibition is recorded in *B. subtilis*. The ethyl acetate extract of the same plant showed good effect on *S. aureus*, the ethanol : ethyl acetate extract is very good inhibitory effect on *E. coil* and *S. aureus*. In the case of antifungal activity, the ethanolic extract had no effect on *C. albicans* even in the highest concentration, but on *A. niger* the extract showed good inhibitory effect (13mm). The ethyl acetate extract inhibits the growth of both *C. albicans* and *A. niger* growths only in the highest concentration of the extract. Both ethyl acetate and ethanol mixture extract inhibits the growth of *A. niger* and C. *albicans* only in the highest concentration. In the previous findings the leaf extracts of *Cassia alata* species using various solvents showed a range of activity against several bacteria and protozoa and on the other hand, *Cassia alata* showed only slight activity against bacteria such as *S. aureus* and *B. Subtilis* [18]. Methanol extract of leaves of *Cassia alata* showed antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus*. Somchit et al [19] also tested the whole plant parts of *Cassia alata* and showed activity in the leaves against *Staphylococcus aureus.*

The ethanolic extract of leaf of *Rhinacanthus nasutus* is also inhibits all the test bacterial strains in the higher concentrations of the extract like the ethanolic extract of *C. surattensis* the highest zone of inhibition is recorded in *S. aureus* and *K. pneumoniae.* The results of the present study agreed essentially with the report of these previous

investigators because it revealed activities against *K. pneumonia* which is one of causative agent of pneumonia and bronchitis. *C. occidentalis* have been used by local people for the treatment of bronchitis in the Peruvian amazon as reported by [20].

The ethyl acetate extract of the same plant leaves had shown good effect on *E. coil* and *P. aeruginosa* at the highest concentration. *B. subtilis* and *K. pneumoniae* are resistant to all the concentration of this extract, but *S. aureus* is only resistant to lower concentration of this extract. The ethanol and ethyl acetate mixture extract had highly inhibits the growth of *S. aureus* and *E. coli*, the remaining bacterial strains are resistant to this extract. In the case of antifungal activity, the ethanolic and the ethyl acetate extracts highly inhibits both *C. albicans* and *A. niger* only in the highest concentrations. But in contrast, the ethanol: ethyl acetate (1:1) extract had shown no inhibitory activity on both test fungal species even in the higher concentrations. Previous literature also revealed the use of *Rhinacanthus nasutus* decoctions in various skin conditions associated with bacterial infections (21, 22, 23 and 24). *S. aureus* being the most susceptible organism, these drugs could be more effective in infections related to *S. aureus* rather than other bacterial infections (25).

Overall, the present study indicates the antimicrobial properties of leaf extract of *C. surattensis* and *Rhinacanthus nasutus* provides some idea about the extract phytochemical constituents. Both plant's leaf extracts has proven good inhibitory activity on some bacterial and fungal strains, but in total both plant extracts using different solvents active against all the bacterial strains and fungal speices *C. albicans* and *A. niger*. It is however recommended that further attention and research should be conducted to identify the active compound responsible for the antimicrobial activity of both *C. surattensis* and *Rhinacanthus nasutus*.

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Table.1. phytochemical screening of ethanolic extract of medicinal plants.

Sl.no.	Phytochemicals	*Cassia surrattensis*	*Rhinacanthus nasutus*
1.	Alkaloids	-	-
2.	Carbohydrates	-	-
3.	Glycosides	-	-
4.	Phytosterols	+	+
5.	Saponins	-	-
6.	Fixed oils & Fats	-	-
7.	Tannin	+	+
8.	Phenolic compounds	+	+
9.	Proteins	-	-
10.	Free amino acids	+	+
11.	Gum & mucilage	-	-
12.	Flavonoids	+	+
13.	Lignin	-	-
14.	Volatile oil	-	-

(+) – Positive, (-) – Negative.

Table: 2 – Antibacterial activity and zone of inhibition of ethanolic extract of *Cassia surattensis*. Burm.f on selected bacterial pathogenic strains.

Different concentration of ethanolic extract (mg/disc) with negative control	Measurement of zone of inhibition (mm in diameter)				
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*K. pneumoniae*
Negative control	-	-	-	-	-
6.25	6.0	-	6.0	6.0	-
8.75	7.0	-	7.0	9.0	-
10.0	9.0	-	7.0	9.0	-
11.25	9.0	-	9.0	10.0	-
12.5	10.0	17	10.0	11.0	13.0

Table: 3 – Antibacterial activity and zone of inhibition of ethylacetate extract of *Cassia surattensis*. Burm.f on selected bacterial pathogenic strains.

Different concentration of ethylacetate extract (mg/disc)with negative control	Measurement of zone of inhibition (mm in diameter)				
	S. aureus	*B. subtilis*	*E. coil*	*P. aeruginosa*	*K. pneumoniae*
Negative control	-	-	-	-	-
6.25	7.0	-	5.0	7.0	-
8.75	8.0	-	6.0	9.0	-
10.0	10.0	-	8.0	9.0	-
11.25	11.0	-	10.0	10.0	-
12.5	12.0	-	11.0	12.0	-

Table: 4 – Antibacterial activity and zone of inhibition of ethanol : ethylacetate extract of *Cassia surattensis*. Burm.f on selected bacterial pathogenic strains

Different concentration of ethanol : ethylacetate extract (mg/disc) with negative control	Measurement of zone of inhibition (mm in diameter)				
	S. aureus	*B. subtilis*	*E. coil*	*P. aeruginosa*	*K. pneumoniae*
Negative control	-	-	-	-	-
6.25	6.0	-	5.0	6.0	-
8.75	8.0	-	7.0	8.0	-
10.0	8.0	-	9.0	9.0	-
11.25	9.0	-	10.0	10.0	-
12.5	11.0	-	10.0	11.0	-

Table: 5 – Antifungal activity and zone of inhibition of ethanol extract of *Cassia surattensis*. Burm.f on selected fungal pathogens

Different concentration of ethanolic extract (mg/disc) with negative control	Measurement of zone of inhibition (mm in diameter)	
	A. niger	*C. albicans*
Negative control	-	-
6.25	5.0	-
8.75	6.0	-
10.0	8.0	-
11.25	10.0	-
12.5	13.0	-

Table: 6 – Antifungal activity and zone of inhibition of ethylacetate extract of *Cassia surattensis*. Burm.f on selected fungal pathogens.

Different concentration of ethylacetate extract (mg/disc) with negative control	Measurement of zone of inhibition (mm in diameter)	
	A. niger	*C. albicans*
Negative control	-	-
6.25	7.0	5.0
8.75	8.0	5.0
10.0	9.0	7.0
11.25	9.0	7.0
12.5	15.0	20.0

Table: 7 – Antifungal activity and zone of inhibition of ethanol : ethylacetate extract of *Cassia surattensis*. Burm.f on selected fungal pathogens.

Different concentration of ethanol : ethylacetate extract (mg/disc) with negative control	Measurement of zone of inhibition (mm in diameter)	
	A. niger	*C. albicans*
Negative control	-	-
6.25	6.0	7.0
8.75	8.0	9.0
10.0	10.0	10.0
11.25	10.0	10.0
12.5	12.0	12.0

Table: 8 – Antibacterial activity and zone of inhibition of ethanolic extract of *Rhinacanthus nasutus* (L) Kure on selected bacterial pathogenic strains.

Different concentration of ethanolic extract (mg/disc) with negative control	Measurement of zone of inhibition (mm in diameter)				
	S. aureus	*B. subtilis*	*E. coil*	*P. aeruginosa*	*K. pneumonia*
Negative control	-	-	-	-	-
6.25	7.0	-	6.0	6.0	-
8.75	8.0	-	7.0	8.0	-
10.0	9.0	-	7.0	8.0	-
11.25	11.0	-	8.0	8.0	-
12.5	13.0	14	8.0	13.0	8.0

Table: 9 – Antibacterial activity and zone of inhibition of ethylacetate extract of *Rhinacanthus nasutus* (L) Kure on selected bacterial pathogenic strains.

Different concentration of ethylacetate extract (mg/disc)with negative control	Measurement of zone of inhibition (mm in diameter)				
	S. aureus	*B. subtilis*	*E. coil*	*P. aeruginosa*	*K. pneumonia*
Negative control	-	-	-	-	-
6.25	4.0	-	6.0	5.0	-
8.75	7.0	-	7.0	7.0	-
10.0	6.0	-	7.0	7.0	-
11.25	7.0	-	9.0	8.0	-
12.5	10.0	-	15.0	12.0	-

Table: 10 – Antibacterial activity and zone of inhibition of ethanol : ethylacetate extract of *Rhinacanthus nasutus* (L) Kure on selected bacterial pathogenic strains.

Different concentration of ethanol: ethylacetate extract (mg/disc) with negative control	Measurement of zone of inhibition (mm in diameter)				
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*K. pneumonia*
Negative control	-	-	-	-	-
6.25	6.0	-	6.0	4.0	-
8.75	9.0	-	6.0	6.0	-
10.0	9.0	-	8.0	7.0	-
11.25	10.0	-	10.0	8.0	-
12.5	12.0	-	12.0	10.0	-

Table: 11 – Antifungal activity and zone of inhibition of ethanolic extract of *Rhinacanthus nasutus* (L) Kure on selected fungal pathogens.

Different concentration of ethanolic extract (mg/disc) with negative control	Measurement of zone of inhibition (mm in diameter)	
	A. niger	*C. albicans*
Negative control	-	-
6.25	9.0	10.0
8.75	9.0	12.0
10.0	10.0	13.0
11.25	11.0	15.0
12.5	11.0	18.0

Table: 12 – Antifungal activity and zone of inhibition of ethylacetate extract of *Rhinacanthus nasutus* (L) Kure on selected fungal pathogens.

Different concentration of ethylacetate extract (mg/disc) with negative control	Measurement of zone of inhibition (mm in diameter)	
	A. niger	*C. albicans*
Negative control	-	-
6.25	7.0	4.0
8.75	8.0	6.0
10.0	10.0	9.0
11.25	10.0	9.0
12.5	12.0	15.0

Table.13. Antimicrobial activity and zone of inhibition of commercially available antibiotics Gentamycin, Ciprofloxacin (5μg/disc) and Clotrimazole (10μg/disc).

Name of the organisms	Measurement of zone of inhibition (mm in diameter)		
	Gentamycin	Ciprofloxacin	Clotrimazole
S. aureus	7mm	9mm	-
B. subtilis	12mm	30mm	-
E. coli	10mm	15mm	-
P. aeruginosa	13mm	16mm	-
K. pneumonia	15mm	25mm	-
C. albicans	-	-	20mm
A. niger	-	-	10mm

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