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RESEARCH ARTICLE

STATUS AND DIVERSITY OF BIRD SPECIES IN GOVERNMENT COLLEGE CAMPUS IN CHITTUR OF PALAKKAD, KERALA

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ABSTRACT

A study on the status and Diversity of birds in Government College Campus, Chittur, Palakkad, Kerala was conducted from July, 2018 to February, 2019. A total of 35 birds species belonging to 32 genera, 26 families and 9 orders were encountered. The results of the present study confirm the findings of previous studies that local vegetation and habitat characteristics such as densities of shrubs and mixed vegetation in the Govt. College Campus, Chittur influenced bird species richness and diversity.

Keywords: Bird, diversity, richness, checklist, Chittur, Palakkad

1. INTRODUCTION

Birds play an important role in the ecosystem. The birds are cosmopolitan distribution that is found all over the world except South Pole. Birds are highly mobile vertebrates and easily observed indicators of change (Graber and Graber, 1976, Morrison, 1986). They occupy almost all places of highest altitudes, high peaks, deserts, jungles, seas, caves etc. Recently, birds are being studied based on field observations concerning wider domain of avian natural history , amongst others, including diversity, habitat, distribution on local, regional and continental basin etc. Ninety percent of the birds in the World had been discovered and described by 1850 (Fisher 1954). Similarly, the Ali et al (1983) studied detailed study on bird species in the Indian Sub-continent. The bird species diversity and species richness of the Government College Campus , Chittur, in Palakkad district of Kerala has been least studied. The present study was carried out for reporting the avifaunal diversity and richness of this campus with various kinds of ecosystems or habitats and also to prepare a checklist of birds.

2. MATERIALS AND METHODS

The Palakkad district popularly known as the 'ricebowl of Kerala' lies close to the Palghat Gap, the major gap in the Western Ghats which connects Kerala to the plains of the Tamil Nadu in the east Parts of the district experience a dry climate when compared to the other districts of Kerala due to its unique geographical position. The study was done in Government College Campus, Chittur , Palakkad district was located between 10.6890 N 76.72340 E of Kerala, which started functioning in the present forty acre campus on the bank serene Shokanashini (also known Chittur puzha) since 1954. The college is 17km away from Palakkad town where it will take less than one hour to reach the college from town. The Kannadi puzha river (called as Shokanashini) is one of the main tributaries of the Bharathapuzha River, the second largest river in Kerala of Southern India . A portion of River is flowing through the side of the campus. It irrigates a major portion of the Palakkad district and is also a source of drinking water of Chittur Taluk . The study area was selected based on different vegetation types in order to understand the avian diversity and species richness in and around the campus.

Map showing the study area in Govt. College Campus, Chittur, Palakkad.

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Study method

The random transect method was used to study the birds species in varied habitats namely Eucalyptus plantations , Open places (including ground, paddy fields, swamps and mixed vegetation and wetland habitat respectively. The census was started half an hour after sunrise in all the seasons. The birds were observed by using binocular (10 x 25) and photographs also taken for further identified and birds were identified by using field guide (Ali et al., 1983). The checklist of the birds was prepared based on the Asheeh Pittie (2001).

3. RESULTS

A total of 35 species comprises of 26 families and nine orders of terrestrial and semi aquatic birds were recorded during the present study. Among 32 resident species of birds, three winter migrants bird species were recorded (Annexure.1). The highest birds species diversity and richness was recorded the month of October, 2018 (Figure 1).

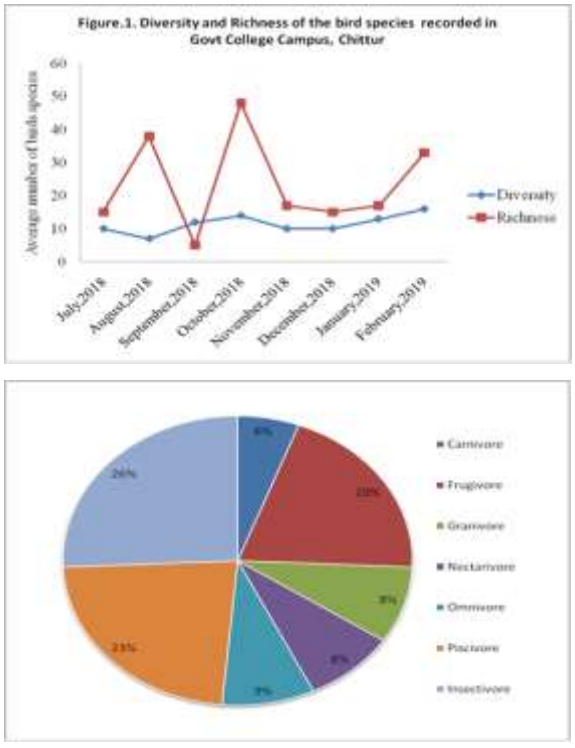

Fig. 2. Pie chart showing percentage of various types of feeding of birds in Govt. College Campus, Chittur, Palakkad, Kerala

The family wise percent occurrence showed that the bird families corvidae, nectariniidae, ardeidae, accipitridae followed by columbidae had value with 8.71 % (Table 1). However based on feeding guilds the maximum number of Piscivores birds were recorded followed by Nectarivores, Frugivores, Granivores and Carnivores, respectively (Figure 2).

Table 1. Family wise percent occurrence of birds species recorded in the Govt College, Chittur, Palakkad, Kerala.

S. No.	Name of the Family	Percent Occurrence
1	Phalacrocoracidae	2.87%
2	Anhingidae	2.87%
3	Ardeidae	5.71%
4	Ciconiidae	2.87%
5	Threskiornithidae	2.87%
6	Accipitridae	5.71%
7	Phasianidae	2.87%
8	Columbidae	5.71%
9	Cuculidae	2.87%
10	Alcedinidae	5.71%
11	Meropidae	2.87%
12	Megalaimidae	2.87%
13	Picidae	2.87%
14	Laniidae	2.87%
15	Corvidae	8.57%
16	Oriolidae	5.71%
17	Monarchinae	2.87%
18	Dicruridae	2.87%
19	Mucicapidae	2.87%
20	Sturnidae	2.87%
21	Pycnonotidae	2.87%
22	Leiothrichidae	2.87%
23	Nectariniidae	8.57%
24	Estrididae	2.87%
25	Chloropseidae	2.87%
26	Acrocephalidae	2.87%

Annexure.1. Checklist of Bird Species was recorded in Govt Arts College Campus of Chittur, Kerala.

S. No.	Family	Common Name	Scientific Name	Feeding Guild	IUCN Status	Migrant Status
		Order : Suliformes				
1	Phalacrocoracidae	Little Cormorant	*Phalacrocorax niger*	P	LC	R
2	Anhingidae	Oriental Darter	*Anhinga melanogaster*	P	NT	R
		Order: Ciconiiformes				
3	Ardeidae	Cattle Egret	*Bubulcus ibis*	P	LC	R
4		Indian Pond Heron	*Ardeola grayii*	P	LC	R
5	Ciconiidae	Asian Openbill-Stork	*Anastomus oscitans*	P	LC	WM
6	Threskiornithidae	Black-headed Ibis	*Threskiornis melanocephalus*	P	NT	R
		Order: Falconiformes				
7	Accipitridae	Black Kite	*Milvus migrans*	C	LC	R
8		Brahminy Kite	*Haliastur indus*	C	LC	R
		Order: Galliformes				
9	Phasianidae	Indian Peafowl	*Pavo cristatus*	O	LC	R
		Order: Columbiformes				
10	Columbidae	Blue Rock Pigeon	*Columba livia*	G	LC	R
11		Spotted Dove	*Streptopelia chinensis*	G	LC	R
		Order: Cuculiformes				
12	Cuculidae	Greater Coucal	*Centropus sinensis*	I	LC	R
		Order: Coraciiformes				
13	Alcedinidae	White –throated Kingfisher	*Halcyon smyrnensis*	P	LC	R
14		Stork-Billed Kingfisher	*Pelargopsis capensis*	P	LC	R
15	Meropidae	Blue-tailed Bee-eater	*Merops philippinus*	I	LC	WM
		Order: Piciformes				
16	Megalaimidae	White-cheeked Barbet	*Psilopogon viridis*	F	LC	R
17	Picidae	Black-rumped Flameback	*Dinopium benghalense*	F	LC	R
		Order: Passeriformes				
18	Laniidae	Brown Shrike	*Lanius cristatus*	I	LC	R
19	Corvidae	Indian House Crow	*Corvus splendens*	O	LC	R
20		Large-billed Crow	*Corvus macrorhynchos*	O	LC	R
21		Rufous Treepie	*Dendrocitta vagabunda*	F	LC	R
22	Oriolidae	Black-headed Oriole	*Oriolus larvatus*	F	LC	RM
23		Indian Golden Oriole	*Oriolus kundoo*	F	LC	RM
24	Monarchinae	Indian Paradise-Flycatcher	*Terpsiphone paradisi*	I	LC	RM
25	Dicruridae	Black Drongo	*Dicrurus macrocercus*	I	LC	R
26	Mucicapidae	Oriental Magpie-robin	*Copsychus saularis*	I	LC	R
27	Sturnidae	Common Myna	*Acridotheres tristis*	F	LC	R
28	Pycnonotidae	Red-whiskered Bulbul	*Pycnonotus jocosus*	F	LC	R
29	Leiothrichidae	White-headed Babbler	*Turdoides leucocephala*	I	LC	R
30	Nectariniidae	Purple-rumped Sunbird	*Nectarinia zeylanica*	N	LC	R
31		Purple Sunbird	*Nectarinia asiatica*	N	LC	R
32		Little Spiderhunter	*Arachnothera longirostra*	N	LC	R
33	Estrididae	Tri-coloured Munia	*Lonchura malacca*	G	LC	R

Feeding Guild:

C-Carnivore; F-Frugivore; G-Granivore;
I-Insectivore; N-Nectarivore; O-Omnivore;
P-Piscivore

Status:
R-Resident; RM-Resident Migrant; WM-Winter Migrant

IUCN Category:
LC-Least Concern; NR-Near Threatened

4. DISCUSSION

Community ecology studies on birds that discuss the need to address structural features of habitat for better understanding of avian communities (Jayson, 2000) are some of the seminal works in this field. Forests are home to 80% of terrestrial biodiversity. In Aves, forest structure is a key feature in habitat selection because it plays an important role in their life history (Cody 1985; Karr 1989). Forest height, tree species diversity, bark textures, snags and dead wood, fruit types, leaf characteristics, other dependent plants, gaps, and edges are some of the structural that influence bird assemblages (Karr 1976). Foliage height diversity has been used to explain increasing diversity of birds in forests with increasing height and vertical structural diversity because plant communities of increasing size, diversity, and structure support greater variety of available niches (Crowell et al.1962). The abundance, richness and diversity of these communities can be related to heterogeneity and complexity of the habitat (Fernandez and Gentile 1999). Similarly the birds were attracted by tree species in the habitat such as *Ziziphus jujube* (jujube tree) were attracted by frugivorous species such as White- headed babbler, Orioles, Rufous treepie , etc., *Albizia saman* (Rain tree), *Eucalyptus globulus* (Eucali), *Mangifera indica* (mango tree) *Terminalia catappa* (Indian almond tree), *Cocos nucifera* (coconut tree), *Azadirachta indica* (Neem trees) and *Tectona grandis* (Teak) were attracted insectivores birds. In addition the wetland and paddy field habitats attracted wetland birds. Soladoye et al (2016) was conducted a study in the Akoka Campus University of Lagos. The wetland habitats of Kerala are under severe stress as seen at the global level. They are unique in the context of their diversity and are a natural abode for several species of birds (Nameer,1994). Recently (Praveen, 2015) gave detailed checklist birds in Kerala. it is observed that 94.28% of birds species fall into the Least Concern Category by IUCN (International Union for Conservation of Nature) while the two bird species like Oriental Darter and Black-headed ibis were recorded in the campus are categorized as Near threatened birds IUCN (2015). This depicts the need for conserving those species at the verge of threats or even may be extinct in the near future.

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RESEARCH ARTICLE

A STUDY ON THE GROWTH CHARACTERISTICS OF *PLEUROTUS SAJOR-CAJU* WITH VARYING SUBSTRATE STERILIZATION METHODS AND DAYS OF SPAWN MATURITY

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ABSTRACT

The research work carried out investigated the influence of various substrate sterilization methods and days of spawn maturity on the growth characteristics of *Pleurotus sajor-caju*, grey oyster mushroom. The paddy straw substrate was sterilized by various sterilization methods such as chemical sterilization, boiling water sterilization and steam sterilization. The spawn of the mushroom produced were used at three different days of maturity viz. 25 days, 35 days and 45 days. The substrate and spawn inoculated beds were maintained at defined environmental conditions in the mushroom cultivation chamber and the growth performance were monitored and the characteristics were recorded. The study revealed that the steam sterilized substrate and the 35 days old spawn gave the maximum yield compared to the beds inoculated with substrates sterilized with other two methods of sterilization and spawn of 25 days and 45 days old. The results gave a reliable information regarding the preference of the substrate sterilization method and the days of spawn maturity for the cultivation of the *Pleurotus sajor-caju*, grey oyster mushroom.

Keywords: *Pleurotus sajor-caju*, spawn run, paddy straw, sterilization, cultivation.

1. INTRODUCTION

Mushrooms are defined as macrofungi with distinctive and visible fruiting bodies that may grow above or below ground (1). Higher Basidiomycetes represent a taxonomically, ecologically, and physiologically extremely diverse group of eukaryotic organisms. Recently, extensive research on these fungi has markedly increased, mainly due to their potential use in a variety of bio¬technological applications, particularly for the pro¬duction of food, enzymes, dietary supplements, and pharmaceutical compounds (2-4). It is estimated that there are approximately 1.5 million species of mushrooms in the world of which approximately 70,000 species are described. About 10,000 of the known species belong to the macrofungi of which about 5,000 species are edible and over 1,800 species areconsidered to have medicinal properties (5).

Mushroom is being widely used as food and food supplements from ancient times. They are increasingly being recognized as one of the important food items for their significant roles in human health, nutrition and diseases (6). Mushrooms are recognized as the alternative source of good quality protein and are capable of producing the highest quantity of protein per unit area and time from the worthless agro-wastes (7). Mushrooms can substantiate the sufferings from malnutrition to some extent, because they produce large quantities in a short time and provide more protein per unit area than other crops (8).

Edible mushroomsare widely consumed in many countries as a food. Owing to their attractive taste, aroma and nutritional values, edible mushrooms are valuable components of the diet, whose culinary and commercial value is mainly due to theirorganoleptic properties such as their texture and flavour, being possible to distinguish edible mushroom species on the basis of their characteristic odour or aroma (9,10). Their nutritional value is due to high protein, fiber, vitamin and mineral contents and a low-fat level (11-14). The amino acid compositions of mushroom proteins are comparable to animal protein (15,16) which is of particular importance to counterbalance a high consumption of protein animal food sources, especially in developed countries. In addition, edible mushroomscharacteristically contain many different bioactive compounds such as eritadenine and phenolic compounds (13,14,17).

Cultivation of edible mushrooms might be the only current process that combines the production of protein-rich food with the reduction of environmental pollution (18). It represents one of the most efficient biotechnological processes for lignocellulosic organic waste recycling (19).

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The present study was designed to study the effect of various substrate sterilization methods viz. chemical sterilization, boiling water sterilization and steam sterilization and days of spawn maturity on the growth parameters of *Pleurotus sajor-caju*.

2. MATERIALS AND METHODS

2.1. Mushroom culture

The culture of *Pleurotus sajor-caju* was procured from Vijaya Mushrooms, Coimbatore. The species was sub cultured and maintained in Potato dextrose agar medium at room temperature as slants and in pertriplates (20).

2.2. Mushroom spawn production (21)

The mushroom spawn was prepared on white sorghum grain. The mature grain procured from local market was well cleaned and boiled in water for 30 min. The boiled grain was mixed with 2% calcium carbonate. 300g of calcium carbonate mixed grain was filled in polypropylene bags of size 11inch x 5 inch and sterilized for 15 psi for one hour. The sterilized bags were cooled to room temperature and inoculated with the mushroom culture maintained in slants. The culture inoculated bags were kept undisturbed at room temperature for mycelium running. After mycelium running, spawn at different age levels viz. 25 days, 35 days and 45 days were used for preparation of mushroom beds.

2.3. Cultivation technology (22)

Paddy straw was chosen as the substrate for cultivation of P.sajor-caju. The cut paddy straw was soaked in water overnight and washed in water thoroughly. Sterilization is being done in three ways. In chemical sterilization of paddy straw, chopped paddy straw was soaked in water containing formalin, bavastin and malathion overnight. In boiling water sterilization, Paddy straw was allowed to boil in water for 45 min. In steam sterilization of substrate, the washed paddy straw was steam sterilized for 45 min and shade dried. The matured spawn of *Pleurotus sajor-caju* was taken and dispersed carefully in a sterile bowl. The polypropylene bag was taken and initially, a handful of paddy straw was taken and dispersed at the bottom of the bag which forms the first layer. A handful of spawn was taken and dispersed over the first layer of paddy straw. Thus, first layer is made. Next, a layer of paddy straw was made with the spawn spreaded over it. Likewise, alternate layers were made with spawn and paddy straw. The packed bag was tied with the rope and hanged in the mushroom unit for mycelium spreading. The mushroom unit was maintained at a temperature of 18 – 23 °C.

Bioefficiency (%) = Yield of fresh mushroom (g) / Total weight of dry substrate used (g) x 100

2.4. Statistical analysis

Statistical analysis was carried out at 5% significance level using SPSS package version 20.0. One way ANOVA followed by DMRT analysis of LSD was done.

3. RESULTS AND DISCUSSION

The cultivation of *Pleurotus sajor-caju* was done by following three different substrate sterilization methods viz. Chemical sterilization, boiling water sterilization and steam sterilization. The spawn used for cultivation were of 25 days, 35 days and 45 days old spawn. The days of spawn run, days of pin headed appearance, days for first harvest, days for second harvest, days for third harvestwere recorded and tabulated as follows.

Table. 1 Effect of various substrate sterilization methods and days of spawn maturity on the days for spawn run of *Pleurotus sajor-caju*

Days of spawn maturity	Chemical sterilization	Boiling water sterilization	Steam sterilization
25	15.17 ± 0.29^{be}	14.83 ± 0.76^{be}	13.83 ± 1.04^{bd}
35	14.17 ± 0.58^{af}	13.33 ± 0.29^{ae}	12.67 ± 0.29^{ad}
45	14.83 ± 0.29^{be}	14.17 ± 0.76^{be}	13 ± 0.87^{abd}

All the values are expressed as mean ± SD; n=6

Mean values in the same column followed by different alphabets (a-c) and mean values followed by different alphabets (d-f) in the same row in the superscripts are significantly different ($P<0.05$, ANOVA, DMRT).

The days for spawn run of *Pleurotus sajor-caju* in the packed mushroom beds varied with different substrate sterilization methods and also with the different age of spawn. In the 25 day aged spawn inoculated beds, spawn run took 15.17±0.29 days in chemically sterilized substrate, 14.83±0.76 days in boiling water sterilized substrate and 13.83±1.04 days in steam sterilized substrate. In the 35 day aged spawn inoculated mushroom beds; the spawn run was on 14.17±0.58days in chemically sterilized substrate, 13.33±0.29days in boiling water sterilized substrate and 12.67±0.29

days in steam sterilized substrate. Similarly, the 45 day aged spawn inoculated beds took 14.83±0.29days, 14.17±0.76 days, and 13±0.87 days for spawn in chemically sterilized substrate, boiling water sterilized substrate and steam sterilized substrate respectively. The days of spawn run in the 35 day aged spawn inoculated beds with paddy straw sterilized chemically was significant with the days of spawn run recorded in 25 day and 45 day aged spawn inoculated beds with substrate sterilized chemically. Similarly, days for spawn run in the 35 day aged spawn inoculated beds with boiling water sterilized substrate was also statistically significant with that of 45 day and 25 day aged spawn inoculated beds. The days of spawn run in the 35 day aged spawn inoculated beds with steam sterilized substrate was almost same with that of 45 day aged spawn inoculated and does show any significance, whereas it was significant with 25 day aged spawn inoculated beds.

Table. 2 Effect of various substrate sterilization methods and days of spawn maturity on the days for pin headed appearance of *Pleurotus sajor-caju*

Days of spawn maturity	Chemical sterilization	Boiling water sterilization	Steam sterilization
25	16.67±0.29^{be}	16.67±0.29^{ce}	15.17±0.76^{bd}
35	15.33±0.29^{af}	14.83±0.29^{ae}	14.33±0.29^{ad}
45	16.67±0.29^{be}	15.67±0.76^{bd}	15.17±0.29^{bd}

All the values are expressed as mean ± SD; n=6

Mean values in the same column followed by different alphabets (a-c) and mean values followed by different alphabets (d-f) in the same row in the superscripts are significantly different (P<0.05, ANOVA, DMRT).

The days of pin headed appearance in the beds with substrate sterilized by three different methods and effect of age of spawn maturity on the same was tabulated in the table.19. The 35 day aged spawn inoculated beds showed pin headed structures soon as compared to 25 day and 45 day aged spawn inoculated beds. 35 day aged spawn inoculated beds showed pin headed structures during 15.33±0.29 days, 14.83±0.29 days and 14.33±0.29 days in chemical, boiling water and steam sterilized substrates respectively, whereas 45 day aged spawn inoculated beds pin headed appearance took place during 16.67±0.29 days, 15.67±0.76 days and 15.17±0.29 days in chemical, boiling water and steam sterilized substrates respectively. Similarly, in the 25 day aged spawn inoculated beds, the pin headed structures appeared in the range of 15.17±0.76days to 16.67±0.29 days. Among the methods of substrate sterilization, steam sterilization was found to be effective as pin headed structures appeared first only in the steam sterilized substrate bed irrespective of spawn age and as age of spawn maturity is concerned, 35 day aged spawn was vigour enough in producing pin headed structures before 45 day and 25 day aged spawn irrespective of substrate sterilization method. The days of pin headed appearance in the 35 day aged spawn inoculated beds with substrates sterilized by all the three methods were statistically different with that of 45 day and 25 day aged spawn inoculated beds of all three substrate sterilization methods.

Table.3 Effect of various substrate sterilization methods and days of spawn maturity on the days for first harvest of *Pleurotus sajor-caju*

Days of spawn maturity	Chemical sterilization	Boiling water sterilization	Steam sterilization
25	17.83±0.76^{be}	17.67±0.29^{ce}	16.67±1.04^{bd}
35	16.5±0.5^{ae}	15.83±0.76^{ad}	15.5±0.5^{ad}
45	17.83±0.29^{be}	16.5±0.5^{bd}	16.83±0.29^{bd}

All the values are expressed as mean ± SD; n=6

Mean values in the same column followed by different alphabets (a-c) and mean values followed by different alphabets (d-f) in the same row in the superscripts are significantly different (P<0.05, ANOVA, DMRT).

Table. 4 Effect of various substrate sterilization methods and days of spawn maturity on the days for second harvest of *Pleurotus sajor-caju*

Days of spawn maturity	Chemical sterilization	Boiling water sterilization	Steam sterilization
25	26.17±1.61^{bf}	24.33±1.04^{be}	22.33±1.04^{bd}
35	23.17±1.04^{af}	21.83±1.04^{ae}	20.17±0.76^{ad}
45	25±0.5^{be}	21.67±0.76^{ad}	21.83±1.04^{bd}

All the values are expressed as mean ± SD; n=6

Mean values in the same column followed by different alphabets (a-c) and mean values followed by different alphabets (d-f) in the same row in the superscripts are significantly different (P<0.05, ANOVA, DMRT).

The days of first, second and third harvest of *Pleurotus sajor-caju* in all the three different substrate sterilization methods and effect of different spawn age on the production was tabulated in Tables 3,4 and 5.

3.1. Chemical sterilization method

In the 25 day aged spawn inoculated beds, the first harvest were during17.83±0.76days, second harvest during 26.17±1.61days and third harvest during 29.17±0.76days, where as in 35 day spawn inoculated beds, the three harvests were on a range from 16.5±0.5 days to 26.83±1.26days, for that of 45 day aged spawn the range of all the three harvests were from 17.83±0.29days to 28.17±0.76days.

Table. 5 Effect of various substrate sterilization methods and days of spawn maturity on the days for third harvest of *Pleurotus sajor-caju*

Days of spawn maturity	Chemical sterilization	Boiling water sterilization	Steam sterilization
25	29.17±0.76^{be}	28.17±0.76^{bd}	27.83±0.76^{bd}
35	26.83±1.26^{ad}	27.5±0.5^{ad}	26.67±1.04^{ad}
45	28.17±0.76^{bd}	27.83±0.29^{abd}	27.5±0.87^{abd}

All the values are expressed as mean ± SD; n=6

Mean values in the same column followed by different alphabets (a-c) and mean values followed by different alphabets (d-f) in the same row in the superscripts are significantly different (P<0.05, ANOVA, DMRT).

3.2. Boiling water sterilization method

The first, second and third harvests in the 25 day aged spawn inoculated beds were during 17.67±0.29days, 24.33±1.04days and 28.17±0.76 days respectively. In 35 day aged spawn inoculated beds; first, second and third harvests were during 15.83±0.76days, 21.83±1.04days and 27.5±0.5days respectively. For 45 days aged spawn inoculated beds, the three harvests ranged from 16.5±0.5days to 27.83±0.29days.

3.3. Steam sterilization method

In the steam sterilization method, the first, second and third harvests of the mushroom in the 35 day aged spawn inoculated beds were during 15.5±0.5 days, 20.17±0.76 days and 26.67±1.04 days respectively. In 25 day aged spawn inoculated beds; first, second and third harvests were during 16.67±1.04 days, 22.33±1.04 days and 27.83±0.76 days respectively. In 45 day aged spawn inoculated beds, the three harvests ranged from 16.83±0.29 days to27.5±0.87 days.

In the first harvest, days of harvest in the 35 day aged spawn inoculated beds with substrates sterilized by all the three methods were almost significant with that of the days of harvest recorded in the 45 day and 25 day aged spawn inoculate beds with substrates sterilized by all the three methods.

In the second harvest also, the days of harvest in the 35 day aged spawn inoculated beds of all the three substrate sterilization methods were almost significant with that of the 45 day and 25 day aged spawn inoculated beds.

Like days of first and second harvest, days of harvest in 35 day aged spawn inoculated beds were almost significant with that of rest of the treatments.

Chang and Hayes, 1978 (23) stated that successful cultivation of mushroom often requires pasteurization of the substrate, prior to inoculation with spawn. Among all the three substrate sterilization methods, steam sterilized substrate gave better results followed by boiling water and chemical sterilized substrates. The better results of *Pleurotus sajor-caju* in steam sterilized substrate can be attributed to the fact that steam sterilized substrate retains all the nutrients and makes it available to the mushroom growth.

4. CONCLUSION

Biological approaches based on industrial and environmental biotechnology is focusing on the development of “clean technologies" which emphasizes on the maximum production, reduced waste generation, treatment and conversion of waste in some useful form (24). Mushroom cultivation is one of such component which addresses the issues of waste utilization, nutritional product formation and economical stability for the population involved in it.

The cultivation of oyster mushrooms is popular in our country and it has more advantages than any other types of mushroom in the way that it is disease resistant, simpler cultivation technology, wide variety of substrate utilization and gained popularity among the common mass. So the substrate sterilization methods also play a role in the growth of the mushrooms. As an inference of this study, it is clear that steam sterilized substrate is better for the optimum growth of mushrooms in a more convenient way.

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RESEARCH ARTICLE

THE STUDY ON FRESHWATER FISH BIODIVERSITY OF UKKADAM (PERIYAKULAM) AND VALANKULAM LAKE FROM COIMBATORE DISTRICT, TAMIL NADU, INDIA

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ABSTRACT

Wetlands of India preserve a rich variety of fish species. Globally wetlands as well as fauna and flora diversity are affected due to increase in anthropogenic activities. The present investigation deals with the fish bio-diversity of selected major wetlands Periyakulam famously called Ukkadam Lake, Singanallur Lake and Sulur Lake of Coimbatore district fed by Noyyal River. Due to improper management of these lentic wetlands water bodies around Coimbatore district by using certain manures, insecticides in agricultural practices in and around these selected areas has polluted the land and these fresh waters creating hazards for major vertebrate fishes which are rich source of food and nutrition, an important and delicious food of man. The results of the present investigation reveals the occurrence of 19 fish species belonging to 5 order, 8 families 18 species recorded from the Ukkadam wetland followed by Singanallur wetland with 5 different orders 7 different families and 14 species. *Ichthyofaunal* diversity of Sulur wetland compressed of 6 families with 14 species. The order *Cypriniformes* was found dominant followed by *Perciformes, Ophicephalidae, Siluriformes* and *Cyprinodontiformes* species in Ukkadam and Singanallur wetland lakes while in Sulur it was recorded as *Cyprinidae* > *Cichlida* > *Ophiocephalidae* > *Anabantidae* > *Bagridae* > *Heteropneustidae*. This study on the freshwater fish species diversity of these wetlands would help in explore the fish fauna status and estimate the factors that may need rectification for fish conservation and management.

Keywords: Freshwater, Fish species, Biodiversity, Valankulam Lake and Ukkadam Lake.

1. INTRODUCTION

Water is the elixir of life, an expensive gift of nature to mankind and millions of other species living on the earth. It is fast becoming a scare commodity in most part of the world. Due to the urbanization and expending economic activities, nearly 13% of the world's populations do not have to access the safe drinking water. Wetlands situated in the vicinity of the cities generally undergo rapid degradation due to various factors related with city development such as waste dumping, industries and large-scale reclamation for other uses. Globally, wetland is estimated to cover 5-10% of the earth's terrestrial surface (Mitsch and Gosselink 2007).Coimbatore being a rapidly developing city in the western part of Tamil Nadu, has several wetlands and lakes in and around its limits. These wetlands have been facing rapid degradation due to liquid or solid waste disposal, filling and reclamation, real-estate ventures and industrial development has open drainage and sewerage systems which joining these lakes without any prior treatment. Hence, the present review was undertaken in Coimbatore on urban lakes wetlands to know about water quality of these water bodies with reference to the pollution from various sources. It is also one of the fastest growing cities in Tamil Nadu, India that has around 28 wetlands in and around the city which are fed by the river Noyyal. These wetlands serve as storage and percolation tanks. They are the major recharge sites for groundwater (Rachna et al., 2010).

Fish constitutes almost half of the total number of vertebrates in the world. They live in almost all conceivable aquatic habitats. They exhibit enormous diversity of size, shape and biology, and in the habitats they occupy. Of the 39,000 species of vertebrates in the world, Nelson (2006) estimated 21,723 extant species of fish under 4,044 genera, 445 families and 50 orders in the world, compared to 21,450 extant tetra pods. Of these 8411 are freshwater species and 11650 are marine. Day (1889) described 1418 species of fish under 342 genera from the British India. Freshwater fish has been identified a suitable tool for biological assessment due to its easy identification and economic value measure has to been take to conserve these bioindicators. In order to preserve the freshwater fish diversity that is declining rapidly each day due to unending anthropogenic stress in the wetland bodies it is necessary to conserve these wetland water bodies of Coimbatore district. Categories and report on the freshwater fish diversity in selected wetlands Ukkadam Lake and Valankulam Lake of Coimbatore district fed by Noyyal River.

Ukkadam lake is spread over an area of 1.295 km^2 (0.500sq mi) and has an average depth of 5.82m

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(19.1 ft) 10 58 54 " N 76 51 17 E in 2010 the lake was taken over by Coimbatore corporation on a 90 years lease from the public works department of the government of Tamil Nadu. The lake is fed by canals derived from Noyyal River. The lake has also receives water from Selvachinthamani Lake located upstream in the north. The lake has an outlet connecting it with Valankulam Lake. The water can be released through four sluice gates located on the south side of the lake. It is situated between Trichy road and Sungam bypass road connecting with Ukkadam. A railway track connecting Coimbatore junction and Podanur passes over the lake. Various birds including little grebes and purple moorhen can be seen in this lake.

Water can be obtained mainly from two sources namely surface water and ground water. Surface water is any water that travels or is stored on top of the ground. This would be the water in rivers, lakes, streams, reservoirs, and even the oceans. Rain water is the purest form of natural water, since it is obtained as a result of evaporation from the surface water. However, its downwards journey through atmosphere it dissolves a considerable amount of industrial gases like CO_2, NO_2, SO_2, etc and suspended solid particles both of organic and inorganic 3 origin. Ponds with water in large quantity for most part of the year are available in Coimbatore and also it has only a very few rivers like Siruvani to supply water. But the quality of water obtained from these ponds is not reliable because it contains suspended matter and number of other impurities. The main source of ground water is rain water. A major part of rain water is absorbed into the earth. The absorbed water percolates into the earth and goes deep down the earth. Water beneath the surface comprises the next largest store of water. Ground water and soil water together make up about 0.5% of all water (by volume).

Lakes situated in the Coimbatore city undergo rapid degradation due to the various factors related with city development, waste dumping, industries and large scale reclamation for other uses. There are around 28 lakes in and the city limits which are largely fed by the river Noyyal, flowing alongside the city. The present study has been carried out to assess the freshwater fish biodiversity of Ukkadam (Periyakulam) and Valankulam Lake from Coimbatore District and to examine the changes in water quality that have happened during the last decades.

2. MATERIALS AND METHODS

2.1 Study area

To study the freshwater fish diversity the major wetland Sukkadam and Valankulam Lake of Coimbatore district. It receives water from rain and also from catchment areas of the catchment area of the pond is about 10.75 sq. Miles. The sides of the ponds are surrounded by human settlements and markets.

Fig 1: Map showing study area

2.2. Data Collection

The study has been conducted for 4 months from October, 2018 to January, 2019. The data has been collected for a period of 4 months. Data were collected from five fishing points where the intensity was high. Data were collected after 15 days interval, twice in a month from 10 selected fishermen by asking questions from a field trailed organized questionnaire.

2.3 Sample Collection

Fish samples were collected every month during the study period from the catchment point and fish landing centres with help of skilled fishermen. Fish samples were collected through experimental fishing using cast nets (dia. 3.7m and 1.0m for collecting fish in shallow areas, monofilamentos gill nets (vertical height 1.0m 1.5m; length 100m - 150m), drag net (vertical height 2.0m) and a variety of traps. Sampling points were distributed throughout the site to cover its whole area.

Catla catla

Cirrihinus mrigala

Labeo rohita

Labeo calbasu

Labeo bata

Channa marulius

Channa striatus

Oreochromis mossambicuss

Mystus vittatus

Clarias batrachus

Gambasia affinis

2.4. Identification of Fish Species

All fishes caught were identified to species level using standard taxonomic Fishes of India, FAO identification sheets, ITIS (integrated taxonomic information system) standard report (http://www.itis.gov), and other reference book using standard keys of jeyaram (1999), Qureshi and Qureshi (1983), Talwer and Jhingran (1991), Day Francis (1994) and Shrivastava (1998).Fish base website was also referred for various aspects of fish fauna (www.fishbase.org).

2.5. Collection of Water Samples

The samples were collected from the surface of the ponds for a period of 4 months [October 2018 to January 2019] with an interval of 1 month. The samples were collected during 12.30 to 1.30 pm and this was uniformly maintained throughout the study period. The samples were

collected in collected in clean white plastic containers.

2.6. Assessment of water quality

The physico-chemical characters were analysed in Ukkadam (Periyakulam) and Valankulam Lake of Coimbatore district.

2.6.1. pH

pH is measured mathematically by, the negative logarithm of hydrogen ions concentration. The pH of natural waters is greatly influenced by the concentration of carbon dioxide which is an acidic gas (Boyd, 1979). The pH in all ponds water was alkaline throughout the experimental period. Different authors have reported a wide variation in pH from 6.7 to 8.3 (Hossain et al. 1997), 7.18 to 7.24 (Kohinoor et al. 1998), and 7.37 to 8.65 (Kohinoor et al. 2004) in fertilized fish ponds and found the ranges productive. The ranges and mean values of pH in the present study were 7.5-8 indicating the productive nature of the fertilized ponds.

2.6.2. Dissolved oxygen

Estimation of dissolved oxygen in the water samples were analysed by the Winklers method. Add 1 ml of manganese sulphate solution followed by 1 ml of alkaline iodine solution, below the surface of the water sample diplacing the water at the top. Shake well and gently allowed the solution to precipitate and settle down into the bottle. After the precipitate forms add 1 ml of concentrated sulphuric acid settling the acid to run down at the neck of the bottle, then stopper it. Shake well and see that the iodine is diffused slowly and evenly distributed, before drawing the sample for titration. Take 50 ml of the above solution is a conical flask and add 1 ml of starch solution in it. Which develops a blue in colour then titrate against 0.025 N sodium thio sulphate solution.

2.6.3. Dissolved dioxide (DO) and Biological Oxygen Demand (BOD)

Dilution water was prepared in glass container by bubling compressed air in distilled water for 30 minutes. 1ml each of phosphate buffer magnesium sulphate, calcium chloride and ferric chloride solution was added for each litre of dilution water and mixed thoroughly.The sample was neutralized to Ph 7.0 by using 1N NaOH or H_2SO_4 Required dilution were prepared in a bucket, mixed thoroughly in and filled in two set of BOD bottles One set of the BOD bottles was kept in BOD incubator at 20^0c for 5 days and another set DO was determined immediately. DO in the sample bottle was determined immediately after the complexion of 5 days incubation Similar procedure followed for the blank (dilution). BOD mgr1=DO1-DO2-BC×100/Persent of sample; DO 1-Initial dissolved oxygen DO 2 –Dissolved oxygen after 5 days of incubation; BC-Blank correction.

2.6.4. Carbonates and Bicarbonates

Take 25 ml of water sample in a clean conical flask to that add 2 drops of phenolphthalein indicator. Then the solution is titrated against 0.02N sulphuric acid taken in the burette. The end point is reached when the carbonate is neutralized and solution become colourless. To this colourless solution add 2 or 3 drops of methyl orange indicator. Now the colour changes into light orange or light pink colour. Then, this is titrated against 0.02N sulphuric acid. Till the light orange changes to dark pink colour.

2.6.5. Total alkalinity

100ml of sample was taken in a conical flask and 2 drops of phenolphthalein indicator was added. If a pink colour appeared the solution was titrate against 0.1N hydrochloric acid. If the solution remained colourless 2-3 drops of methyl orange was added to the same sample and titrate against 0.1N hydrochloric acid until the yellow colour changed to pink as the end point. The gives the total alkalinity.

3. RESULTS

Table 1: Fish diversity of Ukkadam wetland

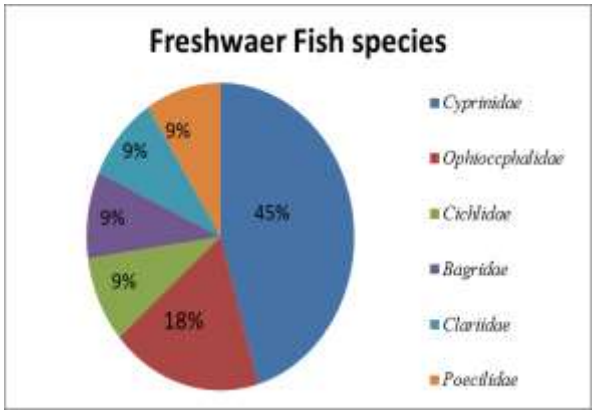

Fig: 1. Diagrammatic representation on family wise fresh water fish composition at Ukkadam Lake

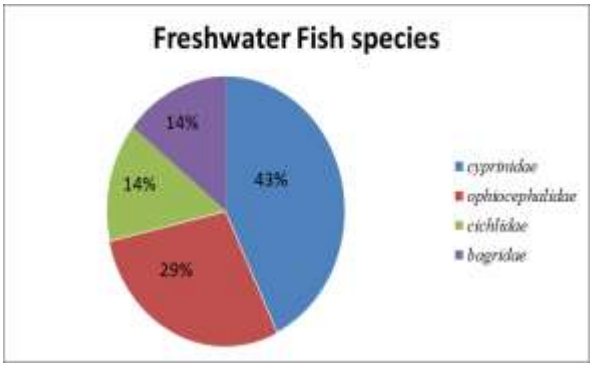

Fig 2: Diagrammatic representation of family wise fresh water fish composition at valankulam lake

Table 2: Fish diversity of Valankulam wetland

S. No	Order	Family	Species
1	*Cypriniformes*	*Cyprinidae*	*Catla catla*
			Cirrihinus mrigala
			Labeo rohita
2	*Ophiocephalif-ormes*	*Ophiocephal-idae*	*Channa marulius*
			Channa punctatus
3	*Perciformes*	*Cichlidae*	*Oreochromis mossambicus*
4	*Siluriformes*	*Bagridae*	*Mystus vittatus*
			Clarias batrachus
5	*Cyprinodontiformes*	*Poecilidae*	*Gambasia affinis*

4. DISCUSSION

The results of the present study revealed the occurrence of 11 fish species belonging to 6 families and 5 orders were recorded from the Ukkadam Lake. The order cypriniformes was dominant with 5 fish species followed by ophiocephaliformes 2 and siluriformes with 2, followed by order perciformes 1 fish species. During the present investigation in the Ukkadam wetland the order of dominance was as follows. Cypriniformes > ophiocephaliformes > siluriformes > perciformes > cyprinodontiformes. The family cyprinidae was represented by 5 species namely catlacatla, cirrhinusmrigala, labeorohita, labeocalbasu and labeobata. The family ophiocephalidae was represented by 2 species namely channamarulius and channastriatus. The order siluriformes was represented by 2 species namely mystusvittatus and clariasbatrachus. The family poecilidae was represented by 1 species namely gambasiaaffinis. The diversity of Valankulam Lake comprises of 4 families namely cyprinidae, ophiocephalidae, cichlidae and bagridae. The sequence of dominance of encountered families is as follows: Cyprinidae > ophiocephalidae > cichlidae > bagridae. On the basis of species richness, order cypriniformes was dominant with (3 species)

followed by ophiocephaliformes (2 species) perciformes (1 species) and siluriformes (1 species). The family cyprinidae was represented by 3 species namely catlacatla, cirrihinusmrigala and labeorohita. The family ophiocephalidae was represented by 2 species namely channamarulius and channapunctatus. The family cichlidae was represented by 1 species namely oreochromismossambicus. The order bagridae was represented by 1 species namely mystusvittatus.

The result of the present study will provide future strategies for development and fish fauna conservation in and around the river Noyyal fed wetlands of Coimbatore district, Tamil Nadu. Proper utilization, developing advanced techniques for fish culturing, banning illegal methods of fishing and care for propagation of fish culture and to prevent further depletion of freshwater fish resource will be highly beneficial for the socially and economically poor people of these areas. The physico-chemical characteristics of an aquatic ecosystem undergoes changes due to the action of tides, inflow of domestic and industrial effluent. During rainfall, consequently, the biological characteristics are also likely to change. Rainfall results in the inflow of fresh water from rivers and estuaries (Hale et al., 1995, 1998; Hale and Guardia, 2001). In the present study, surface water temperature showed variations between stations and increase in temperature was recorded during months of November and February. The surface water temperature in the Nuyal River also showed a corresponding increase even up to P abundance from Valankulam and Ukkadam of Coimbatore district, about ten kilometers up south from Coimbatore.

Generally, higher pH values may be attributed to sea water mixing and redox variations in sediment and water column; while lower pH values observed during month of November may be due to influx of freshwater and tide action (Panigrahy *et al*., 1999). When thermal coolant water was released, variations can be attributed to the release of coolant from the power plant. pH has a positive effect on the biotic component on an aquatic ecosystem. Stress in aquatic systems are mostly related to pH and is thought to alter the biochemical changes in the organisms that make up communities, thereby altering the way of the metabolic function (Linton and Warner, 2003). In the present study, the pH values did not show much variation between the stations and a very little difference was noticed between the seasons, if any, due to coolant water released from the power plant.

Table 3. Data on the seasonal variations in the chemical of the Periyakulam pond Coimbatore for a period of 4 months (Oct 2018 to Jan 2019).

Months	pH	DO 1	DO 2	BOD	Carbonates	Bicorbanates	Total alkalinity
October	7.50	3.50	2.90	2.40	2.33	1.90	9.10
November	7.90	2.20	3.30	2.70	2.33	10.20	12.50
December	8.45	1.28	6.60	3.80	2.80	13.20	16.00
January	8.40	1.30	6.00	3.60	2.75	12.80	15.55

Table 4. Data on the seasonal variations in the chemical of the Valankulam pond Coimbatore for a period of 4 months (Oct 2018 to Oct 2019)

Months	pH	DO1	DO2	BOD	Carbonates	Bicarbonates	Total alkalinity
October	8.50	3.48	2.90	2.40	2.33	1.90	9.10
November	7.50	2.25	3.30	2.70	2.33	10.20	12.50
December	8.20	1.29	6.60	3.80	2.80	13.20	16.00
January	8.47	1.30	6.00	3.60	2.75	12.80	15.55

In general, salinity revealed clear seasonal variations with lowest values in the monsoon season, while the higher values of salinity coincided with the November and February months during the study periods. The salinity was higher at less polluted sites indicating a gradual increase and combined effect of seawater through the creek as observed elsewhere (Padmalal and Seralathan, 1991; Babu *et al.,* 2000). Since the tidal amplitude is comparatively large, the volume of water entering

the system for freshwater run-off is small compared to tidal flushing and could be a result of high salinity zones in the less polluted sites (Hall et al., 19Dissolved oxygen recorded a minimum of 0.134 mg/L from Valankulam sites and a maximum of 0.873 mg/L in less Ukkadam sites. This could be due to the H_2S found in solution and gas effervescence originating from the bottom when disturbed (Raman, 1995). The low DO values also indicate that the waters were highly polluted during low tidal cycle, turbulence and, mixing of seawater. Low DO values may be due to the closeness of the sampling stations to the effluent discharge points in the creek, and due to the combined effect of temperature, photosynthetic action and biochemical degradation of wastes entering the freshwater ecosystem from Valankulam and Ukkadam of Coimbatore district (Ganapathi and Raman, 1973).

5. CONCLUSION

The freshwater fish biodiversity in abundance from Valankulam and Ukkadam lake of Coimbatore district, Tamil Nadu. The main aim of study is to know edible and wild fishes of the particular area and its fishery potential. The data obtained in the present is also important in variety of manners such as to know the present status of fish fauna in the local region it is helpful for the researchers as well as fishermen's to get an idea about the tolerance and diversity of fish found in both research study region from Valankulam and Ukkadam and choose exact variety of fish species for the culture so as to get maximum yield. Moreover the results also suggests that the selected wetlands are important as it provide a wide diversity of piscine fauna with good economic potential and highly significant from fisheries point of view for the fishermen living in its vicinity. Proper utilization, developing advanced techniques for fish culturing, banning illegal methods of fishing and care for propagation of fish culture and to prevent further depletion of freshwater fish resource will be highly beneficial for the socially and economically poor people of these areas. In the light of present study of wetland of Coimbatore District, Tamilnadu it is time to conserve, protect, make proper policies and take necessary steps to implement so that the future generation can get the fishes lively on earth rather than photographs in literature.

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RESEARCH ARTICLE

STRUCTURAL EVALUATION AND INSILICO STUDY OF PROTEINS OF *ASTERIAS RUBENS* - "STARFISH AS NEW SOURCE TO MARINE PROTEINS"

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ABSTRACT

Marine sources have received great attention recently; research on marine-derived molecules has discovered new bioactive compounds with vital properties increasing their applicability as nutraceuticals in the food and supplement industries. Most notably Hippocrates, the "father of modern medicine", is recorded as describing the therapeutic effects of various marine invertebrates and their constituents on human health. Astreias is an important marine of the family Asteriidae known for its variety of medicinal properties. Functional characterization of a protein sequence is one of the most frequent problems in biology. This task is usually facilitated by accurate three-dimensional (3-D) structure of the protein. The number of protein sequences that can be modeled, as well as the accuracy of the prediction, is increasing steadily because of the growth and number of known protein sequences and structures as well as improvements in the modeling software. It is currently possible to model, with useful accuracy. Significant parts of approximately one half of all known protein sequences. This research report deliver an innovative summary of bioinformatics study of *Asterias rubens* with emphasis on the current development and future directions, which shall provide tools and resources necessary to understand and uphold advances in this important field. The aim of the present study, 10 proteins of *Asterias rubens* were analysed using bioinformatics tools. Structural prediction and functional characterization of proteins of *Asterias rubens* were done using Expasy Protparm server, 3D structure was done using SWISS MODEL. The important enzymes present in *Asterias rubens* involved Reproductive function and proper growth and development of human body.

Keywords: *Asterias rubens,* Computational tools, Algae, Therapeutic effects, Bioinformatics

1. INTRODUCTION

Pharmaceutical market is growing rapidly and continuously. But, still the demand for new drug discovery is encouraged. The reason behind this motivation can be the growing numbers of drug–resistant infectious disease and more and more upcoming disorders. The terrestrial resources have been greatly explored and thus academic and industry researchers are striving to get lead molecules from the inner space of oceans. The marine resources are nowadays widely studied because of numerous reasons. One of the reason is as the oceans cover more than 70% of the world surface and among 36 known living phyla, 34 of them are found in marine environments with more than 300000+ known species of fauna and flora The rationale of searching for drugs from marine environment stem from the fact that marine plants and animals have adapted to all sorts of marine environments and these creatures are constantly under tremendous selection pressure including space competition, predation, surface fouling and reproduction [1].

Starfish (*Asterias rubens*) are probably the single most significant predator of blue mussels living on the seabed in natural mussel beds, bottom cultures or relaying areas. In areas like these, starfish can cause significant decreases in the number of live mussels that are otherwise fished and used for human consumption [2]. The occurrence of starfish in the inner Danish waters has been increasing and still is [3]. Hence, the mussel fishermen in Limfjorden, Lillebælt and Isefjorden, have reported that the mussel fishery has been plagued by substantial amounts of starfish that negatively affect the fishery. The fishermen have suggested that if they fish the starfish, they could be of benefit elsewhere [4].

Determinations of certain biologically important elements have been made on different species of the genus *Asterias*, found in their respective area in the world. Starfish contains various secondary metabolites including steroids, storoidal glycosides, anthraquinones, alkaloids, phospholipids, peptides, and fatty acids (FA). These chemical constituents exhibit cytotoxic, hemolytic, antiviral, antifungal and antimicrobial activities. Therefore, starfish is of great interest as a natural bioactive marine product and presumably in a wide variety of pharmacological activities [5].

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The sea stars are members of the phylum echinodermata, and belong to the deuterostomes that also include the vertebrates. The echinoderms have no heart, no centralized nervous system, no respiratory organs and no specialized excretory organs. They are exclusively marine and most species are unisexual [6]. *Asterias rubens* is native to the north-east of Atlantic Ocean, and is the most common starfish found in Danish waters. *Asterias rubens* produces the secondary metabolite asterosaponins, that are pentaglycoside or hexaglycoside sulfated steroids. These molecules are interesting because of their hemolytic, cytotoxic, anti-bacterial, anti-fungal, anti-viral and anti-tumor properties [7].

For some echinoderms, as well as in other animal groups, the sacrificing of body parts is a way of escape from predators. The sea star has an autotomy plane, close to the central disc, where it can "release" the attacked arm. *Asterias* has a single autotomy plane for each arm, while other echinoderms have the capacity to autotomize at various points along the whole arm length [8].

In this study ten protein sequences of *Asterias rubens* were selected and analyzed with the help of computational tools. In silico approach provide useful information by identifying the primary, secondary, tertiary structure predictions and ten proteins role in human reproductive system which can be used for further analysis.

Fig. 1. The common sea star *Asterias rubens* with three regenerating arms.

2. MATERIALS AND METHODS

2.1. Se*quence Retrieval*

The FASTA sequence of the proteins [TABLE: 1] were retrieved from Genbank database hosted by the NCBI (http://www.ncbi.nlm.nih.gov) [9].

2.2. Primary Structure Prediction

For Physio-chemical characterization, theoretical Isoelectric Point (pI), molecular weight, total number of positive and negative residues, extinction coefficient, instability index, aliphatic index and grand average of hydropathy (GRAVY) were computed using the Expasy Protparm server[10] (http://us.expasy.org/tools/protparam.html).

2.3. Secondary structure prediction

SOPMA (Self Optimized Prediction Method with Alignment) was used for the secondary structure prediction.

2.4. Functional characterization

SOSUI and TMHMM v.2.0 tools were used to characterize whether the protein is soluble or trans membrane in nature. Inter Pro is an integrated resource for protein families, domains and functional sites. Inter Pro incorporates the major protein signature databases into a single resource. These include: PROSITE, which uses regular expressions and profiles, PRINTS, which uses Position Specific Scoring Matrix-based (PSSM-based) fingerprints, ProDom, which uses automatic sequence clustering, and Pfam, SMART, TIGRFAMs, PIRSF, SUPERFAMILY, Gene3D and PANTHER, all of which use hidden Markov models (HMMs). Superfamily and molecular function were predicted by Inter pro protein sequencing and classification [11]. (http://www.ebi.ac.uk/interpro/).

2.5. Sequence Alignment

Sequence alignment of Maturase K (ALN49191.1.) was performed using pair wise sequence alignment tool (NCBI- BLAST) (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and multiple sequence alignment was done using the EBI-CLUSTAL OMEGA (http://www.ebi.ac.uk/Tools/msa/clustalo/) tool. Clustal Omega also has powerful features for adding sequences to and exploiting information in existing alignments, making use of the vast amount of precomputed information in public databases like Pfam [12]. The emphasis of this work was to find the regions of sequence similarity, which in other words allows us to yield functional and evolutionary relationships among the proteins considered in this study.

3. RESULTS AND DISCUSSION

Asterias rubens (family Asteriidae) It has been well-known for decades and around the world, that starfish are a serious pest of both

mussels and oyster-bed [13]. Reported that starfish was a serious pest of oyster-beds in the coastal waters of the north-eastern United States, and were captured in considerable quantities by fishermen engaged in oyster culture. The starfish were here grounded into meal and sold for either food for farm animals or used as fertilizer.

Table 1. Proteins of *Asterias rubens*

S. No.	Accession Number	Protein	Length
1	Q9SV11	Cytidine Mono phosphate-N-acetylene –N-acetyl neuraminic acid hydroxylase	653
2	PO2286	Histone H2B gonadal	122
3	A8DX82	Glyceraldehyde -3 –po4-Dehydrogenase	312
4	Q8IA45	Alpha Amylase	492
5	AOAOU2J6Z0	Cholecytpkinin type	`163
6	AOAOU2PX76	Tyrotrophin – releasing hormone	225
7	AOAOU2Q685	Corticotrophin releasing hormone type	130
8	AOAOU2NQTS	Gonadotropin releasing hormone	121
9	Q38JJ7	Peptidoglycon recognition Protein	195
10	AOAODMEJ9	NPS/CCAP type receptor	449

The primary structure prediction was done with the help of protparam tool (Table 2). The parameters were computed using Expasy's protparam tool which revealed that the molecular weights for two different proteins as 26249.79 (Ribulose bisphosphate carboxylase), 2590.85 (PsbA). The pI of two protein was less than 7 which indicated that they are acidic in character. The proteins are found to be compact and stable at their pI [14]. Among the two proteins one is showed instability index lesser than 40, indicating that the protein are stable.

Aliphatic index of the proteins ranged between 73.74-90.78. The computed extinction coefficients help in the quantitative study of protein–protein and protein–ligand interactions in solution. The range of GRAVY (Grand Average of Hydropathicity) of *Asterias rubens* proteins was found to be -0.324 to -0.534. The lowest value of GRAVY indicates the possibility of better interaction with water and only one protein indicates the less interaction with water. (NPS/CCAP type receptor 0.229) [15].

The secondary structure prediction of *Asterias rubens* proteins (Table-3) was analyzed by SOPMA which revealed that alpha helix, extended strand, beta turn and random coil, were more predominant. In all the two proteins alpha helix dominates which is followed by random coil, extended strand and beta turn. The secondary structure were predicted by using default parameters (Window width: 17, similarity threshold: 8 and number of states: 4). TMHMM v.2.0 and SOSUI predicted that 2 proteins were soluble protein.

The secondary structure prediction of *Asterias rubens* proteins (Table-3) was analyzed by SOPMA which revealed that alpha helix, extended strand, beta turn and random coil, were more predominant. In all the two proteins alpha helix dominates which is followed by random coil, extended strand and beta turn. The secondary structure were predicted by using default parameters (Window width: 17, similarity threshold: 8 and number of states: 4). TMHMM v.2.0 and SOSUI predicted that 2 proteins were soluble protein.

Secondary structure prediction of proteins by SOPMA revealed that α – helix, random coil, β – turn and extended strand were more prevalent. In ten protein of *Asterias rubens*, α – helix predominates, random coil region was frequent (Table: 3). In proteins, extended strand dominates followed by random coil and α – helix. Domains are evolutionary units, often identified as recurring sequence or 3D structure. Inter pro tool analysis of proteins of *Asterias rubens* revealed its super family, molecular function (Table 4).

Table 2. Parameters computed using expasy's protparam tool

S. No.	Protein	Accession number	Length	Mol.wt	PI	-R	+R	EC	II	AI	GRAVY
1	Cytidine Mono phosphate-N-acetylene –N-acetyl neuraminic acid hydroxylase	Q9SV11	653	75335.51	5.32	98	75	138240	45.96	73.74	-0.534
2	Histone H2B gonadal	PO2286	122	13591.82	10.52	9	28	7450	40.13	64.84	-0.795
3	Glyceraldehyde -3 –po4-Dehydrogenase	A8DX82	312	33628.57	7.84	35	36	31525	24.97	90.32	-0.054
4	Alpha mylase	Q8IA45	492	55186.24	5.89	51	40	112800	34.48	77.24	-0.279
5	Cholecytpkinin type	AOAOU2J6Z0	`163	18340.28	5.51	26	21	28085	36.49	72.39	-0.726
6	Tyrotrophin – releasing hormone	AOAOU2PX76	225	26645.23	8.75	38	41	90870	54.27	47.29	-1.395
7	Corticotrophin releasing hormone type	AOAOU2Q685	130	14792.01	9.57	15	19	3105	65.72	97.62	-0.419
8	Gonadotropin releasing hormone	AOAOU2NQTS	121	13872.77	5.88	16	14	23490	46.99	78.10	-0.618
9	Peptidoglycon recognition Protein	Q38JJ7	195	21339.01	9.20	12	20	32930	42.33	69.95	-0.324
10	NPS/CCAP type receptor	AOAODMEJ9	449	50056.29	9.58	25	44	75790	27.27	90.78	0.229

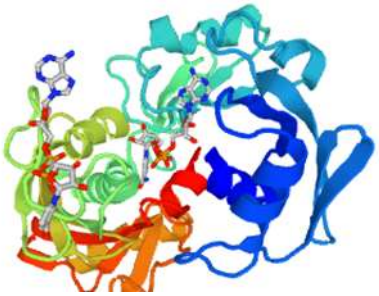
Mol. Wt – molecular weight (Daltons), pI – Isoelectric point, -R - Number of negative residues, +R – Number of Positive residues, EC – Extinction Coefficient at 280 nm, II – Instability Index, AI – Aliphatic Index, GRAVY – Grand Average Hydropathicity.

Table 3. Secondary structure results of proteins of *Asterias rubens*

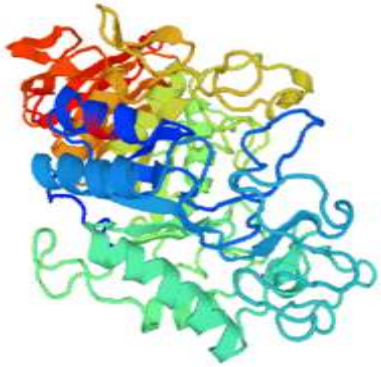
S. No.	Secondary structure	Q9SV11	PO2286	A8DX82	Q8IA45	AOAOU2J6Z0	AOAOU2PX76	AOAOU2Q685	AOAOU2NQTS	Q38JJ7	AOAOD5MEJ9
1	Alpha helix	23.74%	43.44%	32.05%	25.61%	28.22%	33.78%	28.46%	55.37%	28.72%	30.51%
2	Extended strand	24.04%	6.56%	24.36%	26.42%	21.47%	15.11%	19.23%	10.74%	17.95%	28.06%
3	Bend turn	13.02%	9.02%	12.82%	12.80%	12.88%	7.11%	43.08%	29.75%	43.08%	31.18%
4	Random coil	39.02	40.98%	30.77%	35.16%	37.42%	44.00%	43.08%	29.75%	43.08%	31.18%

1) Cytidine Mono phosphate-N-acetylene –N-acetyl neuraminic acid hydroxylase

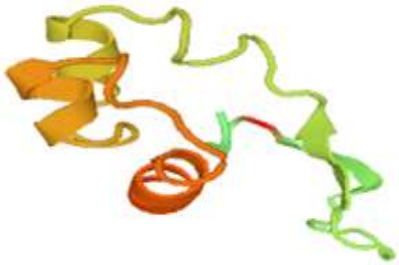
2) Histone H2B gonadal0



3) Glyceraldehyde -3 –po4-Dehydrogenase

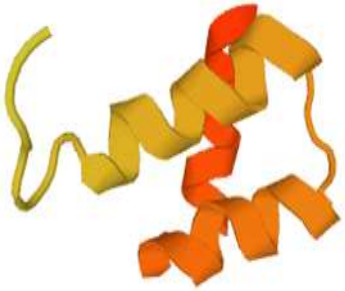


4) Alpha Amylase



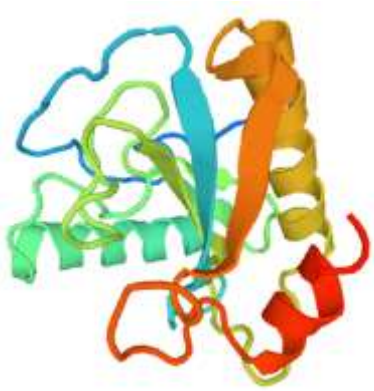
5) Cholecytpkinin type

6) Tyrotrophin – releasing hormone



7) Corticotrophin releasing hormone type

8) Gonadotropin releasing hormone



9) Peptidoglycon recognition Protein

10) NPS/CCAP type receptor

ENZYMES OF *ASTREIAS RUBENS* AND THEIR ROLE IN HUMAN

Cytidine Mono phosphate-N-acetylene –N-acetyl neuraminic acid hydroxylase:

Sialic acids are a family of more than 50 naturally occurring acidic nine-carbon backbone monosaccharides. The predominant sialic acids in mammals are N-acetylneuraminic acid (Neu5Ac) and N-glycolylneuraminic acid (Neu5Gc), which commonly occupy the terminal positions of various glycan chains. The only known biosynthetic pathway for generation of Neu5Gc takes place in the cytosol and is catalyzed by the cytidine monophospho- N-acetylneuraminic acid hydroxylase (Cmah). The Cmah enzyme is the only known biosynthetic pathway to generate the major mammalian sialic acid Neu5Gc. Besides namH, a bacterial hydroxylase, it is the only known enzyme capable of catalyzing the generation of an Nglycolyl group in nature [16].

HISTONE H2B GONADAL:

Posttranslational histone modifications are essential for proper cell function. The N-termini of histone tails contain amino acid residues that are affected by methylation, acetylation, phosphory-lation, ubiquitylation and sumoylation. The sum of these modifications and the information they communicate is referred to as the histone code. Methylation is one of the most prevalent histone posttranslational modifications. It is monitored by histone methyltransferases [HMTases] and is generally associated with gene silencing. Methylation of H3K9, for example, is a classic indication of gene silencing and is commonly found in heterochro- matin, as well as silenced promoters. In some cases, however, methylation of arginine and lysine residues can lead to gene activation. For example, methylation of histone H3K4 is implicated in gene expression. More than one methyl group may be transferred to a single amino acid residue [17]. The location and number of methyl groups in a region of DNA convey a specific epigenetic signal Histone acetylation is associated with increased levels of transcription and is modulated by both histone acetyl transferases (HATs) and histone deacetylases (HDACs). HATs activate gene expression, while HDACs inhibit gene expression. Acetylated lysines are specifically recognized by bromodomain-containing proteins and act to enhance chromatin remodeling. Phos- phorylation of histones occurs on serine residues and generally leads to gene activation [18].

GLYCERALDEHYDE 3-PHOSPHATE:

Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is a glycolytic enzyme which catalyzes the conversion of glyceraldehyde 3-phosphate to 1, 3-diphosphoglycerate. The most common form is the NAD+-dependent enzyme found in all organisms studied so far and which is usually located in the cytoplasm. In addition to its metabolic function, studies have demonstrated that GAPDH is present on the surface of several microbial pathogens and may facilitate their colonization and invasion of host tissues by interacting directly with host soluble proteins and surface ligands. Surface localization of GAPDH was first demonstrated in the Gram-positive pathogen, *Streptococcus pyogenes*. In this organism, surface-exposed GAPDH binds several mammalian proteins including the uPAR/CD87 membrane protein on pharyngeal cells], regulates intracellular host cell signalling events and contributes to host immune evasion. In addition, surface localization of GAPDH has been reported in enterohemorrhagic (EHEC) and enteropathogenic (EPEC) *Escherichia coli*; the

protein of these pathogens has been observed to bind to human plasminogen and fibrinogen, suggesting a role in pathogenesis. [19].

α –AMYLASE

α–Amylase are ubiquitous enzymes that catalyze the hydrolysis of internal α-1,4-glucosidic bonds in starch and related polysaccharides. They belong to the glycoside hydrolase family 13, one of the largest families of enzymes whose members are widespread in all three domains of life. The alpha-amylases are the calcium metalloenzymes which can't function in the absence of calcium. There are many digestive enzymes in humans and among them the most important one is pancreatic alpha-amylase, that act as a catalysis in the reaction which involves the hydrolysis of the alpha-1,4 glycosidic linkages of the starch, amylopectin, amylose, glycogen, and numerous maltodextrins and is responsible for starch digestion. The other important enzyme is alpha-glucosidase or maltase hich catalyzes the final step of the digestive process of carbohydrates mainly starch by acting upon 1,4-alpha bonds and producing glucose as the final product. The large molecules like starch cannot cross the blood brain barrier as glucose has to reach the brain thus; to overcome this problem alpha-amylase cleaves the large starch molecules into smaller fragments of sugars in order to cross the blood brain barrier. If there will be excess conversion of starch to sugars, it will increase the sugar level in blood, then the role of insulin will come into action by ordering cells to metabolize the excess sugar moieties and store as energy sources i. e. glycogen. This cycle is endlessly happening in a healthy person. But in some cases, due to excess activity of amylase enzyme and insulin deficiency or resistance to insulin, level of blood glucose arises which might results in hyperglycaemia. To control hyperglycaemia several studies on inhibition of amylase enzyme activity is being studied. However, if there will be excessive inhibition of pancreatic alpha-amylase, it might cause abnormal bacterial fermentation of undigested carbohydrates in the colon resulting in flatulence and diarrhea [20].

CHOLECYSTOKININ:

Cholecystokinin is a hormone whose primary action is to stimulate the enzyme release from pancreatic acinar cells. After food is consumed and broken down in the stomach, chime then moves to the small intestine. There are varying amounts of fat in the chyme; this fat triggers the release of hormone Cholecystokinin. The peptide hormone CCK then sends chemical signals to the brain and pancreas where it reaches specific receptors. Cholecystokinin is a small peptide hormone that helps regulate many different functions in the body. CCK is located in the duodenum, the first part of the small intestine. CCK receptors however are located in various locations of the body, in order to conduct different biological functions in the body. CCK receptors are located on the brain, gallbladder, liver, pancreas acinar cells, and in the central nervous system. Cholecystokinin is very important for normal bodily functions. It is well known in the digestive system to help break down protein and fat in chyme. CCK is also linked to functions in the central nervous system. A major role that Cholecystokinin plays in the body is to trigger a feeling of satiety [21].

THYROTROPIN-RELEASING HORMONE (TRH):

Thyrotropin-releasing hormone (TRH) is a tripeptide (pGlu- His-Pro-NH2; where pGlu stands for pyroglutamic acid) that is released from the hypothalamus and transported via the portal vascular system to the anterior pituitary where it stimulates the release of thyroid-stimulating hormone (TSH) and prolactin from the anterior pituitary . In addition to its classical function as a releasing hormone, the distribution of TRH and its receptor indicates supplementary roles. TRH and TRH receptor (TRHR) are found distributed throughout the central and peripheral nervous systems as well as in other tissues including thymus and small intestine epithelial cells , consistent with its proposed role in the immune system [22].

CORTICOTROPIN-RELEASING HORMONE (CRH):

Corticotropin-releasing hormone (CRH), a 41 amino acid neuropeptide, is likely involved in all three types of stress-response. Behavioral responses may involve CRH present in the cerebral cortex and amygdala. Autonomic responses are controlled in part by brainstem fibers descending from the locus coeruleus, which receives CRH-containing fibers from the amygdala and paraventricular nucleus. Hormonal responses center on activation of the hypothalamic–pituitary–adrenal (HPA) axis, which is initiated by CRH present in the paraventricular nucleus of the hypothalamus (PVH) [23].

GONADOTROPIN-RELEASING HORMONE (GNRH)

The secretion of gonadotropin-releasing hormone (GnRH) by GnRH neurons is vital for reproductive competence in all mammalian species. GnRH neurons are unique in that they arise from progenitor cells located outside of the central

nervous system, in the olfactory placode. These neurons migrate through the nasal septum into the basal forebrain, where they establish their final

underlying the migration of the hypothalamic GnRH-producing neurons from the nasal region along the olfactory placode axons into the brain are still not fully understood. A detailed understanding of the process of GnRH migration clearly requires a complete cellular and molecular characterization of the GnRH neuron during early development.

4. CONCLUSION

The potential medicinal uses of *Asterias rubens* were supported by the presence bioinformatics activities. *Asterias rubens* has been exploited by the secondary compounds are further confirmed by bioinformatics studies, which conclusively and comprehensively validates its therapeutic potential. Hence, the need to exploit the potentials of these plants especially in areas of traditional medicine and pharmaceutical industries arises. Further analysis is required for drug target identification.

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RESEARCH ARTICLE

MOLECULAR IDENTIFICATION AND PHYLOGENETIC ANALYSIS OF *SUAEDA MARITIMA* FROM PARANGIPETTAI COASTAL AREAS, SOUTHEAST COAST OF INDIA

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ABSTRACT

Conventional taxonomy is limited with delineating species and controversies arise with DNA barcoding based identifications. Hence, an alternative supporting approach is very much needed to identify species and differentiate them within the species based on the genetic material. 18S rRNA genes have been particularly helpful in analyzing phylogeny at the species level. In addition, bioinformatics which represents a new, growing area of science uses computational approaches to answer biological questions. Salt tolerant costal salt marsh plant of *Suaeda maritima* was selected for 18s rRNA sequencing to solve the ambiguity in its species level identification. Similarity search of study species shared 99% similarity with 5 species of *Atriplex canescens clone* s128*, Atriplex torreyi var. griffithsii clone* p508*, Spinacia oleracea, Oenothera laciniata clone, Beta vulgaris*. Phylogenetic tree infer that *S.maritima* is closely related to *Spinacia oleracea* and *Oenothera laciniata*. *Atriplex canescens* (fourwing saltbush**)**, *Atriplex torreyi and Phaulothamnus spinescens, Celosia argentea* found to be closely related and are in one group. Hence, this study result clearly shows thus study species evaluated from angiosperm and provides key step in understanding the evolution of salt tolerance in angiosperm.

Keywords: *Suaeda maritima*, Molecular identification, 18s rRNA sequencing, Phylogenetic tree

1. INTRODUCTION

Coastal habitats represent important ecosystems in terms of biodiversity; they can be broadly classified into four distinct types: salt marshes and mangroves, sand dunes, vegetated shingle and rocky cliffs. Of these, salt marshes are particularly important habitats, in terms of their ecosystem value. The plants that grow on coastal marshes must be tolerant to the varying saline conditions. Colonies areas characterized by regular inundation, shifting sedimentation patterns and the hydraulic power of waves such sites are typically found behind sandbars and spits and in estuaries (Little, 2000).

Halophytic plants are generally located in coastal areas and they also exist as in land salt marshes in appropriate geological and climatological settings around the globe. Halophytes represent salt-tolerant species that thrive in the inhospitable habitats of inland and coastal salt marshes, dunes, beaches, deserts and salt flats (Flowers *et al*., 1986, W.H.O, 1990). Different physiological and anatomical traits of halophytes vary between species to survive in saline habitats. Anatomical structures of plant organs, especially of leaves, thus enabling plant adaptation to its environment. Many leaf traits have been recognized to provide a protection against various environmental conditions and stresses including drought, high air, temperature and high concentration of salt in soil. The morpho-anatomical alterations of succulent halophytes include increase of cell volume, especially of spongy and water paenchma increase of leaf thickness and decrease in number of stomata (Strogonov, 1973).

Recent advances in sequence analyses methods and softwares have revolutionized the phylogenetic studies. Phylogeney is an evololutionary tree that shows how species are related to each other, and this model has greatly facilitated the discussion and testing the hypotheses also this technique considered as a powerful tool for addressing many different evolutionary questions (Elliott *et al*., 2006). The identification of one species and its homologs in several species indicates that these genes divergence from their common ancestor. Because of the adaptive characteristics the salt marsh plants have been extensively studied in terms of morphology, pharmacology and cytology.

To date, traditional taxonomy relies mostly on diagnostic morphological characters, requiring expert knowledge to identify specimens. In this regard, DNA sequencing and phylogenetic analysis has proved to be a useful and alternative method for rapid global biodiversity assessment providing an accurate identification system for living organism. But molecular and bioinformatics studies on the vital coastal salt marsh are largly unexplored. Therefore phylogenetic analysis was carried out to study the homologs of study species with other species. This can aid in our understanding of the

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evolution of species and could serve to develop model systems for studying the species for future analysis. The main goal of this study was to identify the plant species and to predict the homologs with other species through a phylogenetic analysis.

2. MATERIALS AND METHODS

2.1. Isolation of Genomic DNA

In the present study, samples for PCR analysis (leaf tissue of *Suaeda maritima*) were collected into a sterile eppendorf tube. The tissue was softened for 15s at room temperature using a sterile tip as grinder without any buffer, 500µl of extraction buffer (200mM Tris-HCl (pH 7.5), 250mM NaCl, 25mM EDTA, 0.5% SDS) (Edwards *et al*., 1991) was added to the tube and mixed for 5s. In order to disrupt plant cells completely, the tube was placed in a water bath at 60ºC for 30min. Subsequently, an equal volume of chloroform:iso-amyl alcohol (24:1) was added to the sample, which was then mixed and centrifuged at 15,000g for 5min at 4ºC. The aqueous supernatant was transferred to a new eppendorf tube, to which an equal volume of isopropanol was added, mixed and incubated at -20ºC for 30min. Following centrifugation at 15,000g for 5min, the pellet was dried and dissolved in 100µl DEPC water. Finally, for precipitation of starch and other insoluble polysaccharides, the tube was placed on ice for 5min and centrifuged at 15000g for 2min; the resulting white pellet was mostly starch (Deshmukh *et al*., 2007). The supernatant containing the DNA was stable at 4ºC.

2.2. Quantitation and Quality Assessment of DNA

The DNA stock samples was quantified using Nanodrop spectrophotometer at 260 and 280nm using the convention that one absorbance unit at 260nm wavelength equals 50µg DNA per ml. The Ultra violet (UV) absorbance was checked at 260 and 280nm for determination of DNA concentration and purity. Purity of DNA was judged on the basis of optical density ratio at 260:280 nm. The DNA having ratio between 1.8 to 2.0 was considered to be of good purity. Concentration of DNA was estimated using the formula. Concentration of DNA (mg/ml) = OD 260 x 50 x Dilution factor Quality and purity of DNA were checked by agarose gel electrophoresis. Agarose 0.8% (w/v) in 0.5X TAE (pH 8.0) buffer was used for submarine gel electrophoresis. Ethidiumbromide (1%) was added at 10µl /100ml. The wells were charged with 5µl of DNA preparations mixed with 1µl gel loading dye. Electrophoresis was carried out at 80V for 30min at room temperature. DNA was visualized under UV using UV transilluminator. The DNA was further used for PCR analysis.

2.3. Polymerase Chain Reaction

18SrRNA gene fragment was amplified by PCR from genomic DNA using 18S gene universal primers: 1F:5'CTGGTGCCAGCAGCCGCGGYAA3' and 4R: 5'CKRAGGGCATYACWGACCTGTTAT3'. PCR was carried out in a final reaction volume of 25µl in 200µl capacity thin wall PCR tube in Eppendorf Thermal Cycler. Composition of reaction mixture for PCR is given in Table 1. PCR tubes containing the mixture were tapped gently and spin briefly at 10,000rpm. The PCR tubes with all the components were transferred to thermal cycler. The PCR protocol designed for 30 cycles for the primers used is given in Table 2.

Table 1. Composition of reaction mixture for PCR

Components	Quantity	Final
DNase-RNase free water	7.50 µl	--
2X PCR master mix (MBI Fermentas)	12.50 µl	1X
Forward Primer (10 pmole/µl)	1.00 µl	10 pmole
Reverse Primer (10 pmole/µl)	1.00 µl	10 pmole
Diluted DNA (30ng/µl)	3.0 µl	--
GRAND TOTAL	25.00 µl	--

Table 2. Steps and conditions of thermal cycling for PCR

Steps	Temperature	Time	Cycles
Initial Denaturation	95°C	2 min	1
Final Denaturation	94°C	30 Sec	
Annealing	50°C	30 Sec	30
Extension	72°C	90 Sec	
Final Extension	72°C	10 min	1

2.4. Visualization of PCR Product

To confirm the targeted PCR amplification, 5µl of PCR product from each tube was mixed with 1µl of 6X gel loading dye and electrophoresed on 1.2% agarose gel containing ethidium bromide (1 per cent solution at 10µl /100ml) at constant 5V/cm for 30min in 0.5X TAE buffer. The amplified product was visualized as a single compact band of expected size under UV light and documented by gel documentation system (Biorad).

2.5. Purification of PCR product

Amplified PCR product was purified using Qiagen Mini elute Gel extraction kit according to the manufacture's protocol.

2.6. Sequencing of Purified 18S rRNA Gene Segment

The concentration of the purified DNA was determined and was subjected to automated DNA sequencing on ABI 3730xl Genetic Analyzer (Applied Biosystems, USA). Sequencing was carried out using BigDye® Terminator v3.1 Cycle sequencing kit following manufacturer's instructions.

2.7. Cycle Sequencing

Cycle sequencing was performed following the instructions supplied along with BigDye® Terminator v3.1 Cycle Sequencing Kit. The reaction was carried out in a final reaction volume of 20µl using 200µl capacity thin wall PCR tube. The cycling protocol (Table 3) was designed for 25 cycles with the thermal ramp rate of 1°C per second. After cycling, the extension products were purified and mixed well in 10µl of Hi-Di formamide. The contents were mixed on shaker for 30min at 300xg. Eluted PCR products were placed in a sample plate and covered with the septa. Sample plate was heated at 95°C for 5min, snap chilled and loaded into autosampler of the instrument.

Table 3. Cycling protocol for sequencing reaction

Step	Temperature	Time
Denaturation	96°C	10 sec
Annealing	50°C	5 sec
Extension	60°C	4 min
***Repeat step 1 to 3 for 25 cycles**		

2.8. Electrophoresis and Data Analysis

Electrophoresis and data analysis was carried out on the ABI 3730xl Genetic Analyzer using appropriate Module, Basecaller, Dyeset /Primer and Matrix files.

2.9. Sequence analysis

The sequence was compared for detecting homologous sequences found in databases using Basic Local Alignment Search Tool (BLAST) (Altschul *et al*., 1990). BLAST from NCBI was used to compare the query sequence with the database sequence to find its homologues.

2.10. Data analysis

To better present the diversity of the isolates obtained in this study and to assess the genetic relationship of the novel ones here to the known ones, intraspecific phylogenies were reconstructed with the neighbor-joining (NJ) and maximum likelihood (ML) methods in MEGA7.0 program. NJ and ML trees were constructed using 1000 and 100 bootstrap replicates, respectively (Tamura *et al*., 2011; Nei and Kumar, 2000).

2.11. Wet Lab methodology

Quantitation of DNA was estimated in two different absorbances and the average was taken into consideration. OD values with its average and the concentration of the DNA in µg/µl and ng/µl is shown in Table 4. The isolated genomic DNA of the study species is shown in Fig 1.

Table 4: Quantitation of DNA

Sample	A_{260}	A_{280}	A_{260}/A_{280}	Conc. (µg/µl)	Conc. (ng/µl)
S. maritima	0.435	0.233	1.86	2.175	2175

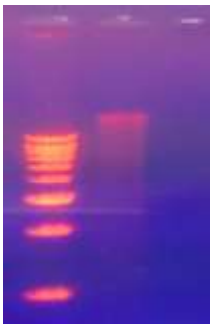


Fig. 1. Genomic DNA isolation of the study species

2.12. Electrophoresing PCR amplicons

The PCR Products (5µl) was separated by electrophoresis in 1.2% Agarose gels containing ethidium bromide (1 per cent solution at 10µl /100ml). Electropherogram obtained after electrophoresis of the PCR amplicons is shown in Fig 2. The amplified product was visualized as a single compact band of expected size against 100bp DNA ladder (Fermentas make). No overlapping of the band in the case of test organism was observed and so the band was clear.

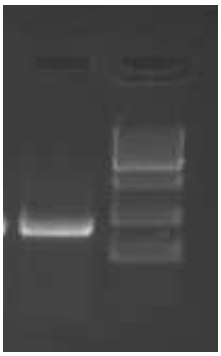


Fig. 2. The electropherogram obtained after electrophoresis of the PCR

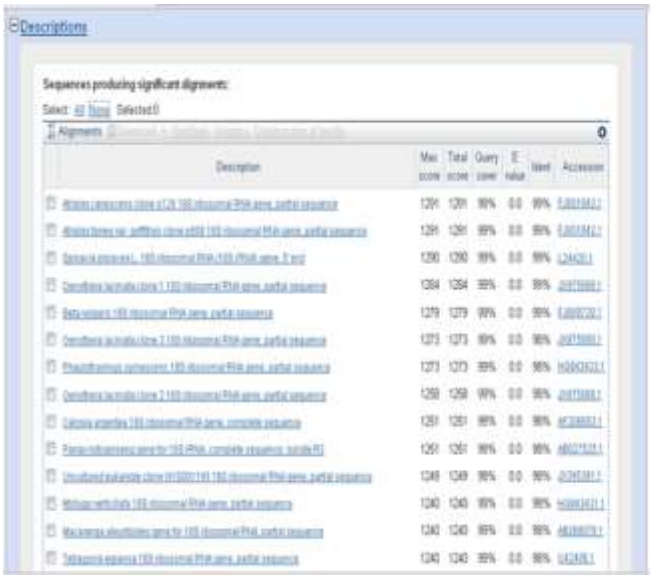
2.13. Sequence analysis

The DNA sequence was analyzed with bioinformatic tools and softwares with statistical applications. Simultaneously the DNA sequences were submitted to NCBI for obtaining the accession number. The DNA sequence was aligned in FASTA format and shown as follows

AGGTTGTTTGCAGTTAAAAAGCTCGTAGTTGGACCTTGGGGTGGTACGACCGGTCCGCCTCACGGTGTGCA
CCGGTCGTCTCGCCTCTTTCGCCGGCGATGCGCTCCTGGCCTTAACTGGCCCGGGTCGTGCCTCCGGCACT
GTTACTTTGAAGAAATTAGAGTGCTCAAAAGCAAGCCTACGCTCTGTATACATTAGCATGGGATAACATTATA
GGATTCCGGTCCTATTGTGTTGGCCTTCGGGATCGGAGTAATGATTAACAGGGACAGTCGGGGGCATTCGTA
TTTCATAGTCAGAGGTGAAATTCTTGGATTTATGAAAGACGAACAACTGCGAAAGCATTTGCCAAGGATGT
TTTCATTAATCAAGAACGAAAGTTGGGGGCTCGAAGACGATCAGATACCGTCCTAGTCTCAACCATAAACG
ATGCCGACCAGGGATCGGCGGATGTTACTTTTAGGACGCCGCCGGCACCTTATGAGAAATCAAAGTTTTTG
GGTTCCGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTAG
CCTGCGGCTTAATTTGACTCAACACGGGGAAACTTACCAGGTCCAGACATAGTAAGGATTGACAGACTGAG
AGCTCTTTCTTGATTCTATGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGCGATTTGTCTGGTTAATTC
CGTTAACGAA

2.14. Dry lab methodologies

As a general rule, a top match with a sequence similarity of at least 98% was used as a criterion to designate potential species identifications (Barbuto et al., 2010). The top sequences producing similar and significant alignments was identified for the study species from BLAST and listed. Maximum identity of 99% with a maximum score of 1291 and the identity varied from 97-99% with the alignments derived from NCBI. Family and their accession numbers of sequences closely related to the test organisms used in the analysis were given in.



Species	Accession Number	Family
Atriplex canescens clone s128	FJ601843.1	Amaranthaceae
Atriplex torreyi var. *griffithsii* clone p508	FJ601842.1	Chenopodiaceae
Spinacia oleracea	L24420.1	Amaranthaceae
Oenothera laciniata clone 1	JX975688.1	Onagraceae
Beta vulgaris	FJ669720.1	Amaranthaceae
Phaulothamnus spinescens	HQ843433.1	Achatocarpaceae
Celosia argentea	AF206883.1	Amaranthaceae
Mollugo verticillata	HQ843431.1	Molluginaceae
Halophytum ameghinoi	HQ843429.1	Halophytaceae

Fig. 3. Sequences Producing Significant Alignments from BLAST

2.15. Multiple Sequence Alignment

The close phylogenetic relationships were found within the samples. This was clear evidence in both Multiple Sequence Alignments and Molecular Phylogenetic analysis by Maximum Likelihood and NJ methods. The alignment was easy and clear because no gaps were found in our sequence. Read lengths were about 724bp long, although in some instances some base calls were uncertain. No insertions, deletions or stop codons were observed (Fig. 4).

2.16. Evolutionary relationships of taxa by Neighborhood joining Method

18srRNA partial sequence of study species shared 99% similarity with 5 species of Atriplex canescens clone s128, Atriplex torreyi var. griffithsii clone p508, Spinacia oleracea, Oenothera laciniata clone, Beta vulgaris in genbank database. In order to strenghthen the results, phylogenetic analysis was included in the present study. Phylogram of 18SrRNA gene was built with 9 closely related 18SrRNA sequences were collected from Genbank by referring BLAST results. The

evolutionary relationship was inferred using the neighbor joining method (Saitou and Nei, 1987). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to branches (Felsenstein, 1985). The tree was drawn to scale with branch lengths in the same units as those of the evolutionary distances were computed using the Maximum Likelihood method (Tamura et al., 2004) and in the units of the number of base substitutions per sites. All positions containing gaps and missing data were eliminated from the dataset (complete deletion options). Phylogenetic analysis was conducted in MEGA 5 (Tamura, 2011).

Fig 4. Multiple Sequence Alignment

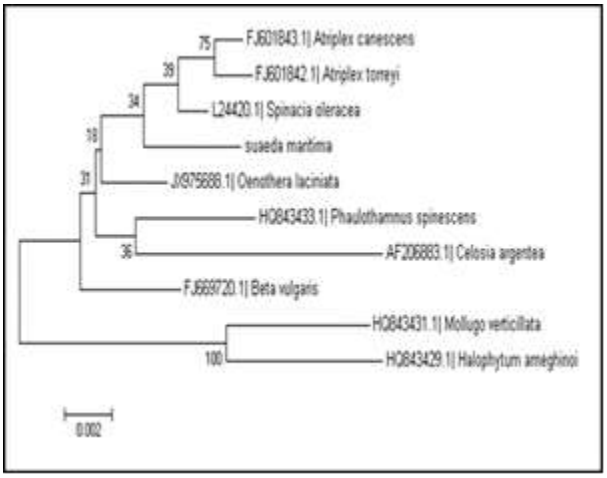


Fig. 5. Evolutionary relationships of taxa by NJ Method

Phylogenetic tree infer that *S.maritima* is closely related to *Spinacia oleracea* and *Oenothera laciniata*. Along with these two species *S.maritima* forming monophyletic group with *Beta vulgaris* (Sea beet or Sub sp.*maritima*), *Atriplex canescens* (fourwing saltbush**)**, *Atriplex torreyi, Phaulothamnus spinescen and Celosia argentea* found to be closely related and were fallen in one group. Phylogram showed distinct clade with *Mollugo verticillata* and *Halophytum ameghinoi* (Succulant annual plant) with strong bootstrap support value of 100%.

3. RESULTS AND DISCUSSION

Halophytes have evolved to cope with a wide variety of climatic conditions, all around the world, and they are found at varying altitudes, although in greatest numbers at sea level. (Khan *et al*., 2006a). The number of halophytic species is an area of some debate and will remain so while there are no highly specific definitions for levels of salinity or for the mechanisms employed in salt tolerance. Lieth *et al*., (2008) claimed the occurance of 1,560 terrestrial halophyte species. However, Khan *et al*., (2006b) stated that, the number could be as high as 6,000 for 6 terrestrial halophytes (including tidal zones). Flowers *et al*., (1986) found that about one third of all the angiosperm families contain halophytic genera, but the glycophytes would have lost salt-tolerance through evolutionary processes. It is now accepted that there are approximately 20 significant families containing some 500 halophytic genera. The most widespread and well-studied family is one of the Dicotyledoneae: Chenopodiaceae, which, according to Flowers *et al*., (1986) includes 44 halophytic genera with 312 halophytic species; these 44 genera represent 44% of the family. Perhaps the most important genera within the research community are *Atriplex* sp, *Salicornia* sp and *Suaeda* sp; this last one is to play a central part in salt tolerance research (Flowers *et al*., 1986).

Of the *Suaeda* genus, the current research was focused particularly on *Suaeda maritima* (L.) also known as sea blite, which is an annual obligate halophyte that has the distinctive feature in that it thrives in both coastal and inland salt marshes. It is a model plant and its salt-tolerance has been extensively investigated (Flowers *et al*., 1977; Yeo, 1981; Greenway and Munns, 1983; Hajibagheri *et al*., 1985; Clipson, 1987). For molecular identification salt marsh plant of *S. maritima* was selected for 18sDNA sequencing to solve the ambiguity in its species level identification.

BLAST algorithm used to compare study sequence with the sequences available in the GenBank database. Maximum identity of 99% with a maximum score of 1291 and the identity varied from 97-99% with the alignments derived from NCBI. On

the contrary, currently there is no 18S sequence data of *S.maritima* deposited in GenBank. Hence, this is the first report of partial 18srRNA sequence of *Suaeda maritima*. According to these intra generic differences between molecular and morphological results, this study extend to sequencing with distinct markers to identify these isolates in future studies. In addition, the absence of sequence data for *Suaeda maritima* (only *Suaeda maritima* isolate 6, 7 and 8 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence available in GenBank) made our molecular identification harder. Incase of similarity search of study species shared 99% similarity with 5 species of *Atriplex canescens clone* s128*, Atriplex torreyi var. griffithsii clone* p508*, Spinacia oleracea, Oenothera laciniata clone, Beta vulgaris* are belongs to angiosperm.

In order to classify and trace out evolutionary path among related species, it is important to investigate them at the molecular level for better understanding of evolution of plants. 18S gene is one of the useful phylogenetic marker which one can use in understanding phylogenetics. Phylogenetic tree infer that *S.maritima* is closely related to *Spinacia oleracea* and *Oenothera laciniata*. Along with these two species *S.maritima* forming monophyletic group with *Beta vulgaris* (Sea beet or Sub sp.*maritima*). *Atriplex canescens* (fourwing saltbush**)**, *Atriplex torreyi and Phaulothamnus spinescens, Celosia argentea* found to be closely related and are in one group. Phylogram showed distinct clade with *Mollugo verticillata* and *Halophytum ameghinoi* (Succulant annual plant) with strong bootstrap support value of 100% (Fig: 5). Current study results clearly shows thus study species evaluated from angiosperm. Flowers *et al*., (1977) and (2010) reported that, halophytes come from a wide range of angiosperm lineages, suggesting that the adaptations involved in salt tolerance have arisen repeatedly during angiosperm evolution. This result provides a key step in understanding the evolution of salt tolerance in angiosperm.

The results of the present study highlight the identification of plant species by investigating them at the molecular level with the use of molecular markers for a better and wide understanding of halophytic plants**.**

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RESEARCH ARTICLE

BIO-ASSISTED SYNTHESIS OF FERRIC SULPHIDE NANOPARTICLES FOR AGRICULTURAL APPLICATIONS

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ABSTRACT

Herein, Ferric sulphide nanoparticles were prepared by co-precipitation (green synthesis) method. Structural study (XRD) confirms the crystalline nature of prepared Ferric sulphide nanoparticles. The crystallite size was estimated and it was found to ~2.0 nm. The surface morphology of the Fe2S3 nanoparticles shows the agglomeration and is sponge and dried algae like structure. EDS analysis reveals the presence of Fe, S, O and C elements in the prepared Ferric sulphide nanoparticles. FTIR spectrum of Ferric sulphide shows the characteristic peaks that confirms the presence of Fe and S in the sample. Moreover, the plant growth study proves that Ferric sulphide nanoparticles could be used as a fertilizer to enhance the agricultural production.

Keywords: Green synthesis, Ferric sulphide, Agglomeration, Morphology

1. INTRODUCTION

The nanotechnology has got intensive applications for the enhancement of agricultural yield, along with the promising technologies for example biotechnology that including plant breeding, fertilizer technology, genetics and disease control. To overcome the challenges in sustainable production and food security, considerable scientific innovations have been made in the field of agricultural [1, 2]. Using the natural and synthetic resources, agricultural innovations are very essential to resolve the increasing food demand all around the global population.

Therefore, nanotechnology has a great impact to give very effective solutions to the several agricultural associated issues and a significant research has been carried out to highlight its important applications in agriculture region in the recent times [3, 4]. In addition to that, nanoparticles can provide a huge scientific curiosity in comparison to that of bulk materials. Though fertilizers have the important role to enhancement of the agricultural production, the excess usage proved to be change in the chemical ecology of soil and reduction in the existing area for crop production. So that, sustainable agricultural involves a minimum use of agrochemicals which can ultimately protect the environment. In particular, nanomaterials improve the crops production by increasing the effectiveness of agricultural inputs to produce the controlled delivery of nutrients. Furthermore, the main concern in agricultural production is to ensure the adaptation of plants with climate change issues like water deficiency, salinity, excessive temperatures and environmental pollution [5].

Moreover, agricultural land can be one of the main areas of concern for the discharged metal based nanoparticles (NP_s). To observe the effect of metal based NP_s in the field of agriculture ecosystem, it is very necessary to evaluate their revolution in agricultural environment. Plants are the major part in agriculture practice which has active interactions with metal based NP_s and many reviews have been reported on the stability and aggregation of NP_s in environment [6].

Considering the above facts, Iron (Fe) is an important nutrient for all organisms [7]. Iron deficiency is a rising issue with many different crops all over the world. Generally, Fe content in soil is extremely high, but a high percentage is fixed to soil particles [8,9]. Further, Fe is mostly in the form of insoluble Fe^{3+}, particularly in high pH and aerobic soils. So that, these soils are typically deficient in the existing form Fe^{3+} that lead Fe-deficient in plants where Fe participates in several physiological processes that including respiration, chlorophyll biosynthesis and redox reactions [8, 10]. Though, Fe deficiency not only affects the growth or expansion of plants, Fe deficiency results anemia in animals and human as well [11]. Therefore, it is very essential to enhance the utilization efficiency of Fe fertilizers. The purpose to use the Fe fertilizer is still very unique method to enhance Fe deficiency that could lead to increase the crop yield and rise the quantity as well. Fe_2O_3 nanoparticles have been

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utilized in the research filed of catalysis, biomedicine, water treatment and other fields. Not much work is reported on Fe_2O_3 nanoparticles used as fertilizer for agricultural production, especially Ferric Sulphide (Fe_2S_3). Therefore, the applications of Fe2S3 as a fertilizer still an important focus for many researchers. Here, a new type of fertilizer (Fe_2S_3) has been used to enhance the growth of cowpea plant, which can overcome such Fe deficiency in traditional fertilizer.

2. MATERIALS AND METHODS

2.1 Synthesis procedure to prepare Ferric sulphide nanoparticles using plant extract:

In the present work, the green synthesis of Ferric sulphide nanoparticles was prepared by Co-Precipitation method. Fe_2S_3 nanoparticles were synthesized via green synthesis by adding Ferric Chloride, Na_2S, to the solution of plant extract J*usticia-adhatoda*. Primarily, 5 ml of plant extract is added to 20 ml de-ionized water and stirred for 15 minutes using magnetic stirrer. Then, 6.48g of Ferric chloride is mixed in 40 ml of de ionized water and it is added drop wise for 15 minutes to the extract solution. Finally Na_2S (1.56 g) is added drop wise to the same solution and constantly stirred for 1hour. The solution is centrifuged and dried in an oven over 1 hr. The dried powder is crushed well into nano sized particles and the final product is calcination at 600˚C for 4 hours to get fine ferric sulphide nanopowder. The structural, morphological, composition of the prepared nanoparticles was characterized using X-ray diffraction (XRD), Field emission scanning electron microscopy (FESEM), and Fourier transform infrared spectrum analysis (FTIR). Further, the application of prepared sample was studied by applying it with water and analyzes its role as the plant growth promoter.

3. RESULTS AND DISCUSSION

3.1 Structural analysis

The structural properties of Ferric sulphide nanoparticles were examined using XRD analysis. The sharp dominant diffraction peaks at 2θ = 33.22°, 37.22°, 40.55° and 54.75° corresponding to the (101), (111), (200) and (130) orientation planes confirms the orthorhombic crystalline nature of the prepared nanoparticles as shown in Fig.1.

The observed results are consistent with the standard JCPDS card no (03-065-2567). The crystallite size of the Fe_2S_3 nanoparticles was estimated using Scherer's formula and the estimated average crystallite size was found to be 2.0 nm. Moreover, the observed peaks confirm the polycrystalline nature of the Fe_2S_3 nanoparticles.

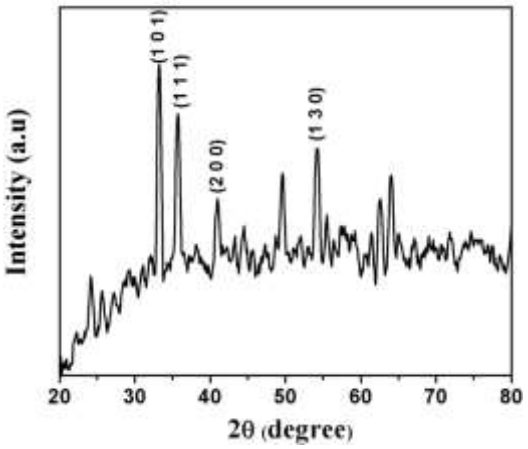

Fig. 1. XRD pattern of prepared Fe_2S_3 nanoparticles.

3.2. Field Emission Scanning Electron Microscope (FESEM)

The prepared Ferric sulphide nanoparticles were analyzed using FESEM (Quanta-200F) to know the surface morphology of the sample. Fig.2 shows the very clear surface morphology that signifies nanoparticles are well agglomerated and the surface appears to be spongy dried algae and sphere shaped.

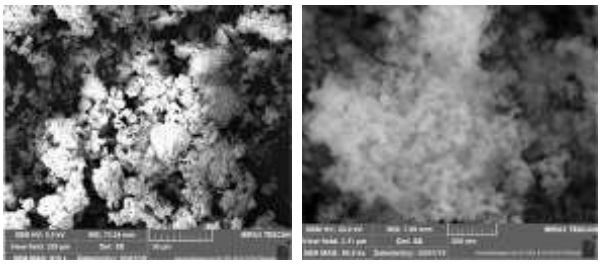

Fig. 2. FESEM images of prepared Fe_2S_3 nanoparticles in different magnifications

3.3 Elemental compositional analysis

The elemental composition of the prepared nanoparticles was carried out by Energy dispersive X-ray spectroscopy (EDS) to confirm the elements present in the system. It is evident from the peaks that the sample is composed of C, O, Fe and S elements. It also reveals the absence of any impurities in the prepared nanoparticles (Fig. 3).

3.4 Fourier Transform Infrared Spectroscopy

The FTIR spectrum of the prepared Ferric sulphide nanoparticles was performed using a

Thermo Scientific instrument, Nicolet 10 using a KBr pellet technique. Fig.4. represents the FTIR spectrum of ferric sulphide nanoparticles and it shows the characteristic peaks at 3136.25 cm-1 is due to the = C-H stretch i.e alkynes. Additionally, the peak at 1627.92 cm-1 confirms the presence of amide or amine. Further, the noticeable peak at 1402.25 cm-1 corresponds to O-H bond, peak at 1128.38 cm-1 corresponds to the alcohols (C-O stretch) compounds.

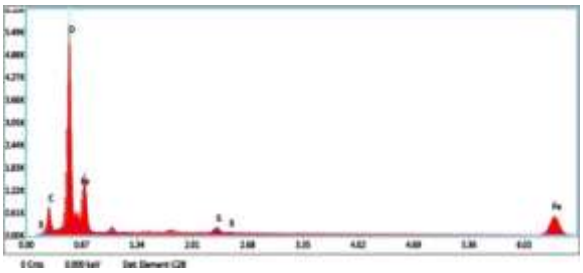

Fig. 3. EDS spectrum of Ferric Sulphide nanoparticles

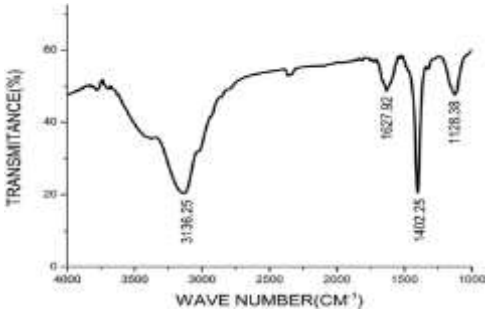

Fig. 4. FTIR spectrum of Ferric sulphide nanoparticles

3.5 Application of Ferric Sulphide nanoparticles in plant growth

Nanotechnology revolutionizes a new intensive technique in agriculture field by enhancing the capability of plants to absorb nutrients. Here, an attempt has been made to perform the application of the prepared Ferric sulphide nanoparticle as plant growth promoter.

After 7 days of observation, a very significant plant growth is observed in the nanoparticle applied sample compared to that of reference sample. The shoot length, root length, leaf and weight are well studied for both the samples. The results are proved that the sample with nanoparticle applied has notable increase in all parameters in comparison to that of the reference sample.

From the Table.1, it is very evident that the shoot length, root length, leaf length and number of leaves are low values for the reference sample (Fig. 5a) in comparison with the nanoparticle treated samples. Moreover, the observed seed growth is more in nanoparticle treated samples (Fig. 5b) which eventually lead to utilize such a kind of fertilizer for better enhancement in agricultural production.

Table.1 Effect of nanoparticles in plant growth

Objectives	Plant grown in pure water	Plant grown using NPs
Length of the shoot	1.3 cm	5 cm
Length of the root	2.7 cm	4.5 cm
Length of leaves	1 cm	3.5 cm
Number of leaves	2 No's	7 No's

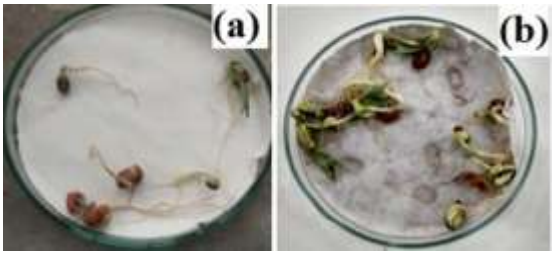

Fig. 5. Seed growth of cowpea plant (a) the reference sample (b) nanoparticle treated samples

4. CONCLUSION

Ferric sulphide nanoparticles were prepared by very simple co-precipitation method. Structural studies reveal Ferric sulphide nanoparticles are in polycrystalline nature. The estimated average crystalline size was found to ~2.0 nm. The surface morphology of the Fe2S3 nanoparticles shows the agglomerations of nanoparticles and are in sponge and dried algae like structure. EDS analysis confirms the presence of Fe, S, O and C elements in the prepared Ferric sulphide nanoparticles. FTIR spectrum shows the presence of Fe and S characteristic peaks in the sample. It is concluded that, the plant growth study proves Ferric

sulphide nanoparticles could be sed as a fertilizer to enhance the agricultural production.

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RESEARCH ARTICLE

SCREENING OF OSMOTOLERANT MICRO ORGANISMS IN THE DRIED SALTED FISH SOLD IN KANYAKUMARI DISTRICT, TAMILNADU

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ABSTRACT

Fish is one of the most important sources of animal protein available in the tropics and has been widely accepted as a good source of protein and other elements for the maintenance of healthy body. Salting and drying is an ancient and simple method to preserve fish and in India about 17% of the total catch is being used for salting and drying. The present investigation was aimed to analyse the presence of osmotolerant microbes in the dry fishes. The commercially important marine edible salted dry fishes were collected from the study area Pallam. Four common edible dry fishes like (Nethili, Sardines, Ribbon fish and Prawn) were selected for the screening of microbial population. The highest TFC value was reported in Sardine and Ribbon fish (5×10^3) than the other fishes like Netthili and Prawn (4×10^3 and 2×10^3). The moisture content was higher (45%) in Prawn followed by Ribbon fish (40%), Sardines (29%), Netthili (26%). In this study, Prawn had high moisture content (45%) and high microbial load (5.3×10^4 cfu/g). Total five fungal species were isolated from the selected dried fishes. The result of isolation of human pathogens such as *Salmonella* and *Vibrio* identified from the selected dry fish samples. The nutritive value of raw fish in found to be good. The sensory characteristics such as colour, odor, texture, insect infestation showed that the Sardine and Prawn was good in quality, while the netthili had decreased the quality. The study showed that salted and sundried fishes sold in study area are contaminated with pathogenic bacteria and fungal agents. Spoilage of dried fish products was found and this might be due to unhygienic handling of the fisher folks, improper processing and unhygienic vendors and vending areas.

Keywords: fishes, infestation, marketing, sundried and vendors

1. INTRODUCTION

Fish is one of the most important sources of animal protein in the tropics and has been widely accepted as a good source of protein and other elements for the maintenance of healthy body. Drying of fishes is susceptible to many types of spoilage which can affect the quality and shelf life. Physical and organoleptic qualities of many traditional sun-dried products are unsatisfactory for human consumption (Nowsad *et al*., 2005). Damages occurring due to flies and insects are of great significance in open sun drying and this is a serious problem in traditional drying. Major problem with respect to distribution of seafood of fishery products is their susceptibility to spoilage, mainly due to the contamination of spoilage and pathogenic microorganism (Gram and Hus, 1996). The study area Pallam in Kanyakumari District situated in the Southern tip of peninsular India is under strategic location and has rich diversity of flora and fauna scattered over the hills and hillocks of the district. However, published data on assessment of the hygienic quality of dried salted fish sold in an around Pallam of Kanyakumari district, Tamil Nadu, South India are meagre. Reports on the assessment of the hygienic quality of dried salted fish sold in an around Kanyakumari district, Tamil Nadu, South India in Kanyakumari district is very scanty. Hence the present study was undertaken to assess the hygienic quality of dried salted fish sold in selected area.

2. MATERIALS AND METHODS

2.1. Study area

Pallam is a beautiful village with green coconut trees along with a beach worth spending sometimes and the churches that resembles the spirituality of the people. Pallam is a fishing village in Kanyakumari District of Tamil Nadu and is located 9 km away from Nagercoil. Villagers mainly depend on fishing for their livelihood. St Mathew church is here. Here is a wounderful beach that is known as Sanguthurai beach which is a very fine picnic place in Kanyakumari District. Pallam has a total

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population of 2,019 peoples. There are about 497 houses in pallam village.

2.2. Sample collection

The commercially important marine edible salted dry fishes were collected from the study area and transported to the laboratory (Suganthi Devadason Marine Research Institute Tuticorin) in clean polythene covers for further microbiological analysis. Four common edible dry fishes (Nethili, Sardines, Ribbon fish and Prawn) were selected for the screening of microbial population. The selective fishes were identified according to fish Base, 2010.

2.3. Preparation of sample

About 1 gm was taken from each fish homogenate was made in 10 ml distilled water. The solution was serially distilled ten folds. 0.1 ml of (10^{-10}) dilution was spread on the Zobell Marine Agar in duplicate and incubated for 18-24 hrs at 37°C.

2.4. Microbiological analysis

Enumeration of bacterial load was done using plate count agar by using spread plate. Ten gm of the sample was mixed with 90ml saline water. Appropriate dilutions of fish homogenate were spread on plate count agar and incubated at 37°C for 24-48 hours and the colonies were counted for total bacterial count (TBC) (FDABAM, 2001).

Fungal count was done (AOAC, 1998) by using Rose Bengal Chloramphenical (RBC) agar. Twenty five gm of the sample was blended with 225ml of 0.1% peptone water and 0.1ml of the appropriate dilutions of the sample was spread on the surface of the medium and incubated at room temperature (28 ± 1°C) for 3-5 days. Fungal colonies were sub cultured on potato dextrose agar (PDA) and the fungal cultures were stained by using wet mount with lacto phenol cotton blue. Identification of the fungi is mainly based on morphology and can be carried out by standard keys. Sensitivity of the fungal isolates to sodium chloride was done by inoculating fungal colonies on PDA containing 0, 10, 14 and 18% NaCl.

The MPN Technique was used to determine the level of total coliforms, fecal coliforms, fecal streptococci and *E.coli* in dry fish samples. Dry fish homogenate was transferred to lauryl sulphate tryptone broth (LSTB) tubes and incubated and 37°C for 24 hours for the estimation of total *coliforms*. Samples from positive LSTB tubes were transferred *coliform* presence or absence broth tubes and cubated at 44.4 ± 0.5°C for 18-24 hours for the estimation of fecal *coliforms*. Samples from positive PA broth tubes were transferred to EC broth tubes and incubated at 37°C for 24-48 hours for E.coli. Samples from positive EC broth tubes were treated on to eosine methylene blue agar plate to isolate *E.coli* culture of feacal *streptococci* was done in glucose azide broth and confirmed by KF agar.

For the isolation of *Salmonella*, 25g of sample was homogenized and enriched in 225 ml of alkaline peptone water (APW) at 36°C for 24 hours. Selective isolation of *vibrio* was carried out in thiosulphate citrate bile salt sucrose agar (TCBS). Presence of vibrio shows yellow closed colonies.

2.5. Estimation of biochemical composition

Biochemical parameters like proteins, lipids, carbohydrates and free fatty acids were estimated by following standard methods. The amount of proteins in the sample was estimated by Biuret Method (Raymont *et al*., 1964). Carbohydrate content was estimated by the phenol sulphuric acid method (Dubois *et al*., 1956), lipid was estimated according to the method of Bligh and Dyer (1959) and the free fatty acid, which is an indication of the quality of fat, was estimated by the method of Ke *et al*., (1976).

2.6. Physical characteristics

Physical characteristics such as colour, odor, texture and insect infestation of the traditionally sun-dried fishes were examined by sensory test on the basis of the method described by Roy, (2013) with help of five member panels of experts.

2.7. Proximate composition analysis

The compositions of moisture, protein, crude lipid and ash content of dried fish were analysed in triplicate according to the standard procedure given in association of official analytical chemists (AOAC, 1995).

2.8. Determination of lipid oxidation

To analyse the degree of lipid oxidation of dried fish, lipids was extended and then peroxide value and the acid value was determined.

2.9. Extraction of lipid

Total lipid was extracted from dried fish samples with a solvent combination of chloroform, methanol, distilled water according to the method of Bligh and Dyer, (1959) with slight modification making those final rations 10:5:3, v/v/v. Then the extracted lipid was dissolved in chloroform and stored at 20°C until further analysis.

2.10. Determinaion of pH

About 10g of samples was weighed in a beaker using electronic balance. Then the sample was homogenized using tissue homogenizer with 10

volumes of distilled water and the pH was measured by a pH meter.

2.11. Salt content

Salt content was determined as chloride where the ions are precipitated with silver nitrate and the excess silver ions are determined by titration with potassium thiocyanate (Pierson, 1999). All analysis are performed in duplicate.

3. RESULTS AND DISCUSSION

3.1. Total plate count (TPC)

In the fish sample, the highest total plate count (TPC) (5.3×10^4 CFU/g) was observed in Prawn followed by Netthili, Sardines and the lowest TPC in ribbon fish (2.5×10^3CFU/g) (Table 2). In fresh fish, the acceptable limit is 5×10^5/g at 37°C but for cooked or dried fish, the permissible limit is 1×10^4/g at 37°C (Surendram *et al*., 2006). In this study, prawn had the highest TPC 5.3×10^4 which exceed the permissible limit. Similar works carried out in dried fishes of Tuticorin market have recorded high bacteria count in Prawn 3.5×10^3 (Ashok kumar, 2008). In Cochin market, the bacterial count in dried fishes was found to be less than $4.1\times10^7 g^{-1}$ (Sanjeev, 1997). In Nigerian market count of dried fish samples was 4.6×10^6 g^{-1}. (Adesiyan and Kaminjola, 1992). The present study results revealed high bacterial load and the mean temperature of the period was 27±3°C. In this season, the dried sea foods absorb moisture from the atmosphere and this leads to the spoilage of the products. The least bacterial load was observed during summer season and this is due to high temperature, low moisture and adequate drying. This results agrees with the direst relationship between the microbial count and moisture content of the sample (Lilabati *et al*., 1999). The highest TFC value was reported in Sardine and Ribbon fish (5×10^3) than the other fishes like Netthili and Prawn (4×10^3& 2×10^3) (Table 2).

3.2. Moisture

The moisture content was higher (45 %) in Prawn followed by Ribbon fish (40%), Sardines (29%), Netthili (26%). Moisture content of seafood's plays on important role in spoilage and lowering of moisture relards the spoilage (Sbanshy, 1963). In the present study, high moisture content and microbial load were observed during monsoon season. There was a direct relationship between the microbial counts and moisture content of the sample.

The seasonal variation in moisture content of dried seafood could be the results of variable in drying time, environmental changes and level and type of salt used for curing (Anihouvi *et al*., 2006). However, the moisture content seems to be an exact indicator of the susceptibility of a product to undergo microbial spoilage. In the present study the moisture was increased during monsoon season and it was considerably lowered in summer season and this result agrees with the result of earlier reports (Chakrabasrti *et al*., 1999).

In this present study, Prawn had high moisture content (45%) and high microbial load (5.3×10^4 Cfu/g). Visible fungal colonies appeared on the sea foods during monsoon season due to high moisture content of the samples and high relative humidity of the atmosphere. In this present study also high fungal count noted with high moisture in monsoon season.

3.3. Fecal indicator bacteria

Pathogenic or indicator bacterial may not be present insufficiently in large numbers in water of food to be detected by planting methods. In such cases, MPN methods are used, where large volumes of samples can be used for inoculation. MPN is only a statistical approximately on the test bacteria in the given sample and not the actual number. MPN method is used to detect the *coliform* bacteria in water or food (Surendran *et al*., 2006).

In the present investigation, the MPN value of the seafood samples varied with different season. The total *coliforms* and feacal coliforms duing summer varied from 95-45 and 70-20/100 ml respectively (Table 2). Total coliforms group can be sub grouped as fecal and non fecal coliforms. The fecal coliform subgroup is derived from feces of human and other warm-blood animals such as cows, sheep, poultry, etc. The non fecal subgroup is frequently found on vegetation and in the soil; some are plant pathogens. The presence of fecal coli-from organisms indicates recent and possibly hazardous fecal pollution. The most common fecal coliform species is *Escherichia coli* (Kabler *et al*., 1960, Sherman 1937). Fecal *streptoscocci* are non pathogenic organisms but commonly occur in the intestines of man and other warm-blooded animals which make them a useful group of indicator of fecal contamination. (Litsky *et al*., 1955). Washing the catches in polluted coasted water definitely add the fecal indicator bacterial. Drying done in unhygienic way also added fecal indicator bacteria in fish and coastal waters has already been reported to be high along Tuticorin fish landing centres (Sugumar, 2002). However, the fecal poullution at Bhuranagar coast was reported to be of human origin based on the fecal diseases.

Sewage imparts considerably to the fecal microorganisms which are considered as a good

indicator of the extent of fecal pollution in the environment. In our present study high MPN values were observed in the monsoon season and it may be duets unhygienic handing in processing and inadequate drying. In our present study also E.coli count showed variations, high in monsoon season. Levels of focal indicator bacteria were also reported to be high both in fish and dehydrated fish from Cochin fisheries and retail markets of Mumbai (Kimura and Kiamukura, 1934).

3.4. Total fungal count

The results of fungal counts in different sun dried sea food were presented in table 4. Total five fungal species were isolated from the dried sea food. The dry fish samples were free from visible fungal colonies during post-monsoon and summer seasons while visible fungal colonies were noted on the fishes during monsoon season. In monsoon season, visible fungal colonies appeared quickly due to the moisture content of the fish samples and high relative humidity of the atmosphere. Even through visible fungal colonies were not noted in post monsoon and summer season but enrichment in RBC broth and plating on RBC agar recovers almost all the fungal flora. The antibiotic chloramphenicol was added to the media to arrest the growth of bacteria.

3.5. Pathogenic bacteria

The result of isolation of human pathogens such as *Salmonella* and *Vibrio* from the selected dry fish samples are shown in Table 2. Both the bacteria are noted in netthili, sardine, prawn except ribbon fish. *Vibrio* is a halophilic bacterium usually present in the marine environment, but in the case of *Salmonella*, it does not occur naturally in marine waters and is presence is usually due to unhygienic handling carries or polluted costal water (Christolite, 2004). Contamination of fish and fishery products with *salmonella* and *vibrio* has been reported in different parts of India. Incidence of pathogens in the samples of fish market may be attributed to external contamination (Lyer and Shrivastava, 1989) and poor handling at ambient temperature (Jedah *et al*., 1998).

The study showed that salted and sun dried fishes in Pallam sample markets were contaminated with pathogenic bacterial and fungal agents. Spoilage of dried fish products was food and this might be due to the unhygienic handling of the fisher folks, importance of quality products and to avail products by hygienic processing of the fishes and air tight packing of the final products up to marketing of the products.

Physico-chemical parameters like proteins, fatty acids Carbohydrates, pH, Sodium chloride and lipid were estimated and the results are given in Table 3. The nutritive value of raw fish in found to be good. But the salt dry fishes had a reduced values Protein 8% (netthili), 7% (sardiae), 9% (ribbon fish) and 6% (prawn). Low fatty acid content (0.015% netthili, 0.010% sardine, 0.011% ribbon fish and 6 % prawn). Carbohydrate is also reduced (13% netthili; 15% aardine; 12 % ribbon fish, 11% prawn). pH also loss in Sardine (6), ribbon fish (6.5) and prawn (6) of 7 in netthili. Low nutritive value in commercially sun dried fish samples is due to improper handling, inadequate preservation and unhygienic mode of drying the fish samples. The lowest pH values of dried products may enhance microbial inhibition and contribute to extend the shelf life of dried fish by inhibiting the activity of the endogenous proteases. On the other hand, an increase in pH indicates the loss of quality in fishes (Farid *et al*., 2014). Sensory characteristics of sea dried selected samples are depicted in Table 1. The sensory characteristics such as colour, odor, texture, insect infestation showed that the Sardine and Prawn was good in quality, while the netthil had decreased the quality. Ribbon fish lost their colour, texture, and odor from their original characteristics. Netthili and Prawn are free from insect infestation and Sardine and ribbon fish are slightly infected. High quality of broken pieces might be the results of using poor quality raw material, excess drying or improper drying of handling of due to moisture reconstitution (Mansur *et al*., 2013).

In the quality assessment of salted dry fishes, the sensory evaluation is most important. As quality deterioration progresses, several off-odours can be noticed. Many different odour compounds can be perceived by some are having very low odour. The microbiological analyses also showed variations among the samples. The changes in enumeration of total viable bacteria (Total plate count, T
PC), Total fungal count (TFC), *E.coli* and pathogenic bacteria, were assessed and the result are given in Table 5. The pathogenic bacteria colonies were tested using the methods of APHA (1992). The highest count of TPC and *E.coli* in commercially sun dried fish sample revealed the unhygienic condition prevailed during the drying process.

Fish acts as protein supplement for the people living in 63% of countries (Bangladesh DOF, 2001). The increase in drying time and loss and moisture content leads to protein de-naturation. The protein content was higher in sew fish than the sun dried fish and it is mainly due to protein de-naturation of reducing moisture level.

The free fatty acid levels (1% oleic acid) of all the sun dried fish samples were higher than the raw fish. In the present study, commercially sun dried sample (Prawn) shows high fatty acid content due to long storage period. The lower fatty acid in raw fish (Sardines) was due to the less degree of oxidation.

The total plate count and *E.coli* counts were found to be higher in commercially sun dried fish which is available in Tuticorin local market than the experimentally sun dried fish. High counts of TPC and *E.coli* in commercial same was due to high content of moisture and humidity in the environment and unhygienic method of preparation.

The biochemical and microbial analysis showed that the quality of experimentally sun dried fish sample was good than the commercially sun dried fish sample. Experimentally sun dried fish was properly handled and well exposed to sun light and moving air and it dried quickly and the end product was clean hygienic.

Water reconstitution of dried fish products the water reconstitution of dried fish products are presented in generally, water holding capacity of dried fish products are increased with the increase of water temperature and soaking time. The rehydration ability of dried fish products was depended on elevated soaking temperature and extended period of time. It has been reported that there was a positive relationship between rehydration ability and physical properties of dried fish products (Reza *et al*., 2005). This might be due to the fact that increased temperature of water opens the internal structure of fish muscle which maximizes the scope of rapid rehydration (Tunde-Akintund, 2008). Moreover, rehydration ability of dried fish products depends on the variation of species beside time and temperature (Nurullah, 2005).

The pH values were ranged from 6-7. The highest pH value was found in Netthili while the lowest was observed in Ribbon fish. The lowest pH values of dried products may enhance microbial inhibition and contribute to extend the shelf lite of dried fish by inhibition the activity of the endogenous proteases. On the other hand an increase in pH indicates the loss of quality in fishes (Farid *et al*., 2014).

The moisture content of the dried fishes was observed in the range of 29%-44% with the highest value obtained from Prawn and the lowest value from netthili. The variation of moisture content among thee dried fishes may occur due to improper storage, improper drying, unawareness of processors etc. According to Kamruzaman (1992), when salt is added to the fish before drying, less water needs to be removed to achieve the same effect, and the product with a water content of 35% 45%, depending on amount of salt present, is often dry enough to inhibit the growth of moulds and bacteria under most climatic condition. It has been reported that the water activity increases with the water absorption from environment, which enhances the microbial growth and reduces the loss of nutrient and shelf life of dried products (Nowsad, 2005). Sometimes fish processors keep comparatively higher moisture content in the dried fish products to gain more weight for economic benefit.The crude protein content ranged from 8%-8.5% with the highest value Ribbon fish and Prawn and the lowest in Netthili.

According to Edema and Agbon (2010), the most common sources of fish deterioration is fungal, which have the ability to grow on substrates with low water activity down to 0.6 (Thiam, 1993) and are thus important in determining fish quality. Geetha *et al*. (2014) isolated *Staphylococcus aureus* in all dried fish samples and concluded *Staphylococous* was the most predominant organisms. *Staphylococcus aureus*can grow in the presence of salt. *Stahylococcus aureus* counts in this study was lower than those reported by Goja (2013) about fresh fish. *Staphylococcus aureus* has also been detected during the process drying and subsequent smoking of eels in Alaska in 1993 (Etlund *et al*., 2004). *Salmonella* sp. was no detected in the sample collected from Bandar torkman. But *Salmonella* contamination was detected in other samples and *Shigella* was observed in all of the samples. The absence of *Salmonella* was similar to the results Oulai *et al*. (2007); Dodds**,** (1992). However, Djinou (2001) found that 0.8% of their samples had *Salmonella*.

E.coli is responsible for the production of histamine in the dried fishes (Loges *et al*., 2012). In rare cases *Samonella* and *Staphylococus* species produce histamine residue (Iduang *et al*., 2010). So safety measures should be taken to reduce the contaminations and insect infestations.The presence of *vibrio* sp., in the fish can cause pathogenic infection to the consumer. In the present investigation, *Vibrio* sp., was studied qualitatively and found in all the samples. According to recommendation of International Association of Microbiology Societies, fresh and frozen fish should be free of *Vibrio* (0/gm). The present study revealed that microbial quality was not good due to presence of *Vibrio* sp., in all the samples.

It is important to state that majority of the fungal agents isolated were of medical significance. The occurrence of *Aspergillus* spp., *Penicillium* spp., and *Candida* spp could lead to mycotoxin

elaboration and when consumed, they induce gastro intestional and metabolic disturbances (Martin,

Table 1. Sensory characteristic of sun dried salty fishes collected from Pallam

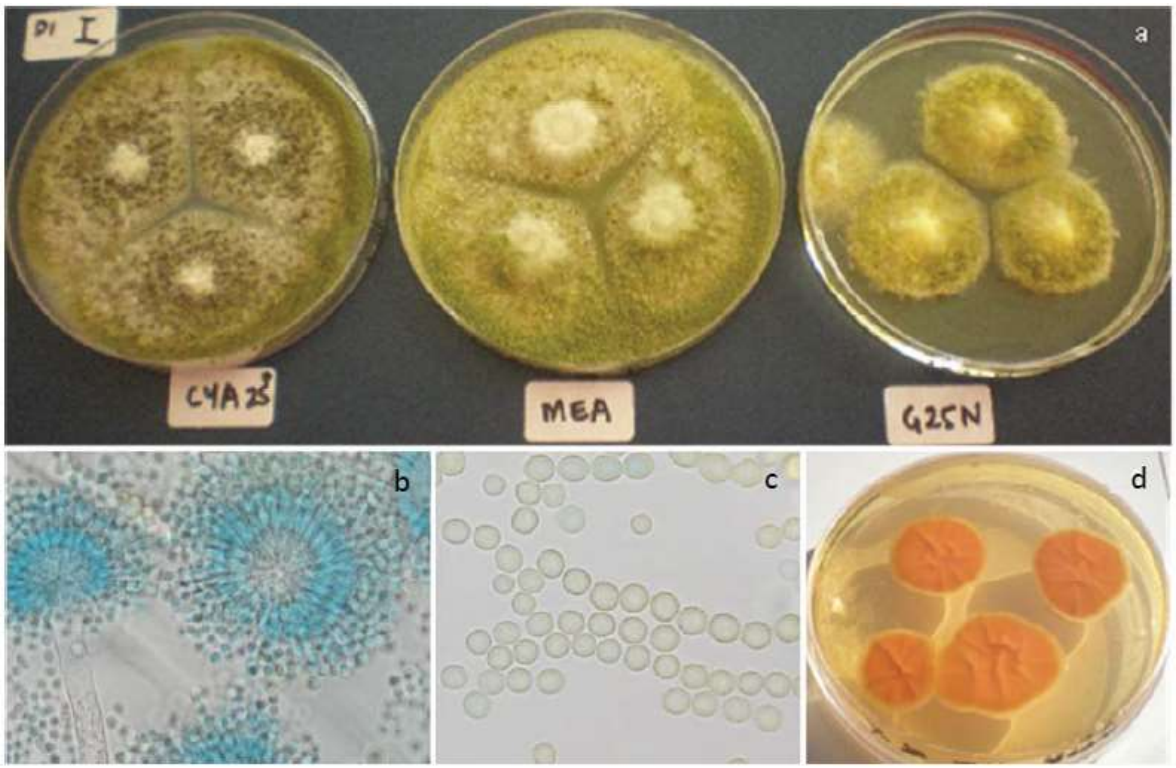
Samples	Colour	Odour	Texture	Insect infestation	Overall quality
Netthili	Brownish	good	firm & flexible	Slightly	acceptable
Sardines	Brownish	good	firm & flexible	Slightly	acceptable
Ribbon fish	Slightly dark	characteristic	Loss of firmness	Nil	good
Prawn	whitish	Firm and flexible	Firm and flexible	Slightly	good

Table 2. Microbial characteristic of selected sun dried salty fishes collected from Pallam

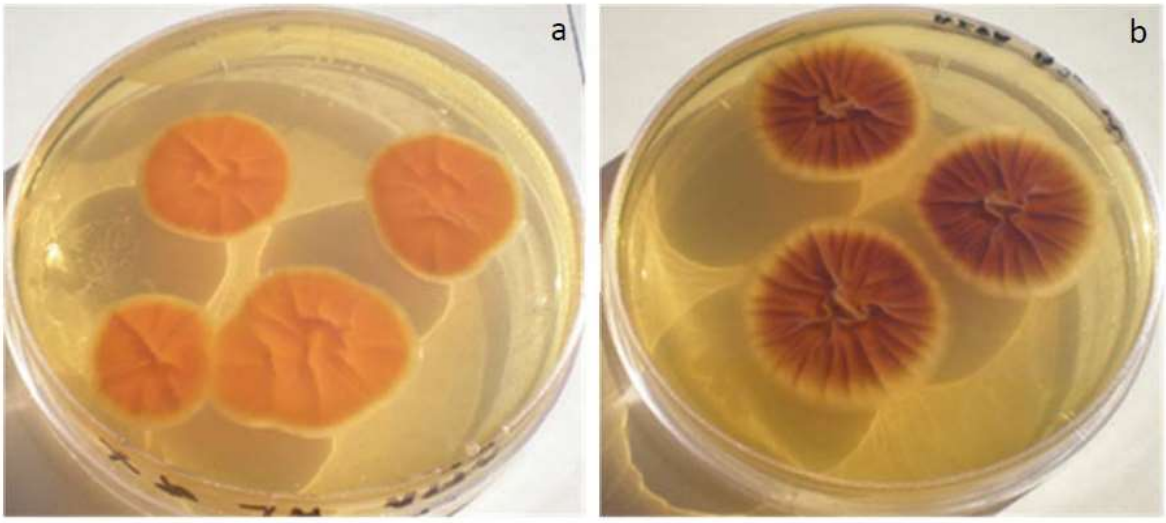
Samples	TPC Fu/g	TFC cfu/g	Moisture	Total coliform	Fecal coliform MPN/100ml	*E.coli* MPN/100ml	Fecal Streptococci MPN/100ml	Salmonella 25g	Vibrio 25g
Netthili	3.9×10^3	4×10^3	26%	60	25	8	95	Present	Present
Sardines	3.8×10^3	5×10^3	29%	65	30	6	90	Present	Present
Ribbon fish	2.5×10^3	5×10^3	40%	45	20	11	115	Absent	Absent
Prawn	5.3×10^4	2×10^3	45%	95	70	15	75	Present	Present

Table 3. Physicochemical analysis of selected sun dried salt fishes collected from Pallam

Parameters	Netthili	Sardines	Ribbon fish	Prawn
Carbohydrates (%)	13	15	12	11
pH	7	6	6.5	6
Fatty acid conlent (%)	0.015	0.010	0.011	6
Protein content (%)	8	7	9	6
Sodium chloride (%)	15	16	16.5	17
Lipids (%)	1.5	1.00	1.20	1.60



Aspergillus niger a) on identification media at 25°C for 7 days, b) light microscopy of condial heads on MEA, c) conidia showing characteristic thick and rough, spiny walls, and d) deep brown reverse colouration on AFPA agar



Orange yellow reverse colouration of A. niger b) deep brown reverse colouration of A. niger, on AFPA agar

Fig. 1. Identified fungi isolation and identification of fungi

2008). Fungi isolated from the present study are in consonance with findings by other authors however, Rafai., *et al*. 2010 reported the *Penicillium* spp., *Aspergilus* spp., and *Rhizopus* spp. are normal mycoflora present in most fish. Not with standing, many fungal genera have virulence factor which cause toxin elaboration under favourable predisposing environment. Ecology is also an important factor which influences the diversity of fungus genera on fish and their eggs. According to Pailwal *et al*., diversity of water moulds depends upon the interaction of physicochemical factors.

Not all fungi which recur in fish are considered deleterious. Moulds are one of the important caused of spoilage of salted dried fish products and they produce mycotoxins and they are able to grow in salt concentrations between 5 and 26% (Reilly, 1986). It is clear from the results than an increasing trend was shown during the storage period. TFC was more in gunny bugs stared products as compared to corrugated boxed. A significant fungal growth was recorded after 4the month of storage and this may be due to increase in a_w, moisture content and salt content. Products deteriorate by growth of moulds if the water content is approximately 15% (Gandotra *et al*., 2012). These observations were in close agreement to the present study. The rapid reduction in the water activity (a_w<0.75) is the most important factors in controlling fungi/mould contamination of the fishery products during storage (Kolakowska, 2002).

Table 4. Identified fungal species in salted dried fishes of selected samples

Samples	Name of the fungi
Netthili	*Aspergillus flavus, A. niger, Mucor*
Sardine	*Penicillium sps, A. niger*
Ribbon fish	*A.niger, A. oryzae, A. flavus*
Prawn	*A.niger, A.flavus, A. oryzae*

The observed results indicated that dry fishes are prone to contamination by pathogenic microorganisms. As fishes to be dried are usually handled with bared hands and drying process is carried out in the open atmosphere, this unhygienic practice might cause the contamination of dried fishes posing a risk of food born disease. The level of microorganisms associated with these foods can be decreased by irradiation that depends upon the absorbed dose of radiation. Ashokkumar (2008) studied the total heterotrophic bacterial count from the dried fishes of tuticorin drying yards. Azam *et al*. (2003) studied the total coliform count in the monsoon season as well as summer and they found more number of coliform in the monsoon because of moisture. The fungus *Aspergillus flavus* is responsible for the products of *alflotoxin* and it is also found that it cause food borne intoxication which leads to serious health hazards. Hashem (2011) have studied the mycotoxins from the fishes and recorded that *Aspergillus* is the main genus that commonly involved in the production of mycotoxins. Present of different types of fungi and bacteria in dried fishes has been reported by several workers. (Ashok-kumar, 2008; Gupta and Samule, 1985; Philips and Wallbridge, 1976). Moisture level of fish also plays an important role in the spoilage and lowering of moisture retards the spoilage (Ashok-Kumar, 2008). This issue is not common throughout the year. During the monsoon season, this problem occurs very severely. This leads to the quality issue and infested with pathogenic microbes leads to the dry fish unit for consumption. For the large scale drying, bamboo made racks of 0.6-1.2 m height from the floor should be used (Samad *et al*., 2009). During the monsoon season, bamboo splits made mat is used on the rack where the raw fishes were spread for drying. The microbial stability of dried fish products during processing and storage is depends upon their moisture content (Scott, 1957; Waterman, 1976; Troller and Christian, 1978). When the moisture is high during the drying of fishes, if favors the growth of microbes and there is a change of infestation with files. Khan and Khan (2001) studied the insect infestation in the dried fishes and control measures using the saturated brine solution. Using the pesticide on the dried fish to control the files, leads to the health hazards to the dry fish consumes, so fishermen should be aware of these things. The requirement of the satisfactory dried product is highly desirable and to achieve this scientific drying method should be practiced in all the drying process (Samad *et al*., 2009). In some of the cases, the food borne illness such as scobroid poisoning is observed in dry fishes mainly due to the chemical agent, histamine. It is also called as histamine poisoning, *E.coli* is responsible for the production of histamine in the dried fishes. In rare cases, *Salmonella* and *Staphylococcus* species are also produce histamine residence (Hyanga *et al*., 2010). So safety measures should be taken to reduce the contaminations and insect infestations.

4. CONCLUSION

In a general note, health education/enlightenment will be of great significance of fishermen, fish handlers, sellers and buyers that good processing and availability of storage facilities are crucial to minimize general microbial contamination. The study showed that salted and sundried fishes sold in four places (study area) were contaminated with pathogenic bacteria and fungal agents in the different seasons. Spoilage of dried fish products was found and this might be due to unhygienic handling of the fisher folks, improper processing and unhygienic vendors and venting area.

Hence control measures such as used of good quality raw material, hygienic handling practices, potable water, good quality packaging material, hygienic processing lace may be considered to improve the microbial quality of the dried fish product. Proper cooking procedures should be emphasized to climate or reduce the microorganisms to an acceptable level.

To the best of our knowledge this is the first study of the shelf life of salt treated sun-dried fish stored at refrigeration temperature. Based on the presented data (TVB-N, FFA, pH values, and microbial load counts) the optimal shelf life of sun-dried salted fishes is approximately 24 to 32 months for refrigeration (4°C) storage. The results also indicated that dried fish have greater nutritive value in terms of percentage crude protein for maintain human health. Also the effect of heat and dryness associated with open sun-drying reduces the water activity of the fish thereby limiting microrganisms, a prerequisite of spoilage. Fish processing by the combination of salting and drying is recommended for used because it gives relatively greater percentage protein and fat. Hence, it is suggested that low storage temperature (4°C) and the traditional preservatives like NaCl (Table salt) which are easily available and chapter cost wise, along with traditional sun drying process can be used by the fisher folk to arrest the growth of bacterial in fishes, thereby avoiding fish poisoning.

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RESEARCH ARTICLE

PERFORMANCE ROUTING WITH SHORTEST PATH EXPLORATION IN WIRELESS MESH NETWORKS

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ABSTRACT

In wireless mesh network the nodes are dynamically self-organized and self- configured networks create a changing topology and keep a mesh connectivity to offer Internet access to the users. The shortest path problem is one of the most fundamental problems in networking. This problem can be solved by many techniques and algorithm. In this paper we find the shortest path by using the fittest nodes in the network. By using the fittest node we can send the packets to the destination without packet loss, delay in packets. Average end to end delay is decreased by increasing bandwidth and the results are shown.

Keywords: Wireless mesh networks shortest path, packet loss, delay in packets, average end to end delay

1. INTRODUCTION

A wireless mesh network (WMN) is a mesh network created through the connection of wireless access points installed at each network user's locale. Each network user is gathered information and receives signal strength with load and bandwidth of the cluster node and also a provider with energy signal with the access point to forwarding data to the next node. The networking infrastructure is decentralized and simplified because each node need only transmit as far as the next node. Wireless mesh networking could allow people living in remote areas and small businesses operating in rural neighbourhoods to connect their networks together for affordable Internet connections. In a full mesh topology, every node communicates with every other node, not just back and forth to a central router. To overcome the reverse path forwarding & packet- loss to protect and in another variation, called a partial mesh network, nodes communicate with all nearby nodes, but not distant nodes. All communications are between the clients and the access point servers. The client/server relationship is the basis for this technology [1].

A wireless mesh network (WMN) is a communications network made up of radio nodes organized in a mesh topology. Wireless mesh networks often consist of mesh clients, mesh routers and gateways. The mesh clients are often laptops, cell phones and other wireless devices while the mesh routers forward traffic to and from the gateways which may, but need not, connect to the Internet. The coverage area of the radio nodes working as a single network is sometimes called a mesh cloud. Access to this mesh cloud is dependent on the radio nodes working in harmony with each other to create a radio network. A mesh network is reliable and offers redundancy. When one node can no longer operate, the rest of the nodes can still communicate with each other, directly or through one or more intermediate nodes. Wireless mesh networks can self form and self heal. Wireless mesh networks can be implemented with various wireless technology including 802.11, 802.15, 802.16, cellular technologies or combinations of the massive nodes with active balancing more than one type. The principle is similar to the way packets travel around the wired Internet— data will hop from one device to another until it reaches its destination. Dynamic routing algorithms implemented in each device allow this to happen. To implement such dynamic routing protocols, each device needs to communicate routing information to other devices in the network. Each device then determines what to do with the data it receives either pass it on to the next device or keep it, depending on the protocol. The routing algorithm used should attempt to always ensure that the data takes the most appropriate (fastest) route to its destination [2].

The shortest path problem is the problem of finding a path between two vertices (or nodes) in a graph such that the sum of the weights of its constituent edges is minimized. This is analogous to the problem of finding the shortest path between two intersections on a road map: the graph's vertices correspond to intersections and the edges correspond to road segments, each weighted by the length of its road segment. The shortest path problem can be defined for graphs whether undirected, directed, or mixed. It is defined here for undirected graphs; for directed graphs the definition of path requires that consecutive vertices be connected by an appropriate directed edge.

Two vertices are adjacent when they are both incident to a common edge. A path in an undirected graph is a sequence of

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n
$v_1 = v$
vertices such that v_i is adjacent to v_{i+1} for $1 \leq i < n$. Such a path P is called a path of length from v_1 to v_n. (The v_i are variables; their numbering here relates to their position in the sequence and needs not to relate to any canonical labeling of the vertices). Let $e_{i,j}$ be the edge incident to both v_i and v_j. Given a real-valued

• A* search algorithm solves for single pair shortest path using heuristics to try to speed up the search.

• FloydWarshall algorithm solves all pairs shortest paths.

• Johnson's algorithm solves all pair's shortest paths, and may be faster than Floyd– Wars hall on the vertex point including hot sport sparse graphs.

weight function $f : E \to \mathbb{R}$, and an undirected (simple) graph G, the shortest path from to v' is the path $P = (v_1, v_2, \ldots, v_n)$ (where and $v_n = v'$) that over all n possible minimizes the sum $\sum_{i=1}^{n-1} f(e_{i,i+1})$. When each edge in the graph has unit weight or $f : E \to \{1\}$, this is equivalent to finding the path with fewest edges. The problem is also sometimes called the single-pair shortest path problem, to distinguish it from the following variations:

• The single-source shortest path problem, in which we have to find shortest paths from a source vertex v to all other vertices in the graph.

• The single-destination shortest path problem, in which we have to find shortest paths from all vertices in the directed graph to a single destination vertex v. This can be reduced to the single-source shortest path problem by reversing the arcs in the directed graph.

• The all-pairs shortest path problem, in which we have to find shortest paths between every pair of vertices v, v' in the graph [3].

The most important algorithms for solving this problem are:

• Dijkstra's algorithm solves the single-source shortest path problem.

• Bellman–Ford algorithm solves the single- source problem if edge weights may be negative

• Viterbi algorithm solves the shortest stochastic path problem with an additional probabilistic weight on each node. There is lots of Algorithm used to find the shortest path in the network from source to destination. In this paper we find the shortest path using the fittest node in the network to overcome the packet loss and the delay in sending packets. We also overcome Average end to end delay by increasing the bandwidth.

2. RELATED WORKS:

Recent researches have dealt with mainly three techniques: highway hierarchy, bit vector, and transit node routing [4–8]. The highway hierarchy is similar to ours and the ordinary layer network approach. The layer network approach considers the set of edges which are frequently used when going to farther destinations and uses the network as a highway.

However, it has no assurance of optimality; hence it definitely differs from more recent algorithms despite it likely being the most popular method in modern car navigation systems Suppose that an edge e is included in the shortest path from v to u, and an endpoint of e is the kvth closest vertex to v , and the other endpoint is the kuth closest vertex to u. If both kv and ku are larger than a threshold value h, we call e a highway. . The highway hierarchy method [6,7] constructs a highway network composed of highway edges. We start by executing Dijkstra's algorithm on the original network, then go to the highway network after h steps. The highway network is usually sparser than the original network; thus the computational time is shortened. Moreover, by constructing highway network upon highway network recursively, the computational time becomes much shorter. In contrast to the layer method, the highway hierarchy method does not lose optimality. Short pre processing time is also an advantage of this method.

The highway hierarchy method finds the edges common to the middle of the shortest paths. In contrast to this, bit vector finds a common structure to the shortest paths between a vertex and a region. When the source vertex is close to the region including the target vertex, the edges to be examined become many, and hence computational time increases. By increasing the number of the regions q , the bit vector can increase the efficiency instead of increasing memory usage.

Moreover, the pre processing of the bit vector needs to solve the all pairs shortest paths problem, and thereby it takes a long time. The third method is transit node routing [8]. In the pre processing phase, it selects the transit their shortest path. Then, we compute all pairs shortest paths of the transit vertices, and these are stored as a data structure. When we execute the Dijkstra's algorithm, we can directly move from a transit vertex near the source to a transit vertex near the

destination. In practice, every node has a transit node in its neighbors; thus we can find the shortest path in a short time (usually in O(1) time).[9]

3. SHORTEST PATH EXPLORATION:

The idea behind the shortest path exploration is the optimum route (OR) is that route between a source node and a destination, providing the following specifications: We create multi-hop neighbour from source to destination The fittest node is found using the higher energy in the network. The node which has more energy will be found and those nodes are used to find the shortest path from source to destination.

It is the shortest route between the source anddestination.The same destination, unless the joint node is one of the Destination's neighbours. (No joint node (JN) or joint link (JK)).The shortest path problem is the problem of finding a path between two vertices (or nodes) in a graph such that the sum of the weights of its constituent edges is minimized. The shortest path problems are solved by different algorithms and techniques. In this paper we find the shortest path algorithm using the fittest node in the given mesh networks from source to destination. The fittest node is found using the higher energy in the network. The node which has more energy will be found and the nodes are used to find the shortest path from source to destination. The idea behind using the fittest node we can overcome the packet loss, delay in sending packets due to less energy in nodes. We increase the bandwidth for the nodes to overcome the Average end to end delay. As the bandwidth increases the average end to end delay is decreased.

4. IMPLEMENTATION

In Wireless mesh networks we find the shortest path managing the proactive and pre-active using the fittest node in the network. The fittest node is calculated using the higher energy in the networks along with bandwidth and transmission rate of packets arrival. The node which has more energy will be found and those nodes are used to find the shortest path from source to destination. By using the fittest node we can overcome the packet loss, delay in sending packets due to less energy in nodes. We increase the bandwidth for the nodes to overcome the Average end to end delay. As the bandwidth increases the average end to end delay is decreased. The implementation results is shown in Fig. 1.

Fig. 1. Decrease in Average end to end delay

5. CONCLUSION

The shortest path problem is one of the most fundamental problems in networking. This problem can be solved by many techniques and algorithm. In this paper we find the shortest path by using the fittest nodes in the network. The node which has more energy will be found and those nodes are used to find the shortest path from source to destination. By using the fittest node we can overcome the packet loss, delay in sending packets due to less energy in nodes. We increase the bandwidth for the nodes to decrease the Average end to end delay. As the bandwidth increases the average end to end delay is decreased.

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RESEARCH ARTICLE

EVALUATE THE ASSOCIATION OF ARBUSCULAR MYCORRHIZAL FUNGI IN SOME MEDICINAL PLANTS GROWN IN NOYAL RIVER BED, TIRUPPUR DISTRICT, TAMIL NADU

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ABSTRACT

To evaluate the rhizosphere soils and ten medicinal herbs polluted soils were tested for the association of arbuscular mycorrhizal fungi and determined the impact of the physico chemical factors in relation to the quantitative and qualitative assessment of AM fungi in polluted soils. Forty species of AMF belonging to five genera such as *Glomus, Acaulospora, Gigaspora, Sclerocystis* and *Scutellospora* were recorded and identified. *Glomus fistulosum* was noticed as the moist dominant in the polluted. In the non-polluted soils, all the plant species were colonized with AM fungi. Where as in polluted soils, eight herb species only were colonized and the percentage of root colonization was less.

Keywords: Arbuscular mycorrhizal fungi (AMF), Polluted soils, AMF root colonization, *Glomus fistulosum*

1. INTRODUCTION

Arbuscular mycorrhizal fungi are a natural constituent of the soil of most ecosystems. They interact with the root of more than 80 % of terrestrial plants and can be considered functional extensions of plant roots considerably enlarging the volume for nutrient up take. Arbuscular mycorrhizal (AM) fungi provide an attractive system to advance plant-based environmental cleanup. During symbiotic interaction the hyphal network functionally extends the root systems of their hosts. AM fungi occur in the soil of most ecosystems including polluted soils by acquiring phosphate micronutrients and water and delivering a proportion to their hosts they enhance the nutrition state of their hosts. Identifying the most sustainable disposal route an important issue in almost all industrialised countries due to a range of legislative, environmental and economic and social drivers (Awotoye, *et al.,* 2009).

Noyyal River is a seasonal river and it flows through the two urbanized and well known industrial cities namely Coimbatore and Tirupur before it reaches River Cauvery as a tributary. More than 6250 textile dyeing and bleaching units are situated in Tirupur region across the river basin release partially or untreated effluents into Noyyal River (Udayakumar *et al.,* 2011). Most of the industries dump their liquid waste in streams and river producing changes in physicochemical and biological conditions of water and soil. Major industrial pollution sources in the country are mills of textiles, leather, dyes, chemicals and number of other industries, in addition to municipal and domestic waste effluents. Textile industry in one of the foreign exchange earner in India. Total annual discharge of waste water is 9,420,000m^3 (Jogdand, 1995) and is disposed into river and on land. In order to find out the potential use of the tannery effluent, an experiment was conducted at polluted soils where the textile liquid wastes mixed with the Noyal river bed, Tirupur District, Tamil Nadu.

Heavy metals (HMs) occur naturally in the environment and constitute a potential hazard for waters, soils, plants and sediments. Numerous studies have indicated that agroecosystems receive inputs of HMs from the increased use of agrochemicals, the application of metalcontaining wastes such as sewage sludge, pig manure, coal and wood ashes to soils, and from atmospheric deposition (Mhatre and Pankhurst, 1997). Although some of these metals are essential plant micronutrients and are required or are beneficial for plant growth and development (Zn, Cu, Fe, Mn, Ni, Mo, Co), high contents and/or long-term presence of HMs, in soils, are generally considered a matter of concern to society as they may adversely affect the quality of soil and water, and compromise sustainable food production (Pandolfini *et al.,* 1997; Keller *et al.,* 2002; Voegelin *et al.,* 2003; Kabata-Pendias and Mukherjee, 2007). Arbuscular mycorrhizal fungi (AMF) are one of the important endophytic fungi living in the roots of most terrestrial plants. This symbiosis confers benefits directly to the host plant's growth and development through the acquisition of phosphorous and other mineral nutrients from the soil by the fungus. In addition, they may also enhance the plant's resistance to biotic and abiotic stresses (Harrier and Sawczak, 2000).

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Now a day, it has been shown that improvement of the interactions between beneficial

significantly lower the stress placed on plants by the presence of HMs, increase the availability of metal for plant uptake and subsequently are considered to be an important tool for phytoremediation technology (Glick, 2003, 2010).

2. MATERIALS AND METHODS

2.1. Study area

Tirupur is located at 11.1075°N 77.3398°E. It has an average elevation of 295 metres (967 feet). Tirupur is situated at the banks of Noyyal River, helping the textile business to grow well. As a textile city Tirupur is full of Dyings and Garments. Its location as a center of other cities like Erode, Coimbatore makes it easier to get cotton and other stuffs for the business to be done. The Noyyal River runs through the city and forms the southern boundary of corporation. The cities fast development leads to growth to its status as Tirupur district (Figure-1). The southern part of the city enjoys more rainfall Due to the surrounding of Western Ghats of the city. The mean maximum and minimum temperatures for Tirupur city during summer and winter vary between 35 to 22 °C (95 to 72 °F). The alternative perspective of historical geography is used to understand how Tirupur's industry is an outcome of processes liking work and investment across sectors in a form of regional industrialization based on small town.

Fig. 1. The map showing study area of Tirupur district.

2.2. Experimental soil

rhizosphere microorganisms and plants can

The physicochemical characteristics of the soil used for experiment were tested in Department Soil Science testing laboratories at Tamil Nadu Agricultural University, Coimbatore, Tamilnadu, India.

2.3. Rhizosphere effect in soil (Subbarao, 2000)

The quantitative rhizosphere effect of the plants was calculated using the formula:

$$R/S = \frac{\text{Number of microorganism per gram of rhizosphere soil}}{\text{Number of microorganism per gram of non-rhizosphere soil}}$$

2.4. Mycorrhizal status in plant roots

Results were processed using Phillips *et al.,* technique to study the percent of root colonization. Arbuscular mycorrhizal fungal spores were isolated by wet sieving and decanting method (Gerdemann and Nicolson, 1963).

2.5. Phosphorus content

The phosphorus content in the shoots was determined by the vanado-molybdate phosphoric acid yellow color method outlined by (Jackson, 1973).

3. RESULTS AND DISCUSSION

The contamination of nature compartments by heavy metals has become a serious environmental problem. The worldwide release of heavy metals has reached 1,350,000 tons Zn in 2002 (Singh *et al.,* 2003). This AMF-induced plant nutrient uptake is of more importance in alkaline and calcareous soils of arid semiarid regions in which the bioavailability of P and most of the cationic micronutrients is limited. Calcareous soils have also lower water holding capacity due to the presence of carbonates (Khodaverdiloo *et al.,* 2011). Furthermore, (Khodaverdiloo and Homaee, 2008 and Davari *et al.,* 2010) reported a significant reduction in plant transpiration with an increase in soil HM concentration. It has been suggested that heavy metals, such as Cd, can affect root hydraulic conductivity by multiple mechanisms operating on the apoplastic and the symplastic pathway (Shah *et al.,* 2010). Physico-chemical properties of soil samples of both the study sites were presented in (Table-1). Both the control and polluted soils of sandy clay loam and the pH of the non-polluted soil ranged from 7.3 - 7.2 while that of the polluted soils ranged from 6.2-5.4. Both study sites were deficient in phosphorus and nitrogen.

Phytoremediation cannot be done alone by the plant, just as there is always a close interaction between the microorganisms in the rhizosphere and the plant which leads to an increased activity related

to soil remediation (Compant *et al.,* 2010). Overall a searching for and application of hyper accumulating plants in combination with a beneficial rhizo- and endo-spheric microbial community holds great promise for low cost cleaning of contaminated sites. Arbuscular mycorrhizal fungi (AMF) are one of the important endophytic fungi living in the roots of most terrestrial plants. This symbiosis confers benefits directly to the host plant's growth and development through the acquisition of phosphorous and other mineral nutrients from the soil by the fungus. In addition, they may also enhance the plant's resistance to biotic and abiotic stresses (Harrier and Sawczak, 2000). Potential roles of AMF associations have repeatedly been demonstrated to alleviate metal stress of plants (Hildebrandt *et al.,* 2007).

Microorganisms in the soil are responsible for nitrogen fixation, assimilation, and degradation of organic residues to release nutrients. When HMs is retained in the soil by repeated and uncontrolled additions, they interfere with these key biochemical processes which alter ecological balance. Toxic effects of HMs on microorganisms manifests in numerous ways such as decrease in litter decomposition and nitrogen fixation, less efficient nutrient cycling. The soil microbial community is thought to be a sensitive bioindicator of metal pollution effects on bioavailability and biogeochemical processes. It has been shown that HMs at certain concentrations can have long-term toxic effects within ecosystems and have a clear negative influence on biologically mediated soil processes (Lee *et al.,* 2002).

In all forty AM fungal species were observed in the rhizosphere soils of both non-polluted and polluted sites. The AM fungal species isolated from the study sites belonging to five genera viz., *Acaulospora, Glomus, Gigaspora, Sclerocystis,* and *Scutellospora* (Table-2). The number of AM spores in the root-zone soils ranged from 0-912. The non-polluted soils were rich both in AM spore number and species abundant whereas in polluted soils were less. This is an agreement with earlier findings of (Ramapulla Reddy and Manoharachary, 1990). There was an impact of season on the distribution of AM spores in polluted and non-polluted soil sites (Jagpal *et al.,* 1988). The numbers of AM spores were more in monsoon and summer. There was a certain degree of specificity among the different species in both non-polluted and polluted sites. There are previous reports of such specificity in root zone soils (Mosse, 1981) and the occurrence of forty species of AM fungi in polluted habitats in the present study is a report as (Gildon and Tinker, 1981) have isolated only one species of effluent tolerant AM fungi. In present study, *Glomus fistulosum* was noticed the most dominant effluent tolerant strain of AM fungi in polluted sites.

All the test plant species in non-polluted soil sites examined exhibited AM colonization whereas in soil polluted with effluent sites, eight plant species were positive for AM colonization and one plant species were nonmycorrhizal, results indicated that mycorrhizal condition is the rule and nonmycorrhizal condition is the exception and agrees with the widespread association of AM reported in natural ecosystem (John and Coleman, 1983). The presence of AM colonization in plants of industrially polluted habitats have earlier been reported (Gildon and Tinker, 1981). The percent root colonization was comparatively more in nonpolluted soil grasses than polluted soil plants (Table-2) and (Figure-2&3). The minimum and maximum per cent root colonization was observed in *Aerva lanata* (Amaranthaceae) (45%) and *Phyllanths amarus* (Euphorbiaceae) (76%) respectively in non-polluted sites and in polluted sites the minimum was in *Datura stromonium* (Solanaceae) (22%) and maximum in *Leucas aspera* (Lamiaceae) (96%) (Table-2). The percentage root colonization was high in summer season of plant species in polluted soil sites in the per cent study as supported by (Ramapulla Reddy and Manoharachary, 1990) in industrially polluted soil sites plant species (Table-2). The number of AM spores were more in monsoon and summer seasons in nonpolluted soils and less in polluted with effluents may be due to dilution of nutrients or accumulation of nutrients and optimum moisture level or water stress and increased level of carbon, zinc and iron were noticed in soil polluted with effluents may be caused reduction in the number of AM propagules, as supported by (Ramapulla Reddy and Manoharachary, 1990).

These results suggested that the variation in soil pH, temperature and effluent pollution seems to be the decisive factors in tropical soils influencing distribution of AM fungi. The physico-chemical data revealed that the polluted soil was acidic to neutral with more of carbon. Zinc, Iron and other nutrients in the present study. It can be concluded that tannery effluents significantly alter the occurrence of native AM fungi both quantitatively and qualitatively. The variation in spore population which was generally more or less in effluent site may be attributed to the season, soil edaphic characters particularly to the acidity, high moisture and organic carbon in soil.

Table 1. Physico-chemical characteristics of polluted and non-polluted soils of three different sites in polluted soils in Noyal river bed, Tirupur District, Tamil Nadu

S.No.	Study Sites	pH	Organic matter	N	P	K	Zn	Cu	Mn	Fe
Polluted sites										
1.	Site I	6.26±0.20	5.4±0.4	96.8±4.2	24.2±4.2	348±12.0	2.4±0.2	1.7±0.12	2.04±1.2	30.56±4.2
2.	Site II	5.93±1.24	4.6 ±0.2	89.0±4.6	26.4±2.4	293±10.0	2.98±0.4	1.8±0.12	1.92±1.2	21.58±4.2
3.	Site III	5.4±0.57	3.2±0.4	87.8±4.2	28.4±2.2	24.2±10.20	1.74±0.3	1.9±0.13	1.93±1.0	20.14±4.2
Non- Polluted sites										
1.	Site I	7.30±0.41	2.2±0.02	92.8±12.1	21.3±12.1	121.0±17.0	2.4±0.2	2.1±0.1	5.6±1.2	1.2±0.1
2.	Site II	6.56±0.57	2.8 ±0.2	89.4±14.0	28.4±11.4	135.4±15.2	2.7±0.1	1.9±0.2	5.4 ±1.3	1.2±0.1
3.	Site III	7.21±0.58	2.4±0.02	77.4±12.0	34.0±10.4	140.0±26.0	2.1±0.4	1.8±0.1	4.2 ±1.1	1.3±0.1

NPS-Non-Polluted Soil. **PS**-Polluted soil.

General nutrient Status of the soil.	**N**	**P_2O_3**	**K_2O**
Low :	<140	<24.2	<140.7
Medium:	141-280	24.3-32.2	140.8-281.6
High :	>280	>32.3	>281.6

Table. 2. Precent root colonization spore count and AM fungal species associated in the root zone soil it plants in polluted and non-polluted sites at Noyal river

S.No.	Family and Botanical name.	Study Sites	% of root Colonization		AMF Spores Population/100g Soil		AMF Spores Associated	
			PS	NPS	PS	NPS	PS	NPS
1.	Amaranthaceae *Aerva lanata* L.	S I	45	66	224	534	*Acaulospora sporocarpa* *Glomus fasciculatum* *Glomus delhiense*	*Glomus citricola* *Glomus fistulosum* *Glomus multicaulis*
		S II	32	45	135	463	*Glomus hoi* *Glomus canadense* *Glomus austral*	*Glomus occultum* *Glomus multisubtensum* *Glomus segmentatum* *Aculospora denticulatam*
		S III	25	33	334	765	*Acaulospora denticulatum* *Aculospora thomii* *Scutellospora scutata*	*Glomus multisubtensum* *Glomus microcarpum* *Glomus mososporum*
2.	Solanaceae *Datura stromonium* L.	S I	33	69	210	345	*Glomus fistulosum* *Glomus multicaulis* *Acaulospora denticulatum* *Aculospora thomii*	*Scutellispora heterograma* *Acaulospora thomii* *Glomus claroids*
		S II	22	53	325	657	*Glomus fistulosum* *Aculospora denticulatam* *Glomus pansihalos*	*Acaulospora apendicula* *Glomus clarum* *Glomus claroids*
		S III	33	79	132	432	*Glomus segmentatum* *Aculospora denticulatam* *Scutellospora scutata*	*Glomus flavisporum* *Glomus fistulosum* *Glomus etunicatum*
3.	Lamiaceae *Leucas aspera* L.	S I	56	89	215	765	*Glomus hoi* *Glomus muticaulis* *Scutellospora verrucosa* *Sclerocystis paecaulis*	*Glomus geosporum* *Acaulospora apendicula* *Glomus segmentatum* *Glomus fasciculatum*
		S II	26	78	453	876	*Glomus pansihalos* *Glomus claroids* *Glomus citricola* *Gl.hoi*	*Aculospora thomii* *Scutellospora scutata* *Glomus fistulosum*
		S III	41	95	312	675	*Glomus fistulosum* *Single spore of dimorphicum* *Glomus manihot*	*Glomus austral* *Glomus muticaulis* *Acaulospora rehmii*
4.	Euphorbiaceae	S I	38	73	343	745	*Glomus pansihalos*	*Acaulospora thomii*

	Phyllanths amarus L.						*Glomus geosporum* *Scutellispora heterograma*	*Acaulosopora gdanskensis* *Single spore of dimorphicum*
		S II	25	55	225	564	*Acaulospora thomii* *Glomus magnicaule* *Glomus citricola*	*Glomus microaggregatum* *Glomus versiforme* *Scutellispora heterograma*
		S III	22	76	143	688	*Glomus delhiense* *Glomus monosporum* *Acaulospora sporocarpa*	*Aculospora denticulatam* *Glomus pansihalos* *Glomus geosporum*
5.	Euphorbiaceae *Phyllanths madraspatensis* L.	S I	31	66	233	543	*Acaulospora apendicula* *Glomus clarum* *Glomus claroids*	*Scutellispora heterograma* *Acaulospora thomii* *Glomus claroids*
		S II	45	75	153	456	*Glomus claroids* *Glomus canadense* *Glomus austral*	*Glomus intraradix* *Glomus clarum*
		S III	32	56	122	698	*Glomus austral* *Glomus muticaulis* *Acaulospora rehmii*	*Glomus pansihalos* *Glomus claroids* *Glomus citricola*
6.	Malvaceae *Sida acuta* Burm.	S I	25	65	211	534	*Glomus multisubtensum* *Glomus microcarpum* *Glomus mososporum*	*Glomus geosporum* *Acaulospora apendicula* *Glomus clarum*
		S II	36	78	143	546	*Acaulospora sporocarpa* *Glomus fasciculatum* *Glomus delhiense*	*Glomus delhiense* *Glomus monosporum* *Acaulospora sporocarpa*
		S III	41	58	241	789	*Acaulospora denticulatum* *Aculospora thomii* *Glomus fistulosum* *Scutellospora scutata*	*Glomus fistulosum* *Aculospora denticulatam* *Glomus pansihalos*
7.	Tiliaceae *Corchorus aestuans* L.	S I	42	81	314	543	*Glomus citricola* *Glomus multicaulis*	*Glomus canadense* *Acaulospora thomii*
		S II	21	78	110	876	*Glomus manihot* *Glomus arborense*	*Acaulosopora gdanskensis*
		S III	43	69	143	765	*Scutellispora heterograma* *Glomus claroids*	*Glomus flavisporum* *Glomus fistulosum*

8.	Oxalidaceae *Oxalis carniculata* L.	S I	55	93	322	869	*Glomus austral* *Glomus muticaulis* *Acaulospora rehmii* *Sclerocystis paecaulis*	*Glomus dimorphicum* *Glomus fistulosum* *Glomus microcarpum*
		S II	46	88	309	896	*Glomus etunicatum* *Acaulosopora gdanskensis* *Glomus magnicaule* *Acaulospora thomii*	*Glomus citricola* *Glomus fistulosum* *Acaulospora apendicula* *Glomus rubiformis* *Sclerocystis paecaulis*
		S III	43	80	259	798	*Acaulospora thomii* *Acaulosopora gdanskensis* *Glomus magnicaule*	*Acaulospora sporocarpa* *Glomus fasciculatum* *Glomus delhiense*
9.	Fabaceae *Cassia occidentalis* L.	S I	12	66	103	659	*Glomus geosporum* *Scutellispora heterograma*	*Scutellispora heterograma* *Acaulospora thomii*
		S II	44	83	215	765	*Glomus maculosum*	*Acaulospora thomii*
		S III	23	78	453	876	*Acaulospora apendicula* *Glomus rubiformis*	*Acaulospora denticulatum* *Aculospora thomii* *Scutellospora scutata*
10.	Mimosaceae *Mimosa pudica* L.	S I	36	87	312	675	*Glomus fistulosum* *Glomus canadense* *Glomus invermeyanum* *Glomus manihot* *Glomus arborense* *Glomus macrocarpum*	*Glomus pansihalos* *Glomus geosporum* *Scutellispora heterograma* *Glomus magnicaule* *Glomus radiatum* *Glomus magnicaule* *Glomus citricola*
		S II	45	66	343	745	*Glomus delhiense* *Glomus monosporum* *Acaulospora sporocarpa* *Acaulosopora gdanskensis* *Acaulospora thomii*	*Glomus etunicatum* *Entrospora infrequens* *Acaulosopora gdanskensis* *Acaulospora apendicula* *Glomus magnicaule* *Acaulospora thomii*
		S III	32	45	233	543	*Glomus citricola* *Glomus fistulosum* *Glomus multicaulis* *Acaulospora apendicula*	*Glomus austral* *Glomus muticaulis* *Acaulospora rehmii*

NPS-Non-Polluted Soil. **PS**-Polluted soil.

Fig. 2. Showing the common herb plants collected in Noyal river bed.
A- *Aerva lanata,* B- *Datura stromonium,* C- *Leucas aspera,* D- *Phyllanths amarus,* E- *Corchorus aestuans,* F- *Mimosa pudica*

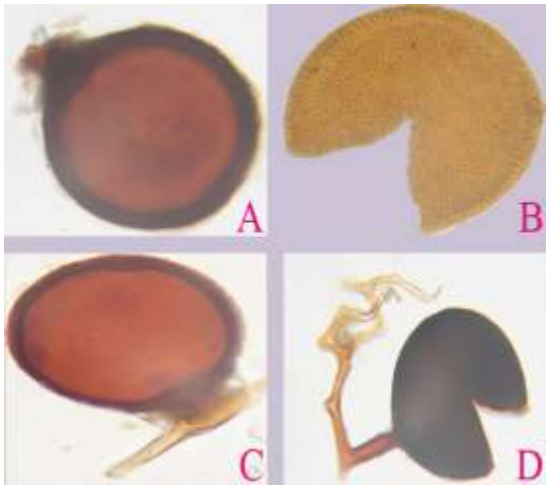

Fig. 3. AM fungal spores/100g soil in both polluted and non-polluted soils of Noyal river
A - *Acaulospora sporocarpa,* B- *Acaulospora denticulatum,* C-*Gigaspora species,* D- *Glomus multicaulis*

4. CONCLUSION

The indigenous AM fungi associated with the dominant plant species growing on the polluted sites, may have assisted plants tirupur in soils that were substantially polluted with toxic metals. In order to utilize native plants for revegetation of metal polluted soils, more detailed investigations are needed to improve our understanding of plant adaptation. It is also essential to identify the potential role of indigenous AM fungi in phytostabilization of toxic metals in contaminated environments, and also in stimulating plant growth so that optimum use can be made of both the plant and fungal symbionts for successful revegetation.

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RESEARCH ARTICLE

KINETIC STUDIES OF PARTIALLY PURIFIED LIPASE FROM MARINE ACTINOMYCETE *STREPTOMYCES ACRIMYCINI* NGP 1

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ABSTRACT

This study was focused on partial purification and characterization of lipase from *Streptomyces acrimycini* NGP 1, isolated from marine sediment of south Indian coastal region. In purification steps, 4.53 fold purification was achieved after 85% ammonium sulphate precipitation with 0.97 percent recovery. In further purification steps, 1.33 fold purification was achieved by Sephadex G-100 chromatography with 1.61 percent of recovery. The specific activity of purified enzyme was 1525 U/mg. Zymogram of crude enzyme on native-PAGE presented bands with lipase activity of molecular weight and Isoelectric point were 50 kDa and 7.4 respectively. These features render this lipase of interest as a biocatalyst for applications such as biodiesel production and detergent formulations.

Keywords: Lipase, Marine sediment, Purification, Application

1. INTRODUCTION

Lipases are serine hydrolases characterized as triacylglycerol acylhydrolases (E.C. 3.1.1.3) and ought to be separated from esterases (E.C. 3.1.1.1) by the idea of their substrate. In reality, a criteria used to recognize these two kinds of catalysts, i.e., actuation by the nearness of an interface, additionally called "interfacial initiation," was discovered unacceptable for the arrangement of such proteins as a few lipases didn't show such marvel. Prominent cases of this phenomenon are Lip4 from *Candida rugosa* (Barriuso *et al*., 2016) and *Candida antarctica* B (Zisis *et al*., 2016). In addition, lipase and esterase accord themes portrayed by PROSITE database (Hulo *et al*., 2006). In addition, lipases are capable of hydrolyzing water-insoluble esters and show a different distribution on the hydrophobic amino acids surrounding the active site (Chahinian and Sarda, 2009). Lipases, in thermodynamic good conditions (i.e., low water movement), likewise catalyze an enormous assortment of blend responses which can be grouped into two principle kinds of responses, i.e., esterification what's more, transesterification (Kapoor and Gupta, 2009).

First lipases were isolated by Eijkman from Bacillus prodigiosus, Bacillus pyocyaneus, and *Bacillus fluorescens*, currently known as *Serratia marcescens*, *Pseudomonas aeruginosa*, and *P. fluorescens*, respectively (Eijkmann, 1901). Lipase are produced by various organisms, including animals, plants and microorganisms. Most animal lipases are obtained from the pancreas of cattle, sheep, hogs, and pigs. Unfortunately, lipases extracted from animal pancreas are rarely pure enough to be used in the food industry (Sharma and Kanwar, 2014). Because of their capacity to utilize fat as the main carbon source, microorganisms delivering lipases have been separated from nourishment waste, where they are answerable for the enhance change of dairy items, for example, cheddar, or from slick situations (sewage, waste dump locales, and oil plant emanating) (Li *et al*., 2014). Due to the importance and wide variety of lipase applications, different techniques have been developed in order to isolate lipases from various sources. Lipase enzyme was purified by sequential methods of ammonium sulphate precipitation and Sephadex G-100 gel column chromatography (Tripathi *et al*., 2014). Further the molecular weight of the enzyme was analyzed and kinetic studies were carried out.

The current study presents sequential optimization strategy to improve lipase production using *S. acrimycini* NGP 1. Partial purification and characterization of the partially purified enzyme were investigated and these parameters are necessary to use the enzyme efficiently in an industrial application.

2. MATERIALS AND METHODS

2.1. Isolation of Actinomycete

The marine sediment was collected from the coastal region of Marina, Tamilnadu, India at 2-3m depth by using grab sampler. The collected

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sediment was subjected for enrichment prior to serial dilution. One gram of enriched sample serially diluted using sterile distilled water and spread over starch casein Agar (SCA) plates and incubated at 28 + 2^{O}C for 7 days (Balagurunathan and Radhakrishnan, 2009).

2.2. Lipase Screening

The marine actinomycete isolate was subjected for rapid screening of lipase production by rapid plate method (Kouker and Jaeger, 1987). The screening of the actinomycete isolates for lipase activity was studied by inoculating them on Rhodomine B medium. Colonies which showed orange fluorescence under UV irradiation indicated lipase activity and was measured by Noomrio *et al*, (1990). The amount of liberated fattyacid was determined by titration with 0.05M NaOH. The enzyme activity was expressed as U/ml (IU = release 1 µ mole of fattyacid in 1 min).

2.3. Genetic Identification

The genetic level identification of potential actinomycete isolates were carried out. Phylogenetic relationships with closely related species were determined by using MEGA version 4.0. Distance matrices were determined and were used to elaborate a dendrogram by the neighbor-joining method (Tamura *et al*., 2007; Teske *et al*., 1996).

2.4. Growth Kinetics and Enzyme Production

To determine the optimum culture conditions for enzyme production, the actinomycete isolates were grown in basal liquid medium in an orbital shaker at 120 rpm.

2.5. Partial Purification of lipase enzyme

The purification procedure was referred to the method of Shu *et al* , (2006). The culture filtrate was collected through whatmann No. 1 filter paper and the filtrate was centrifuged at 4000 rpm for 10 min to obtain crude enzyme. Then the ammonium sulphate was added to the filtrate to give final concentration of 60.0 - 80.0 per cent (w/v) saturation at 4ºC. The precipitate was collected by centrifugation at 4000 rpm for 4 min. The precipitate was then dissolved in 20 ml of double distilled water and dialyzed for 24 hrs at 4ºC against double distilled water and concentrated by lyophilization.

An aliquot of the lyophilized sample (1 ml) was loaded on to a sephadex G-100 column (45 X 1.5 cm) previously equilibrated with 0.02 M phosphate buffer (pH 7.8). Elution of lipase was performed by a linear gradient of NaCl (0-1.0 M/l in equilibrating buffer) at 30 ml/h. The fractions of 5.0 ml each were collected for every one hour (30 fractions) and assayed for lipase and protein activity.

2.6. Optimum Substrate Concentration

The optimum substrate concentration for the maximum activity of the enzyme determined in terms of maximum reaction velocity (V max) and michaelis constant (Km at which reaction velocity is half maximum). For this, various concentrations of specific substrates were prepared and incubated with purified enzyme preparations. For Lipase activity, 1 to 10 mM of olive oil in acetate buffer (20 mM; pH 4.0) was used as substrate (Zhou *et al*., 2012). V max and Km were estimated graphically by plotting substrate concentration in µM on X axis against enzyme activity U/mg protein on the Y axis. The accurate values of Vmax and Km were obtained by double reciprocal Line Weaver-Burk plot and Eadie-Hofstee plot. The protein content was estimated and the molecular weight of the sample was determined by SDS-PAGE (Zouaoui and Bouziane, 2012).

2.7. Determination of Pi Values

The pI values of each enzyme fraction were determined by Iso electric focusing (IEF) technique (Zouaoui and Bouziane, 2012).

3. RESULTS AND DISCUSSION

3.1. Screening and Identification

The actinomycetes growth occured on the SCA plate was subjected for Lipase screening in the Rhodomine B agar medium and it produced maximum enzyme of about 40 U/ml. The genetic level analysis of the 16S rRNA gene is the most important tool for correct identification of microbial species. The isolate was identified as *S. acrimycini* NGP 1 and the sequence was submitted to Gen-Bank (JX843532). A phylogenetic tree constructed by MEGA 4 software based on 16srRNA partial sequence. Similarly, 16SrRNA phylogenetic analysis of actinomycetes isolated from Eastern Ghats was carried out neighbor-joining algorithm (Nithya *et al*., 2012). Similarly, 55 samples from different regions were selected and screened by Rhodamine B flat transparent circle method to observe lipase producing effect, among them, Serratia sp. has the characteristics of fast growth, high enzyme production and stable ability (Gupta *et al*., 2013)

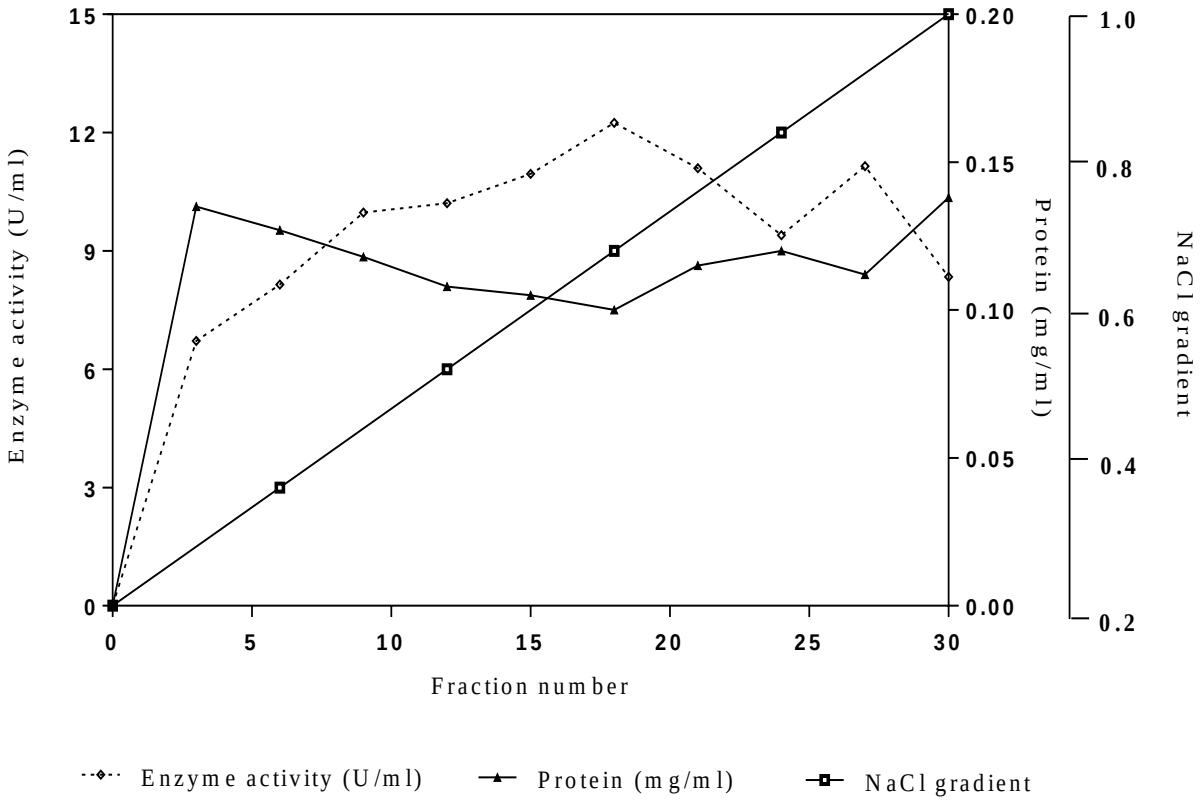
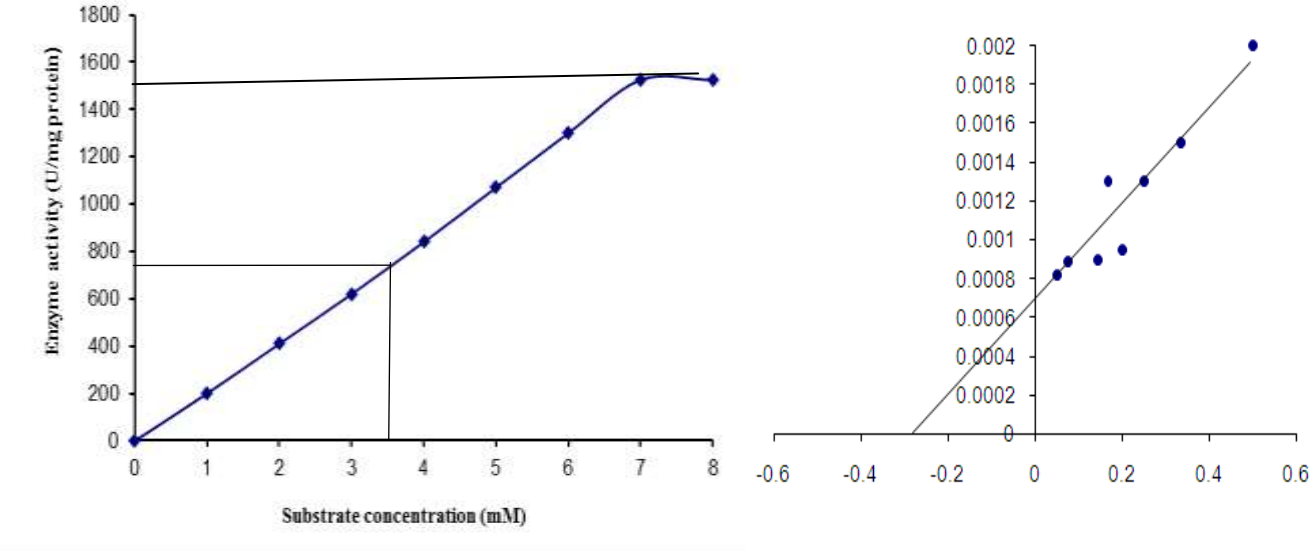


Fig 1: Purification of lipase from *S. acrimycini* NGP 1 on Sephadex G 100 column

Table 1. Purification of lipase from the culture filtrates of *S. acrimycini* NGP 1

Sample	Volume (ml)	Activity (U/ml)	Protein (mg/ml)	Total Activity (U)	Specific Activity (U/mg)	Recovery Yield (%)	Purification factor
***Streptomyces acrimycini* NGP 1**							
Culture filtrate	500	9.15	0.12	4575	76.25	100	1.00
Ammonium sulphate precipitation	20	10.37	0.14	207.4	74.07	4.53	0.97
Column Chromatography Sephadex G - 100							
Fraction 18	5	12.25	0.10	61.25	122.5	1.33	1.61

V max **Double reciprocal plot**



Eadie-Hofstee plot

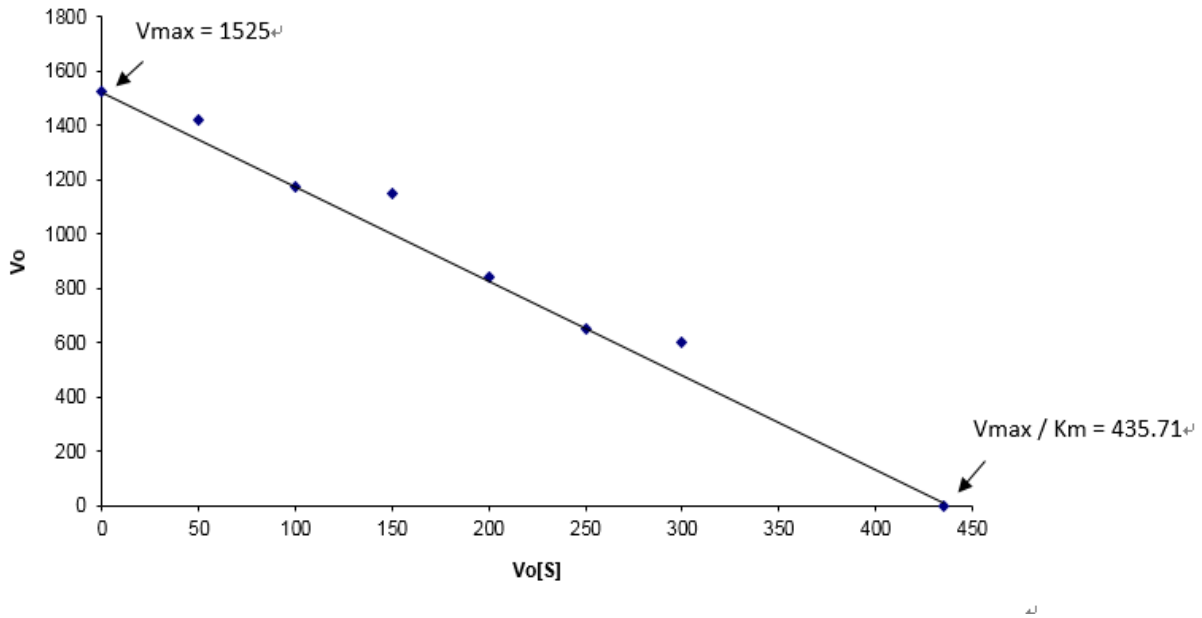


Fig. 2: Kinetics of *S. acrimycini* NGP 1 lipase

3.2. Growth kinetics and Enzyme production

The results showed that the culture filtrate of *S. acrimycini* NGP 1 had lipase total activity of 9.15 U/ml with a protein content of 0.12 mg/ml; the specific activity was 76.25 U/mg protein. When concentrated by ammonium sulphate, the specific activity was decreased to 74.07 U/mg protein with a purification factor of 0.97. The protein content was increased to 0.14 mg/ml. The enzyme recovery was 4.53 per cent. When passed through sephadex G 100 column, the fraction 18 exhibited lipase activity. In fraction 18, the protein content was 0.10 mg/ml; specific activity was 122.5 U/mg proteins, the purification fold and recovery yield were 1.61 and 1.33 per cent (Fig. 1 and Table 1). Similarly, lipase enzyme from *Microbacterium sp*. was partially purified by ammonium sulphate and Sephadex G100 column chromatography. This purification procedure resulted in 2.1fold purification of lipase with a 20.8 % final yield (Tripathi *et al*., 2014)

The kinetic properties of lipase were obtained using 1 to 10 mM of olive oil as substrate in a Lineweaver-Burk plot. In lipase, Vmax and Km values were estimated by using olive oil in acetate buffer (20 mM; pH 4.0); Vmax and Km for *S. acrimycini* NGP 1 lipase were 1525 U/mg protein and 3.5 mM respectively (Fig. 2). The V_{max} of the purified lipase from a newly isolated *Trichosporon coremiiforme* strain was 1800 U/mg protein (Laachari *et al*., 2013). The Km value of the *Serratia marcescens* lipase when tributyrin was used as substrate was 1.35 mM (Abdou, 2003)

3.3. pI and Molecular weight

The enzyme lipase of actinomycete NGP 1 exhibited single fraction of pI. The pI value was found to be 7.4. The molecular weight of the enzyme was determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). A single band was exhibited, had a molecular weight of 50 kDa. Similar to our study, the molecular weight of purified lipase enzyme was 40 kDa on SDS-PAGE from *Microbacterium sp* [9]. According to the report of Priyanka *et al*, (2019) lipase enzyme from *Pseudomonas reinekei* showed 50kDa molecular weight using negative and positive mode anion exchange chromatography.

4. CONCLUSION

The present study revealed the kinetic parameters of lipase produced from actinomycete, isolated from the South Indian coastal region. According to the kinetic studies, the enzyme will be optimized efficiently to carry out the various industrial applications.

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RESEARCH ARTICLE

EFFECTS OF ARTIFICIAL PHOTOPERIOD ON THE BODY COLOURATION IN THE ORNAMENTAL FISH *POECILIA SPHENOPS*

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ABSTRACT

In Orange balloon Molly, *Poecilia sphenops,* the development of chronic stress due to continuous exposure to artificial LED lighting irrespective to photoperiod causes deterioration in the body colour even in the presence of carotenoid rich green water and artificial feed with added additives in the experimental fish tank setup. In the present study, the effects of continuous exposure to lighting and its consecutive development of chronic stress causes the body colouration to fade off in the freshwater ornamental live bearer, *Poecilia sphenops,* though the fishes were provided with colour enhancing as well as stress relieving factors. Several trials showed that, whatever may be the colour enhancing agents provided, artificial lighting for long exposure irrelevant to the normal photoperiod remains as a strong anti-colouration as well as stress inducing factor and remains unaffected to any antagonistic factors. To conclude photoperiod is an essential factor to be concerned for colour enhancement in *Poecilia sphenops.*

Keywords: Photoperiod, Green water, Body colouration, *Poecilia sphenops*

1. INTRODUCTION

Ornamental fish trade is one of the quickly growing markets in the world. More than 125 countries around the globe are involved in this trade either as an importer or exporter. Most of the fishes that are sold by the aquarium are bought by hobbyists (Dey., 2016). Ornamental fishes are bought for their fascinating and attractive skin colour and pattern. The sexual selection acts on colour and patterns leading colourful displays and also more diverse colouration pattern (Endler., 1980). The prices of the fishes are fixed based on their brilliant skin colour and pattern. The body of fishes shows a wide range of skin colours ranging from yellow, orange, blue, purple, green, etc.(Animal colouration / Wikipedia.com). These colourations are due to colour pigments such as carotenoids (Biological pigments / Wikipedia.com). These pigments cannot be stored beneath the skin permanently. As fishes cannot synthesize their own colouring pigments *de novo*, the colouring agents which are synthesized by some plants, algae and microorganisms, need to be incorporated in their diet (Johnson *et al.,* 1991). There are some factors like photoperiod, feeds with added pigments and green water which directly affects the colour pigmentation in the fishes. Some fish feeds with artificially synthesized colour pigments are commercially available in markets to enhance the body colour. Green water which is rich in micro alga, *C. vulgaris* has become a potent pigment source, which imparts yellow/blue hues. The biomass of this alga had already been proved to be useful in the diets of rainbow trout yielding both muscle and skin pigmentation effects (Gouveia *et al.,* 1997). It has also been reported that it contains carotenoid pigments in concentrations of up to 0.4% (dry wt), of which, 80% were potential red hue inducing pigments (Gouveia *et al.,* 1996). Other than green water, commercial feeds with added additives such as astaxanthin is also used to enhance colouration (Gupta *et al.,* 2007). Apart from all those factors, photoperiod has an important effect since it is one of the stimuli that cue the internal biological clock about the seasonal changes in the environment. Light is a key environmental factor that synchronizes all stages of fish from embryo development to sexual maturation (Bairwa *et al.,* 2013).

Photoperiod is most useful in predicting environmental conditions in the future or at distant localities; photoperiod provides a go/ no-go signal that initiates a usually irrevocable cascade of physiological and development processes that culminate in reproduction, dormancy and migration. Day length provides a highly reliable calendar that animals can use to anticipate and prepare for seasonal change (Sarkar *et al.*, 2011). Photoperiod is an important physical factor that affects the growth, reproduction and many other functions of the fish. Light and dark cycle provides internal harmonization for the rhythmic synthesis and release of hormones (i.e. melatonin), whose signal

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affects rhythmic physiological function in fish (Bairwa *et al.,* 2013). This study is carried out to prove that among all factors, photoperiod is a strong factor influencing the colour pigmentation adopting ornamental live bearer, Balloon Molly fish (*Poecilia sphenops*) as the animal model.

2. MATERIALS AND METHODS

2.1. Apparatus setup-

- Size of the tank - 45cm * 25cm * 25cm
- Water column depth - 20cm
- Light intensity - 400lux
- Temperature - 23-27˚ C
- Lamp type - LED

2.2. Animal model-

- Common name - Balloon molly
- Binomial name - *Poecilia sphenops*
- Variety - Balloon Molly
- Colour - Orange
- Number of fishes used - 14 (7 male & 7 female)
- Age of the fish - Adult
- Gestational history:

Gravida	Nil
Parity	Nil

2.3. Methods

To prove photoperiod has a strong influence over body colour, the comfortable method is to compare with other factors that are also engaged in enhancing body colouration in ornamental fishes. Experiments carried out with combination of these factors give us a good platform for comparing their effects. Along with control trial there are six experiments carried out in this study. The three factors that are mostly sought for body colouration are,

- Photoperiod
- Green water
- Commercial feed with artificial pigments

2.3.1. Control

A pair of fishes observed in normal regular environmental condition is made as a control trial to compare the results of the experiments.

- Water – Clear water
- Photoperiod – 12 hrs.. daylight & 12 hrs.. darkness
- Feed – Without artificial pigments
- No. of fishes – 2 (Male – 1 & Female – 1)
- Duration – 1 week
- Lighting – Sun light
- Temperature – 23-27˚ C
- Feeding frequency – 3 times/day

2.3.2. Experiment - 1

- Water – Clear water
- Photoperiod – 24 hrs. LED light & 0 hrs. darkness
- Feed – without artificial pigments
- No. of fishes – 2 (Male – 1 & Female – 1)
- Duration – 1 week
- Lighting – LED lighting
- Temperature – 23-27˚ C
- Feeding frequency – 3 times/day

2.3.3. Experiment - 2

- Water – Green water
- Photoperiod – 12 hrs. daylight & 12 hrs. darkness
- Feed – without artificial pigments
- No. of fishes – 2 (Male – 1 & Female – 1)
- Duration – 1week
- Lighting – Sun light
- Temperature – 23-27˚ C
- Feeding frequency – 3 times/day

2.3.4. Experiment - 3

- Water – Green water
- Photoperiod – 24 hrs. LED light & 0 hrs. darkness
- Feed – without artificial pigments
- No. of fishes – 2 (Male – 1 & Female – 1)
- Duration – 1week
- Lighting – LED lighting
- Temperature – 23-27˚ C
- Feeding frequency – 3 times/day

2.3.5. Experiment - 4

- Water – Clear water
- Photoperiod – 24 hrs. LED light & 0 hrs. darkness
- Feed – without artificial pigments
- No. of fishes – 2 (Male – 1 & Female – 1)
- Duration – 1 week
- Lighting – LED lighting
- Temperature – 23-27˚ C
- Feeding frequency – 3 times/day

2.3.6. Experiment - 5

- Water – Green water
- Photoperiod – 12 hrs. daylight & 12 hrs. darkness

- Feed – without artificial pigments
- No. of fishes – 2 (Male – 1 & Female – 1)
- Duration – 1 week
- Lighting – Sun light
- Temperature – 23-27° C
- Feeding frequency – 3 times/day

2.3.7. Experiment - 6

- Water – Green water
- Photoperiod – 24 hrs. LED light & 0 hrs. darkness
- Feed – without artificial pigments
- No. of fishes – 2 (Male – 1 & Female – 1)
- Duration – 1 week
- Lighting – LED lighting
- Temperature – 23-27° C
- Feeding frequency – 3 times/day

3. RESULTS

The results of the experiments given in this project are the observations made from the trials based on the following criteria.

- Fading or enhancement in body colour.
- Healthy and active swimming
- Courtship behaviour

3.1. Control

In this control trial with normal environmental factors, the colour of the body remains bright with regular body patterns. The fishes were noticed with normal and active swimming all through the trial for complete 1 week. Courtship behaviour between the male and female was spotted several times.

- Colour is bright.
- Healthy swimming is observed.
- Courtship behaviour is seen frequently.
- Sign of copulation is seen.

Fig.1. Body colour deteriorated

Fig.2. Body colour enhanced

Fig.3. Courtship behaviour

Fig.4. Body colour enhanced in green water

3.2 Observations during the Experiments

Based on the observations (Table 1) made during the experimental trials with a combination of different factors, we can conclude the cumulative results with the gained outputs.

Table 1: Observations made during the experimental trials

Trial	Colour	Movement	Courtship
Control	Enhanced	Active	Yes
Exp - 1	Deteriorated	Inactive	No
Exp - 2	Enhanced	Active	Yes
Exp - 3	Deteriorated	Inactive	No
Exp - 4	Deteriorated	Inactive	No
Exp - 5	Enhanced	Active	Yes
Exp - 6	Deteriorated	Inactive	No

With the observations made from the experiments it is easy to conclude whether the fishes are in stress during the trials and on correlating the factors and the observations we can conclude the primary factor that mostly influences the stress and also the body colour pigmentation.

3.3. Correlation between the factors and the observations

Correlation is an effective tool to find which factor influences the result the most by correlating the inputs (photoperiod, green water & pigmented feed) with the observations (colour, movement & courtship behaviour). The factors and the observations are first tabulated to test for correlation (Table. 2).

Table 2: Factors and their corresponding observations

Trial	Photoperiod	Water condition	Feed	Observed colour
Control	Normal	Clear	Non pigmented	Enhanced
Experiment 1	Induced	Clear	Non pigmented	Deteriorated
Experiment 2	Normal	Green	Non pigmented	Enhanced
Experiment 3	Induced	Green	Non pigmented	Deteriorated
Experiment 4	Induced	Clear	Pigmented	Deteriorated
Experiment 5	Normal	Green	Pigmented	Enhanced
Experiment 6	Induced	Green	Pigmented	Deteriorated

3.3.1. Correlation between Water condition and Observed colour

In this calculation, water condition is taken as an independent variable and the observed body colour is considered as a dependent variable since the colouration is either enhanced or deteriorated by the influence of the water condition either green water or clear water.

Pearson's coefficient of correlation, r = -0.1667 (poor relation)

Since the Pearson's coefficient, r = -0.1667 the relation between the independent factor (water condition) has a poor relation with the dependent factor (body colour).

3.3.2. Correlation between commercial feed and Observed colour

In this calculation, pigmented feed is taken as an independent variable and the observed body colour is considered as a dependent variable since the colouration is either enhanced or deteriorated by the influence of the pigmented feed or non-pigmented feed.

Table 3. Correlation between water condition and body colour

Variable X – Independent Variable Y – Dependent

Trial	Water condition Variable X	Observed colour Variable Y
Control	Clear	Enhanced
Exp - 1	Clear	Deteriorated
Exp - 2	Green	Enhanced
Exp - 3	Green	Deteriorated
Exp - 4	Clear	Deteriorated
Exp - 5	Green	Enhanced
Exp - 6	Green	Deteriorated

Fig. 1. Correlation between water condition and body colour

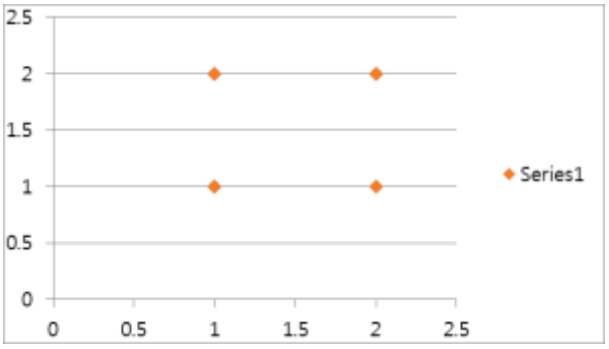
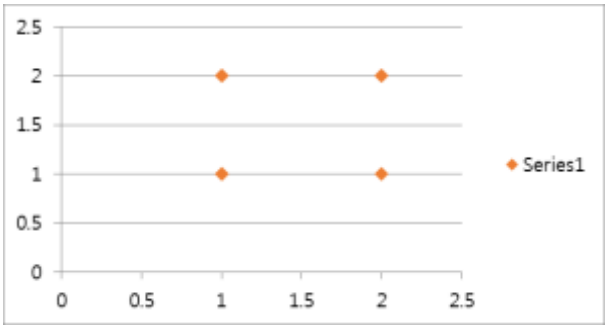


Table 4. Correlation between commercial feed and body colour

Variable X – Independent Variable Y – Dependent

Trial	Feed Variable X	Observed colour Variable Y
Control	Non pigmented	Enhanced
Exp - 1	Non pigmented	Deteriorated
Exp - 2	Non pigmented	Enhanced
Exp - 3	Non pigmented	Deteriorated
Exp - 4	Pigmented	Deteriorated
Exp - 5	Pigmented	Enhanced
Exp - 6	Pigmented	Deteriorated

Fig. 2. Correlation between commercial feed and body colour.



Pearson's coefficient of correlation, r = 0.1667 (poor relation)

Since the Pearson's coefficient, r = 0.1667 the relation between the independent factor commercial feed has a poor relation with the dependent factor body colour.

3.3.3. Correlation between photoperiod and Observed colour

In this calculation, photoperiod is taken as an independent variable and the observed body colour is considered as a dependent variable since the colouration is either enhanced or deteriorated by the influence of the natural photoperiod or induced photoperiod.

Table 5. Correlation between photoperiod and body colour

Variable X – Independent Variable Y – Dependent

Trial	Photoperiod Variable X	Observed colour Variable Y
Control	Normal	Enhanced
Exp - 1	Induced	Deteriorated
Exp - 2	Normal	Enhanced
Exp - 3	Induced	Deteriorated
Exp - 4	Induced	Deteriorated
Exp - 5	Normal	Enhanced
Exp - 6	Induced	Deteriorated

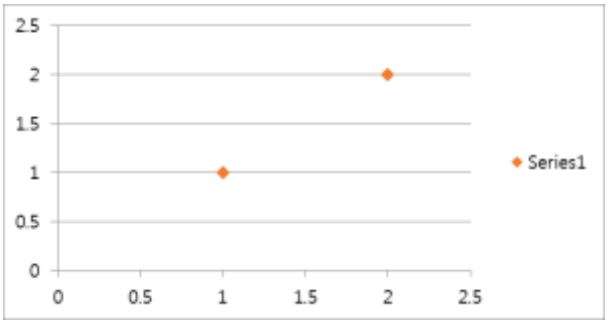


Fig. 3. Correlation between photoperiod and body colour

Pearson's coefficient of correlation, r = 1 (strong linear relation)

Since the Pearson's coefficient, r = 1 the relation between the independent factor commercial feed has a strong linear relation with the dependent factor body colour.

4. DISCUSSION

The results of the present study indicate that, among the considered three factors (water condition, pigmented feed and photoperiod), the correlation between water condition and pigmented feed with body colour is weaker than that of the correlation between photoperiod and the body colour. The correlation graph for water condition (Graph. 1) and the correlation graph for pigmented feed (Graph. 2) show a weak relation since the graphs cannot be interpreted as a strong linear relation but, the correlation graph for photoperiod (Graph. 3) can be interpreted as a strong linear bond. This proves that, among all other factors used in the study, photoperiod has a very strong influence over the body colour pigmentation in the ornamental live bearer *Poecilia sphenops.* Apart from body colouration, the fact that growth of Juveniles of the Anemone fish *Amphiprion melanopus* under 24L: 0D was slower than under 16L: 8D shows that photoperiod also affects the growth of the fishes (Arvedlund *et al.,* 2000). Similar studies were conducted about the exposure of male and female commercially important *P. sphenops* to manipulated photoperiod regimes with a specific time period to assess its effect on the growth and gonadal development along with the utilization of formulated feed. This presumption has a lacuna for stimulation of growth and enhancement of reproductive performance in fingerlings of ornamental fish although it has been tested for species of food fishes (Zutshi *et al*., 2017). These all studies show that apart from colouration, photoperiod has many vital roles to play. This does not mean other factors don't have any significant role in body colouration. In the Experiment 5, the fishes were brighter than in other experiments, which we can conclude by considering the algal carotenoids in account. Significant work has been done on pigmentation of many commercial fish species using carotenoids. In this respect, Mircoalgae such as *Chlorella vulgaris* is as effective as its synthetic counterpart in pigmentation of two most important ornamental fish species, *Cyprinus carpio* & *Carassius auratus* (Das and Biswas, 2016).

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RESEARCH ARTICLE

ON r - DYNAMIC VERTEX COLORING OF SOME GRAPHS

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ABSTRACT

An r - dynamic is an proper vertex k coloring with an function $a : V(G) \to T$ where $|T| = k$ and it is k – colorable. It can be defined as $|a(Neigh(v)| \geq \min\{r, deg_G(v)\}$, for each $v \in V(G)$. The r- dynamic chromatic number of a graph G is the minutest coloring *k* of G which is r-dynamic k -colorable and denoted by $\chi_r(G)$. In this paper, we have obtain the r- coloring results of some special graphs such as Flower graph F_n, Double cone graph $C_{p,q}$, Triangle snake graph TS_n, Helm graph H_m, Crossed prism graph CP_q.

Mathematics subject classification: 05C15

Keywords: r- dynamic coloring; Flower graph; Double cone graph; Triangle snake graph; Helm graph; Crossed prism graph.

1. INTRODUCTION

Let the graph G be undirected connected with m vertices and n edges. In this paper, the study is made on the r -dynamic chromatic number which was first introduced by Montgomery [6]. An r-dynamic coloring is an proper vertex coloring such that coloring of the adjacent vertices should not receives the similar color and each vertex $V(G)$ has neighbors atleast $min\{r, deg_G(v)\}$ different color classes. When $r = 1$ then it is equal to the chromatic number of the graph. The bounds of r - dynamic coloring was given minimum and maximum degree. Furthermore, some of references are given for r - dynamic coloring in the following paper [1], [7], [4], [5] and the bounds are studied from [2] [3]. The most familiar lower bound was given in the following lemma.

Lemma 1. $\chi_r(G) \geq \min\{r, \Delta(G)\} + 1$

The *Flower graph* are derived from the Helm graph by adding pendent edge from pendent vertex to the hub vertex.

The *Double cone graph* is an special case of cone graph $C_m + \overline{K}_n$, $\overline{K} = 2$.

The *Triangle snake graph* is derived from the path graph p_{n-1}, and addi- tionally adding edges (v_{2i-1}, v_{2i+1}) for $i = 1, 2, \ldots, n-1$. It is denoted as TS_n, *n* is odd.

The *Helm graph* is obtained from the wheel graph by adding pendent edge to each vertex. So that each pendent edge has additionally a vertex. [7]

The *Crossed prism graph* is an even graph and it is obtained by taking two disjoint cycle and adding an edges (v_i, v_{2i+1}) and (v_{i+1}, v_{2i}) (v_{i+1}, v_{2i}) for $i = 1, 3, \cdots, n-1$.

2. Results on *r*- dynamic coloring on some graphs

Lemma 2. Let F_a be the flower graph. The lower bound for r -dynamic chromatic number of flower graph is

$$\chi_r[F_a] \geq \begin{cases} \delta + 1, & 1 \leq r \leq \delta \\ \Delta + 1, & \delta + 1 \leq r \leq \Delta \end{cases}$$

Proof : Let $V(F_a) = \{u, u_{i,} t_j : 1 \leq i \leq a, 1 \leq j \leq a\}$ where *u* is the center vertex which joins the vertices u_i and t_j for $1 \leq i \leq a$ and $1 \leq j \leq a$. The minimum degree of F_a is 2 and the maximum degree is 2a. For $1 \leq r \leq \delta$, the

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vertices $V = u, u_1, u_2$ persuade a clique of order $\delta + 1$ in F_a. Thus, $\chi_r[F_a] \geq \delta + 1$. Thus for $\delta + 1 \leq r \leq \Delta$ based on Lemma 1, we have $\chi_r[F_a] \geq \min\{r, \Delta[F_a]\} = \Delta + 1$. Thus, it completes the proof.

Theorem 1. For $a \geq 3$, the r- dynamic coloring of the flower graph F_a are

$$\chi_r[F_a] = \begin{cases} 3, for\ 1 \leq r \leq 2, a\ is\ even \\ 4, for\ 1 \leq r \leq 3, a\ is\ odd \\ 6, for\ 4 \leq r \leq 5, a = 5 \\ r + 1,\ otherwise \end{cases}$$

Proof : The upper bound for the theorem are illustrated in the following cases:

Case : 1 $1 \leq r \leq 2, a\ is\ even$

Based on the lemma 2, we have $\chi_r[F_a] \geq 3$. To find the upper bound cosider the following coloring: Color the middle vertex u with color 3, then color the vertices u_i with color 1 and 2 alternatively for $1 \leq i \leq a$ and next color the vertices t_j with color 2 and 1 orderly for $1 \leq j \leq a$ Thus, $\chi_r[F_a] \leq 3$. Hence, we have $\chi_r[F_a] = 3$.

Case: 2 $1 \leq r \leq 2, a\ is\ even$

Based on the lemma 2, we have $\chi_r[F_a] \geq 4$. To find the upper bound consider the following coloring: Color the vertices u_i with color 1 and 2 alter- natively for $1 \leq i \leq a - 1$, then color the vertex u_a with color 3. Next color the vertices t_j with color 2 and 1 in order for $1 \leq j \leq a$ and finally color the middle vertex u with color 4. Thus, $\chi_r[F_a] \leq 4$. Therefore, $\chi_r[F_a] = 4$.

Case: 3 $4 \leq r \leq 5$, $a = 5$

Based on the lemma 2, we have $\chi_r[F_a] \geq 6$. To find the upper bound consider the following coloring: At this particular case, we color the vertices u_i with separate colors such as $1,2,\cdots,5$ for $1 \leq i \leq a$ Then color the vertices t_j with color 3, 4,5,1 and 2 for $1 \leq j \leq a$. At the last color the center vertex u with color 6. Thus, $\chi_r[F_a] \leq 6$. Hence, we have $\chi_r[F_a] \leq 6$.

Case: 4 otherwise

Based on the lemma 2, we have $\chi_r[F_a] \geq r + 1$. To find the upper bound consider the following coloring: Color the center vertices u_i and t_j with color $1,2,\cdots,r$ based on the r –adjacency condition. But, there is also a need of one more color to satisfy the condition so color the vertex u with color $r + 1$. Therefore, $\chi_r[F_a] \leq r + 1$. Hence, we have $\chi_r[F_a] = r + 1$.

Theorem 2. For $p = 2,\ q \geq 4$, the r - dynamic coloring of the double cone graph $C_{p,q}$ are

$$\chi_r[C_{p,q}] = \begin{cases} 3, for\ 1 \leq r \leq 2, qis\ even \\ 4, for\ 1 \leq r \leq 2\ and\ q\ is\ odd\ 4 \\ 4, for\ r = 3,\ m \equiv 0(mod\ 3) \\ 5, for\ r \neq 3,\ m \not\equiv 0(mod\ 3) \\ 7,\ for\ 4 \leq r \leq 5, m \equiv 2(mod\ 3) \\ r + 2, otherwise \end{cases}$$

Proof: Let the vertices of $C_{p,q} = V(\overline{K}_2) \cup V(C_q)$ i.e., $V(C_{p,q}) = \{p_1, p_2, u_i : 1 \leq i \leq q\}$. The vertices of $\overline{K}_2$ are adjacent to each vertices of C_q but p_1 is not adjacent to p_2 .The edges are $\{u_i u_{i+1} : 1 \leq i \leq q - 1\} \cup \{u_q u_1\} \cup \{p_1 u_i : 1 \leq i \leq q\} \cup \{p_2 u_i : 1 \leq i \leq q\}$. The maximum and minimum degree of $C_{2,q}$ are q and 4.

Case: 1 $1 \leq r \leq 2$

If q is *even*, color p_1 and p_2 with color 1 and the leftover vertices u_i with color 2 and 3 alternatively for $1 \leq i \leq q$. Hence, $\chi_r[C_{p,q}] \leq 3$. Based on the Lemma 1 we have $\chi_r[C_{p,q}] \geq \min\{r, \Delta[C_{p,q}]\} + 1 = 3$. Hence, $\chi_r[C_{p,q}] = 3$.

If q is *odd*, Color p_1 and p_2 with color 1. The remaining vertices u_i with color 2 and for $1 \leq i \leq q - 1$ and the leftover vertex u_q with

color 4. Therefore, $\chi_r[C_{p,q}] \leq 4$. Based on the Lemma 1 we have $\chi_r[C_{p,q}] \geq \min\{r, \Delta[C_{p,q}]\} + 1 = 3$. Thus, $\chi_r[C_{p,q}] \geq 4$. Hence, $\chi_r[C_{p,q}] = 4$.

Case: 2 $r = 3$

If $m \equiv 0(mod\ 3)$, then color the vertices u_i with color 2,3 and 4 orderly for $1 \leq i \leq q$. Atlast color the vertices p_1 and p_2 with color 1. Thus, $\chi_r[C_{p,q}] \leq 4$. Based on the Lemma 1 the lower bound for $C_{p,q}$ are $\chi_r[C_{p,q}] \geq 4$. Therefore, $\chi_r[C_{p,q}] = 4$.

If $m \not\equiv 0(mod\ 3)$, then color the vertices p_1 and p_2 as in the case $m \equiv 0(mod\ 3)$ and color u_i with color 2,3 and 4 sequencingly for $1 \leq i \leq q-1$. But, to satisfy the r -adjacency condition we need of one more color so color the vertex u_q with color 5. Therefore, $\chi_r[C_{p,q}] \leq 5$. Based on the Lemma 1 the lower bound for $C_{p,q}$ are $\chi_r[C_{p,q}] \geq 5$. Therefore, $\chi_r[C_{p,q}] = 5$.

Case: 3 $4 \leq r \leq 5, m \equiv 2(mod\ 3)$

Based on the Lemma 1 the lower bound for $C_{p,q}$ are $\chi_r[C_{p,q}] \geq 7$. The upper bound can be calculated by the following coloring: Color the vertices p_1 and p_2 with color 1, then color the vertices u_i with color 2,3 and 4 alternatively for $1 \leq i \leq q-2$. Next color the vertices u_{q-1} with color 5 and u_q with color 6. But still there is an need of one color so change the color of vertex p_2 with color 7. Hence, $\chi_r[C_{p,q}] \leq 7$. Therefore, $\chi_r[C_{p,q}] = 7$.

Case: 4 Otherwise

Based on the Lemma 1 the lower bound for $C_{2,q}$ are $\chi_r[C_{p,q}] \geq r+2$. The upper bound can be calculated by the following coloring: Other than the about values of r, the result of double cone graph leads to $r+2$. So, color the vertex p_1 with color 1, then color the vertices u_i with color $2, 3, \cdots, r+1$ for $1 \leq i \leq q$ either randomly or sequencingly but the coloring should satisfy the r -adjacency condition. Finally, color the vertex p_2 with color $r+2$. Hence, $\chi_r(C_{p,q}) \leq r+2$. Therefore, $\chi_r(C_{p,q}) = r+2$.

Lemma 3. Let TS_n, be the Triangle snake graph. The lower bound for r- dynamic chromatic number of Triangle snake graph is

$$\chi_r[TS_n] \geq \begin{cases} \delta+1, & 1 \leq r \leq \delta \\ \Delta+1, & \delta+1 \leq r \leq \Delta \end{cases}$$

Proof: Let $V(TS_n) = \{u_i : 1 \leq i \leq n-1\} \cup \{u_{ii+1} : 1 \leq i \leq n-2\}$ where u_i are the vertices of path P_{n-1} and u_{ii+1} are the vertices corresponding to the edges u_i and u_{i+1}. Thus the minimum degree of TS_n are 2 and the maximum degree is 4. For $1 \leq r \leq \delta$, the vertices $V = u_{12}, u_1, u_2$ persuade a clique of order $\delta+1$ in (TS_n). Thus, $\chi_r[TS_n] \geq \delta+1$. Thus for $\delta+1 \leq r \leq \Delta$ based on Lemma 1, we have $\chi_r[TS_n] \geq \min\{r, \Delta[TS_n]\} = \Delta+1$. Thus, it completes the proof.

Theorem 3. For $n \geq 3$, n is odd the r- dynamic coloring of the Triangle snake graph TS_n are

$$\chi_r[TS_n] \geq \begin{cases} 3, & 1 \leq r \leq \delta \\ \Delta+1, & \delta+1 \leq r \leq \Delta \end{cases}$$

Proof: The upper bound for Triangle snake graph are illustrated in following cases:

Case : 1 $1 \leq r \leq \delta$

Based on the Lemma 3 the lower bound of TS_n are $\chi_r(TS_n) \geq 3$. To find the upper bound we consider the following coloring: color the vertices u_i with color 1 and 2 alternatively for $1 \leq i \leq n-1$. Then color the remaining vertices u_{ii+1} with single color 3 for $1 \leq i \leq n-2$. Thus we have $\chi_r(TS_n) \leq 3$. Therefore, $\chi_r(TS_n) = 3$.

Case: 2 $\delta+1 \leq r \leq \Delta$

- Based on the Lemma 3 the lower bound of TS_n are $\chi_r(TS_n) \geq 4$. To find the upper bound we consider the following coloring: when $r = 3$, color the vertices u_i with color 1,2 and 3 orderly for $1 \leq i \leq n-1$ and finally color the last set of vertices u_{ii+1} with color 4 for $1 \leq i \leq n-2$. Therefore, $\chi_r(TS_n) \leq 4$. $\chi_r(TS_n) = 4$.
- Based on the Lemma 3 the lower bound of TS_n are $\chi_r(TS_n) \geq r+1$. To find the upper bound we consider the following coloring: when $r = 4$, color the vertices u_i with the colors as given in the $r = 3$.

Next, color the vertices u_{ii+1} with color 4 and 5 alternatively for $1 \leq i \leq n-2$. Therefore, $\chi_r(TS_n) \leq 5$. Thus we have obtained $\mathrm{r}+1$ colors. Hence, we have $\chi_r(TS_n) \leq \mathrm{r}+1$. $\chi_r(TS_n) = \mathrm{r}+1$.

Theorem 4. For $\mathrm{q} \geq 4$, q is even the r - dynamic coloring of the Crossed prism graph CP_q are

$$\chi_r[CP_q] = \begin{cases} 2, for\ r = 1 \\ 4, for\ 2 \leq r \leq \Delta, q \equiv 0(mod\ 4) \\ 3, for\ r = 2, q \not\equiv 1(mod\ 3) \\ 4, for\ r = 2, q \equiv 1(mod\ 3) \\ 5, for\ r \geq \Delta, q \not\equiv 0(mod\ 4) \\ 6, \ for\ r \geq \Delta, q = 60L + 6, L \in W \end{cases}$$

Proof: The vertex set $V(CP_q) = \{ t_i : 1 \leq i \leq q\} \cup \{x_j : 1 \leq j \leq q \}$ where t_i are the inner cycle of crossed prism and x_j is the outer cycle. The edges are crossed between the vertices t_i and x_{j+1} for i is odd, and the next set of edges are crosses between t_i and x_{j-1} for i is even. The maximum and minimum degree of CP_q are $\delta = \Delta = 3$. Since q is even, we get $q/2$ set of crossed vertices. Here t_1 is the second vertex of first crossed prism and t_2 is the first vertex of second crossed prism. It continues upto q. Similarly, it is same as for x_j.

Case: 1 $r = 1$

Color the vertices t_i and x_j with color 1 and 2 alternatively for $1 \leq i \leq q$. Thus $\chi_r(CP_q) \leq 2$. Based on the Lemma 1 the lower bound for CP_q are $\chi_r(CP_q) \geq 2$. Therefore, $\chi_r(CP_q) = 2$.

Case: 2 $2 \leq r \leq \Delta, q \equiv 0(mod\ 4)$

Color the vertices t_i with color 1,2,3 and 4 alternatively for $1 \leq i \leq q$. Next we need to color the verices x_j which is quite different since, it does not follow any order or sequencing. The coloring of the vertices x_j is dependent on the coloring of the vertices t_i. Since it is an even graph, color the vertex x_1 with the color of the vertex t_2 and color the vertex x_2 with the color of the vertex t_1. Then, color the vertex x_3 with the color of t_4 and the vertex x_4 with the color of t_3. By continuing this way, color the vertex x_q with color of the vertex t_{q-1} and the vertex x_{q-1} with the color of t_q. Thus the coloring are interchanged between every pair of vertices. Thence, $\chi_r(CP_q) \leq 4$. Based on the Lemma 1 the lower bound for CP_q are $\chi_r(CP_q) \geq 4$. Therefore, $\chi_r(CP_q) = 4$.

Case: 3 $r = 2$

Sub case: 1 $q \not\equiv 1(mod\ 3)$, in this subcase there are two subdivisions which are as follows:

If $q \equiv 0(mod\ 3)$, color the vertices t_i with color 1,2 and 3 alternatively for $1 \leq i \leq q$. Similarly, color the vertices x_j with the colors have used in t_i $1 \leq i \leq q$. Therefore, $\chi_2(CP_q) \leq 4$.

If $q \equiv 2(mod\ 3)$, color the vertices t_i with color 1, 2 and 3 orderly for $1 \leq i \leq q-2$. Next, color the vertex t_{q-1} with color 1, 2 and color the vertex t_q with color 2. Finally, color the vertices x_j from the color 1, 2 and 3 either sequencingly or unorderly for $1 \leq j \leq q$ but with an r - adjacency condition. Thus, $\chi_2(CP_q) \leq 4$. Based on the Lemma 1 the lower bound for CP_q are $\chi_r(CP_q) \geq 4$. Therefore, $\chi_r(CP_q) = 4$.

Sub case : 2 $q \equiv 1(mod\ 3)$

Color the vertices t_i with colors 1,2,3 and 4 orderly for $1 \leq i \leq q-2$ and color the vertex t_{q-1} with color 2 and color the vertex t_q with color 3. Next, color the vertices x_j with the same colors as given in t_i unorderly with an 2-adjacency condition. Thus, $\chi_2(CP_q) \leq 4$. Based on the Lemma 1 the lower bound for CP_q are $\chi_r(CP_q) \geq 4$. Therefore, $\chi_r(CP_q) = 4$.

Case: 4 $r \geq \Delta$

- If $q \equiv 0(mod\ 4)$, then color the vertices t_i with color 1,2,3,4 and 5 for $1 \leq i \leq q$. Then the coloring of the vertices x_j is dependent on the coloring of the vertices t_i. The coloring of x_j follows the similar way as given in case-2 but with five colors. Thence, $\chi_r(CP_q) \leq 5$. Based on the Lemma 1 the lower bound for CP_q are $\chi_r(CP_q) \geq 5$. Therefore, $\chi_r(CP_q) = 5$.

- If $q = 60L + 6$, it is an special case of crossed prism graph, since we need to give six different colors to satisfy the Δ-adjacency condition. So color the vertices t_i with color 1, 2 and 3 sequencingly for $1 \leq i \leq q$ and color the vertices x_j with color 4, 5 and 6 orderly for $1 \leq i \leq q$. Therefore, $\chi_r(CP_q) \leq 6$. Based on the Lemma 1 the lower bound for CP_q are $\chi_r(CP_q) \geq 6$. Therefore, $\chi_r(CP_q) = 6$.

Lemma 4. Let H_m be the Helm graph. The lower bound for r-dynamic chromatic number of Helm graph is

$$\chi_r[H_m] \geq \begin{cases} \delta + 1, & 1 \leq r \leq \delta \\ \Delta + 1, \delta + 1 \leq r \leq \Delta \end{cases}$$

Proof: The vertices of H_m are $\{x, x_i, y_i: 1 \leq i \leq m\}$. x is the hub vertex which is connected to vertex of cycle x_i and a pendent edge is add to each vertex of x_i. The vertices at the pendent edge are named as y. The minimum and maximum degree of H_m are $\delta = 1$ and $\Delta = \mathrm{m}$. For $1 \leq r \leq \delta$, the vertices $V = x, x_1, x_2$ persuade a clique of order $\delta + 1$ in (H_m). Thus, $\chi_r[H_m] \geq \delta + 1$. Thus for $\delta + 1 \leq r \leq \Delta$ based on Lemma 1, we have $\chi_r[H_m] \geq \min\{r, \Delta[H_m]\} = \Delta + 1$. Thus, it completes the proof.

Theorem 5. For $m \geq 3$, the r- dynamic coloring of the Helm graph H_m are

$$\chi_r[H_m] = \begin{cases} 4, 1 \leq r \leq 3 \text{ and } m \text{ is odd} \\ 3, 1 \leq r \leq 2 \text{ and m is even} \\ \mathrm{r} + 1, \text{otherwise} \end{cases}$$

Proof : The upper bound for the Helm graph are obtained from the following cases:

Case: 1 $1 \leq r \leq 3$

Based on the Lemma 4, we have $\chi_r(H_m) \geq 4$ To find the upper bound consider the following coloring: color the vertices x_i with color 1,2 orderly for $1 \leq i \leq m-1$ and color the vertex x_m with color 3. Next color the vertices y_i with color 3 for $1 \leq i \leq m-1$ and the vertex y_m with color 1. Finally, the last vertex x with color 4. Hence, $\chi_r(H_m) \leq 4$. Therefore, $\chi_r(H_m) = 4$.

Case : 2 $1 \leq r \leq 2$

Based on the Lemma 4, we have $\chi_r(H_m) \geq 3$. To find the upper bound consider the following coloring: color the vertices x_i with color 1,2 alternatively for $1 \leq i \leq m$ and color the vertex y_i with color 2 and 1 orderly for $1 \leq i \leq m$. The color the vertex x with color 3. Hence, $\chi_r(H_m) \leq 3$. Thus, $\chi_r(H_m) = 3$.

Case: 3 Otherwise

Based on the Lemma 4, we have $\chi_r(H_m) \geq 6$. To find the upper bound consider the following coloring: in this case, there is an special case that is $m = 5$, for $r = 4,5$. At this case we receives six colors. i.e., color the vertices x_i with five different colors for $1 \leq i \leq m$. Similarly, color the vertices y_i with the same colors as given in x_i but in different order with 4, 5-adjacency condition and color the vertex x with color 6. Hence, $\chi_r(H_m) \leq 6$. Thus, $\chi_r(H_m) = 6$.

Based on the Lemma 4, we have $\chi_r(H_m) \geq 5$. To find the upper bound consider the following coloring: next is to receive $r + 1$ colors, at $r = 4$ and $m \neq 5$ color the vertices x_i and y_i for $1 \leq i \leq m$ with colors 1,2,3,4 either orderly or unorderly but with 4- adjacency condition. Even though there is four different colors we also need one more color to satisfy 4- adjacency condition. So color the vertex x with color 5. Therefore, $\chi_r(H_m) \leq 5$. Thus, $\chi_r(H_m) = 5$.

Based on the Lemma 4, we have $\chi_r(H_m) \geq \mathrm{r} + 1$. To find the upper bound consider the following coloring: next at $r = 5$, color the vertices x_i and y_i for $1 \leq i \leq m$ with colors 1,2,3,4 either orderly or unorderly but with 5-adjacency condition. Here also we need one more color, so color the vertex x with color 6. Therefore, $\chi_r(H_m) \leq 6$. Thus, proceeding by this way, at $r = m$ color the vertices x_i and y_i for $1 \leq i \leq m$ with colors $1,2,\cdots,m$ but with r-adjacency condition. Atlast color the hub vertex x with color m+1. Thus, $\chi_r(H_m) \leq \mathrm{m} + 1$. Therefore, $\chi_r(H_m) \leq \mathrm{r} + 1$. Thus, $\chi_r(H_m) = \mathrm{r} + 1$.

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RESEARCH ARTICLE

ON r - DYNAMIC COLORING OF SOME GRAPHS

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ABSTRACT

The *r*-dynamic coloring of a graph H is a proper p-coloring of the vertices of the graph H so that for every vertex $a \in V(H)$ has neighbors in atleast $min\{r, d(a)\}$ distinct classes of color. The least p which provides H an *r*-dynamic coloring with p colors is known as *r*-dynamic chromatic number of the graph H and it is denoted as $\chi_r(H)$. In this paper, we have attained the lower, upper bound and exact *r*-dynamic chromatic number for cocktail party graph Cp_s, *s*-barbell graph Ba_s, windmill graph W_s^q, book graph B_s and pencil graph Pc_s.

Mathematics subject classification: 05C15

Keywords: r-dynamic coloring; cocktail party graph Cp_s; *s*-barbell graph Ba_s; windmill graph W_s^q; book graph B_s; pencil graph Pc_s.

1. INTRODUCTION

Throughout we take into account simple, finite and connected graphs. Montgomery was the pioneer in dynamic coloring. Dynamic coloring [1, 2, 3, 5, 9, 10] of a graph is proper coloring of H so that each and every vertex $a \in V(H)$ has neighbors in atleast two different classes of color. And its generalized version is *r*-dynamic coloring. A mapping $c : V(H) \rightarrow Q$, the set of colors with $Q = |p|$, is known as *r*-dynamic coloring if the following two rules holds:

1) $c(a) \neq c(z)\ for\ az \in E(G)$ and

2) $\left|c\left(N(z)\right)\right| \geq min\{r, d(z)\}$, for each and every $z \in V(H)$ where $N(z)$ denotes the set of neighbors of z, r is a positive integer and $d(z)$ is the degree of the vertex z in H.

The first rule is an indication for proper coloring and the second rule is the *r*-adjacency condition. The *r*-dynamic chromatic number is the least p that allows H an *r*-dynamic coloring with p colors and it is denoted as $\chi_r(H)$. The *r*-dynamic chromatic number does not differ once r reaches the saturation value Δ. There are many open problems one among them was conjectured by Montgomery which states for regular graphs the result $\chi_r(H) \leq \chi_r(H) + 2$. Graph coloring is one among the most challenging problems in mathematics and has many real-life applications.

2. PRELIMINARIES

[7] The **Cocktail Party Graph** Cp_s is a graph with $s = 2q$ vertices $a_j, j = 1, 2, \ldots, 2q$ with a_j non-adjacent to a_{j+q} and adjacent to all other vertices.

[4] The *s*- **Barbell Graph** Ba_s is attained by connecting two copies of complete graph K_s by a bridge. Here, we are connecting the first two vertices of K_s by a bridge.

[12] The **Windmill Graph** W_s^q with $q, s \geq 2$ is constructed by considering q copies of the complete graph K_s with a universal vertex (common vertex). When $q = 2$ and $q = 3$ i.e., W_s^2 and W_s^3 they are the star graph and friendship graph respectively.

[8] The **Book Graph** B_s is the Cartesian product of star graph $K_{1,s}$ and path P_2 i.e., $K_{1,s} \times P_2$.

[6] For $s \geq 2$, the **Pencil Graph** Pc_s is a graph with $2s + 2$ vertices where the vertex set is $\{a_q, b_q : q = 0,1, \ldots, s\}$ and edge set $\{a_q a_{q+1}, b_q b_{q+1} : q = 1, 2, \ldots, s - 1\} \cup \{a_0 a_1, a_0 b_1, b_0 a_s, b_0 b_s\} \cup \{a_q b_q : q = 0, 1, \ldots, s\}$.

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3. OBSERVATIONS

Lemma 3.1. $\chi_r(H) \geq min\{r, \Delta(H)\} + 1$ is a lemma providing the lower bound for *r*-dynamic chromatic number found by Montgomery and Lai [9].

Note 3.2. We can observe easily from the graph Cp_s *that there is a clique of order* $\frac{s}{2} = q$ *hence* $\chi_r(Cp_s) \geq q$.

Note 3.3. From the definition of Ba_s and W_s^q there is a maximal complete subgraph of order *s* hence $\chi_r(Ba_s) \geq s$ and $\chi_r(W_s^q) \geq s$.

4. RESULTS

Lemma 4.1. For $q \geq 2$, the lower bound for *r*-dynamic chromatic number of cocktail party graph Cp_s *is,* $\chi_r(Cp_s) \geq \begin{cases} q, 1 \leq r \leq q-1 \\ r+1, q \leq r \leq \Delta(Cp_s) \end{cases}$

Proof. The cocktail party graph is Cp_s is regular graph with degree 2(q - 1). Then $V(Cp_s) = \{a_j, j = 1, 2, \dots, 2q\}$ and $E(Cp_s) = \{a_j a_k : j, k = 1, 2, \dots, 2q \ where \ j \neq k = j + q\}$. *Also,* $\delta(Cp_s) = \Delta(Cp_s) = 2(q-1)$. The order of Cp_s *is* $|V(Cp_s)| = s = 2q$ and size is $|E(Cp_s)| = s(q-1)$.

By Note 3.2 we have $\chi_{1 \leq r \leq q-1}(Cp_s) \geq q$.

For $q \leq r \leq \Delta(Cp_s)$, by Lemma 3.1 $\chi_r(H) \geq min\{r, \Delta(H)\} + 1$.

Therefore,

$$\chi_{q \leq r \leq \Delta(Cp_s)}(Cp_s) \geq min\{r, \Delta(Cp_s)\} + 1 = r + 1.$$

Theorem 4.2. For $q \geq 2$, the *r*-dynamic chromatic number of cocktail party graph Cp_s *is,* $\chi_r(Cp_s) = \begin{cases} q, 1 \leq r \leq q-1 \\ 2q, q \leq r \leq \Delta(Cp_s) \end{cases}$

Proof. We have two cases: $1 \leq r \leq q-1$ *and* $q \leq r \leq \Delta(Cp_s)$ to consider.

Case 1: When $1 \leq r \leq q-1$.

By Lemma 4.1 we have the lower bound $\chi_{1 \leq r \leq q-1}(Cp_s) \geq q$. Consider the map $c_1 : V(Cp_s) \to \{1, 2, \dots, q\}$ and coloring as follows:

$c_1(a_j) = j, j = 1, 2, \dots, q$.

$c_1(a_j) = k, j = q + k \ and \ k = 1, 2, \dots, q$ since a_j and a_{j+q} are non-adjacent.

By the above coloring $\chi_{1 \leq r \leq q-1}(Cp_s) \leq q$. Hence $\chi_{1 \leq r \leq q-1}(Cp_s) = q$.

Case 2: When $q \leq r \leq \Delta(Cp_s)$.

By Lemma 4.1 we have $\chi_{q \leq r \leq \Delta(Cp_s)}(Cp_s) \geq r + 1$ but inorder to satisfy the *r*-adjacency condition we require 2q colors in total hence we the lower bound have $\chi_{q \leq r \leq \Delta(Cp_s)}(Cp_s) \geq 2q$. The upper bound is given by the coloring below considering the mapping $c_2 : V(Cp_s) \to \{1, 2, \dots, 2q\}$.

$c_1(a_j) = j, j = 1, 2, \dots, 2q \ and \ \chi_{q \leq r \leq \Delta(Cp_s)}(Cp_s) \leq 2q$.

Therefore, $\chi_{q \leq r \leq \Delta(Cp_s)}(Cp_s) = 2q$.

Lemma 4.3. For $s \geq 2$, the lower bound for *r*-dynamic chromatic number of barbell graph Ba_s *is,* $\chi_r(Ba_s) \geq \begin{cases} s, 1 \leq r \leq s-1 \\ s+1, r \geq \Delta(Ba_s) \end{cases}$

Proof. The vertex set of barbell graph $V(Ba_s) = \{a_j, b_j : 1 \leq j \leq s\}$. Here we assume that the vertices a_1 and b_1 are adjacent by a bridge and $E(Ba_s) = \{a_j a_k, b_j b_k : j, k = 1, 2, \dots, s \ and \ j \neq k\} \cup \{a_1 b_1\}$. Also, $\delta(Ba_s) = s - 1$ and $\Delta(Ba_s) = d(a_1) = d(b_1) = s$. And, order of Ba_s *is* $|V(Ba_s)| = 2s$ and size is $|E(Ba_s)| = s^2 - s + 1$.

By Note 3.3 we have $\chi_{1 \leq r \leq s-1}(Ba_s) \geq s$.

For $r \geq \Delta(Ba_s)$, by Lemma 3.1 $\chi_r(H) \geq min\{r, \Delta(H)\} + 1$.

Therefore,

$$\chi_{r \geq \Delta(Ba_s)}(Ba_s) \geq min\{r, \Delta(Ba_s)\} + 1 = \Delta(Ba_s) + 1 = s + 1.$$

Theorem 4.4. For $s \geq 2$, the *r*-dynamic chromatic number of barbell graph Ba_s *is,* $\chi_r(Ba_s) = \begin{cases} s, 1 \leq r \leq s-1 \\ s+1, r = \Delta(Ba_s) \end{cases}$

Proof. We have two cases: $1 \leq r \leq s-1$ and $r = \Delta(Ba_s)$ to consider.

Case 1: When $1 \leq r \leq s-1$.

By Lemma 4.3 we have the lower bound $\chi_{1 \leq r \leq s-1}(Ba_s) \geq s$. The coloring is provided by the mapping $c_3 : V(Ba_s) \to \{1, 2, \dots, s\}$ as follows:

$c_3(a_1, a_2, \dots, a_s) = \{1, 2, \dots, s\}$.

$c_3(b_1, b_2, \dots, b_s) = \{2, 3, \dots, s, 1\}$.

By the above coloring $\chi_{1 \leq r \leq s-1}(Ba_s) \leq s$.

Hence $\chi_{1 \leq r \leq s-1}(Ba_s) = s$.

Case 2: When $r = \Delta(Ba_s)$.

By Lemma 4.3 we have the lower bound $\chi_{r=\Delta(Ba_s)}(Ba_s) \geq s+1$. The upper bound is given by the coloring below considering the mapping $c_4: V(Ba_s) \to \{1, \ldots, s+1\}$.

$c_4(a_1, a_2, \ldots, a_s) = \{1, 2, \ldots, s\}$

$c_4(b_1) = s+1$ and $c_3(b_2, b_3, \ldots, b_s) = \{2, 3, \ldots, s\}$.

$\chi_{r=\Delta(Ba_s)}(Ba_s) \leq s+1$.

Therefore, $\chi_{r=\Delta(Ba_s)}(Ba_s) = s+1$.

Lemma 4.5. For $q, s \geq 2$, the lower bound for *r*-dynamic chromatic number of windmill graph W_s^q *is,* $\chi_r(W_s^q) \geq \begin{cases} s, 1 \leq r \leq s-1 \\ r+1, s \leq r \leq \Delta(W_s^q) \end{cases}$

Proof. The vertex set of windmill graph $V(W_s^q) = \{a_0\} \cup \{a_{j,1}, a_{j,2}, \ldots, a_{j,s-1} : 1 \leq j \leq q\}$ where a_0 is universal vertex adjacent to all other vertices $\{a_{j,k} : 1 \leq j \leq q \text{ and } 1 \leq k \leq s-1\}$. Edge set is $E(W_s^q) = \{a_{j,k}a_{j,i} : \mathrm{k} \neq \mathrm{i} \,\&\, 1 \leq j \leq q \text{ and } 1 \leq k, i \leq s-1\} \cup \{a_0 a_{j,k} : 1 \leq j \leq q \text{ and } 1 \leq k \leq s-1\}$. Also, $\delta(W_s^q) = \mathrm{s}-1$ and $\Delta(W_s^q) = \mathrm{d}(a_0) = \mathrm{q}(\mathrm{s}-1)$. And, order of W_s^q *is* $|V(W_s^q)| = \mathrm{q}(\mathrm{s}-1)+1$ and size is $|E(W_s^q)| = \frac{\mathrm{qs}(\mathrm{s}-1)}{2}$.

By Note 3.3 we have $\chi_{1 \leq r \leq s-1}(W_s^q) \geq s$.

For $s \leq r \leq \Delta(W_s^q)$, by Lemma 3.1 $\chi_r(H) \geq min\{r, \Delta(H)\} + 1$.

Therefore,

$$\chi_{s \leq r \leq \Delta(W_s^q)}(W_s^q) \geq min\{r, \Delta(W_s^q)\} + 1 = r+1.$$

Theorem 4.6. For $q, s \geq 2$, the *r*-dynamic chromatic number of windmill graph W_s^q *is,* $\chi_r(W_s^q) = \begin{cases} s, 1 \leq r \leq s-1 \\ r+1, s \leq r \leq \Delta(W_s^q) \end{cases}$

Proof. We have two cases: $1 \leq r \leq s-1$ and $s \leq r \leq \Delta(W_s^q)$ to consider.

Case 1: When $1 \leq r \leq s-1$.

By Lemma 4.5 we have the lower bound $\chi_{1 \leq r \leq s-1}(W_s^q) \geq s$. The coloring is provided by the map $c_5: V(W_s^q) \to \{1, 2, \ldots, s\}$ as follows:

$c_5(a_0) = 1$.

$c_5(a_{j,1}, a_{j,2}, \ldots, a_{j,s-1}) = \{2, 3, \ldots, s\}$ for $1 \leq j \leq q$.

By the above coloring $\chi_{1 \leq r \leq s-1}(W_s^q) \leq s$. Hence $\chi_{1 \leq r \leq s-1}(W_s^q) = s$.

Case 2: When $s \leq r \leq \Delta(W_s^q)$.

By Lemma 4.5 we have $\chi_{s \leq r \leq \Delta(W_s^q)}(W_s^q) \geq r+1$. The upper bound is given by the coloring below considering the mapping $c: V(W_s^q) \to \{1, 2, \ldots, r+1\}$ for different stages of *r*.

$c(a_0) = 1$ for all cases of *r*.

When *r* = s.

$c(a_{1,1}, a_{1,2}, \ldots, a_{1,s-1}) = \{2, 3, \ldots, s\}$

$c(a_{2,1}, a_{2,2}, \ldots, a_{2,s-1}) = \{s+1, 3, 4, \ldots, s\}$

$c(a_{j,1}, a_{j,2}, \ldots, a_{j,s-1}) = \{2, 3, \ldots, s\}$ $for\ 3 \leq j \leq q$

Hence $\chi_{r=s}(W_s^q) \leq s+1$.

When *r* = s+1.

$c(a_{2,1}, a_{2,2}, \ldots, a_{2,s-1}) = \{s+1, s+2, 4, \ldots, s\}$

$c(a_{j,1}, a_{j,2}, \ldots, a_{j,s-1}) = \{2, 3, \ldots, s\}$ $for\ 1 \leq j \leq q \text{ and } j \neq 2$.

Hence $\chi_{r=s+1}(W_s^q) \leq s+2$.

Proceeding like this at each stage of *r* we introduce the color *r*+1 to the next vertex in the list till $a_{j,s-1}$.

Hence $\chi_{s \leq r \leq \Delta(W_s^q)}(W_s^q) \leq r+1$.

Therefore, $\chi_{s \leq r \leq \Delta(W_s^q)}(W_s^q) = r+1$.

Lemma 4.7. For $s \geq 2$, the lower bound for *r*-dynamic chromatic number of book graph B_s is, $\chi_r(B_s) \geq \begin{cases} 2, r = 1 \\ r+1, 2 \leq r \leq \Delta(B_s) \end{cases}$

Proof. The vertex set of book graph $V(B_s) = \{b_{1,j}, b_{2,j}, x, y : 1 \leq j \leq s\}$ and edge set $E(B_s) = \{xb_{1,j}, \mathrm{y}b_{2,j}, b_{1,j}b_{2,j} : 1 \leq j \leq s\}$. Also, $\delta(B_s) = 2$ and $\Delta(B_s) = \mathrm{d}(x) = \mathrm{d}(y) = \mathrm{s}$. And, order of B_s *is* $|V(B_s)| = 2\mathrm{s}+2$ and size is $|E(B_s)| = 3\mathrm{s}$.

Since the maximal complete subgraph is of order 2 we have $\chi_{r=1}(Ba_s) \geq 2$.

For $2 \leq r \leq \Delta(B_s)$, by Lemma 3.1 $\chi_r(H) \geq min\{r, \Delta(H)\} + 1$.

Therefore, $\chi_{2 \leq r \leq \Delta(B_s)}(B_s) \geq min\{r, \Delta(Ba_s)\} + 1 = r+1$.

Theorem 4.8. For $s \geq 2$, the *r*-dynamic chromatic number of book graph B_s *is*, $\chi_r(B_s) = \begin{cases} 2, & r = 1 \\ 4, & r = 2,3 \\ r+1, & 4 \leq r \leq \Delta(B_s) \end{cases}$

Proof. We have two cases: $r = 1, r = 2, 3$ and $4 \leq r \leq \Delta(B_s)$.

Case 1: When $r = 1$.

By Lemma 4.7 we have the lower bound $\chi_1(Ba_s) \geq 2$. The coloring is provided by the map $c_6: V(B_s) \to \{1,2\}$ as follows:

$c_6(x) = 1$ and $c_6(y) = 2$.

$c_6(b_{1,j}) = 2$ and $c_6(b_{2,j}) = 1$ for $1 \leq j \leq s$.

By the above coloring $\chi_1(B_s) \leq 2$. Hence $\chi_1(B_s) = 2$.

Case 2: When $r = 2,3$.

By Lemma 4.7 we have the lower bound $\chi_{r=2}(B_s) \geq r + 1 = 3$. But since there is presence of C_4 in B_s which leads to the need of an extra color when $r = 2$. So, the lower bound becomes $\chi_{r=2}(B_s) \geq 4$ and by the same lemma we have the lower bound $\chi_{r=3}(B_s) \geq r + 1 = 4$. Now we assign coloring by the mapping $c_7: V(B_s) \to \{1,2,3,4\}$.

$c_7(x) = 1$ and $c_7(y) = 2$.

$c_7(b_{1,j}) = \begin{cases} 3, j \text{ is odd} \\ 4, j \text{ is even} \end{cases}$ and $c_7(b_{2,j}) = \begin{cases} 4, j \text{ is odd} \\ 3, j \text{ is even} \end{cases}$

For $r = 3$ the coloring provided above is sufficient. So, we have the upper bound $\chi_{r=2,3}(B_s) \leq 4$.

Therefore, $\chi_{r=2,3}(B_s) = 4$.

Case 3: When $4 \leq r \leq \Delta(B_s)$.

By Lemma 4.7 we have the lower bound $\chi_{4 \leq r \leq \Delta(B_s)}(B_s) \geq r + 1$. Consider the mapping $c: V(B_s) \to \{1,2,\dots,r+1\}$ which gives the coloring for the vertices.

$c(x) = 1$ and $c(y) = 2$.

$c(b_{1,1}, b_{1,2}, \dots, b_{1,s}) = \{3, 4, \dots, r+1, \underbrace{3,4,\dots}_{s-(r-1)\ terms}\}$.

$c(b_{2,1}, b_{2,2}, \dots, b_{2,s}) = \{4, \dots, r+1, 3, \underbrace{4,5,\dots}_{s-(r-1)\ terms}\}$.

Hence $\chi_{4 \leq r \leq \Delta(B_s)}(B_s) \leq r + 1$. Therefore, we have $\chi_{4 \leq r \leq \Delta(B_s)}(B_s) = r + 1$.

Lemma 4.9. For $s \geq 2$, the lower bound for *r*-dynamic chromatic number of pencil graph Pc_s *is*, $\chi_r(Pc_s) \geq \begin{cases} 3, r = 1,2 \\ 4, r \geq \Delta(Pc_s) \end{cases}$

Proof. The pencil graph Pc_s is *a regular graph with degree 3. The vertex set is defined as* $\{a_q, b_q : q = 0,1, \dots, s\}$ *and* edge set $\{a_q a_{q+1}, b_q b_{q+1} : q = 1, 2, \dots, s-1\} \cup \{a_0 a_1, a_0 b_1, b_0 a_s, b_0 b_s\} \cup \{a_q b_q : q = 0, 1, \dots, s\}$. Also, $\delta(Pc_s) = \Delta(Pc_s) = 3$. And, order of Pc_s *is* $|V(Pc_s)| = 2s + 2$ and size is $|E(Pc_s)| = 3(s + 1)$.

There is a clique of order 3 we have $\chi_{r=1,2}(Pc_s) \geq 3$.

For $r \geq \Delta(Pc_s)$, by Lemma 3.1 $\chi_r(H) \geq min\{r, \Delta(H)\} + 1$.

Therefore, $\chi_{r \geq \Delta(Pc_s)}(B_s) \geq min\{r, \Delta(Pc_s)\} + 1 = \Delta(Pc_s) + 1 = 4$.

Theorem 4.10. For $s \geq 2$, the *r*-dynamic chromatic number of pencil graph Pc_s *is*, $\chi_r(Pc_s) = \begin{cases} 3, r = 1,2 \ \ s \equiv 0,2(mod\ 3), \\ \quad r = 1 \ and \ s \equiv 1(mod\ 3) \\ 4, r = 2 \ and \ s \equiv 1(mod\ 3) \\ 5, r = 3 \ and \ \ s \equiv 0(mod 4) \\ 6, \ \ r = 3 \ and \ otherwise \end{cases}$

Proof. *We have four cases to consider here.*

Case 1: When $r = 1,2 \ and \ s \equiv 0,2(mod\ 3), r = 1 \ and \ s \equiv 1(mod\ 3)$.

Subcase 1: When $r = 1,2 \ and \ s \equiv 0,2(mod\ 3)$

By Lemma 4.9 we have the lower bound $\chi_{r=1,2}(Pc_s) \geq 3$. For upper bound consider the map $c_8: V(Pc_s) \to \{1,2,3\}$.

$c_8(a_0) = 3$

$c_8(a_1, a_2, \dots, a_s) = \{1, 2, 3, 1, 2, 3, \ \dots, 1, 2, 3\}$ when $s \equiv 0(mod\ 3)$

$c_8(a_1, a_2, \dots, a_s) = \{1, 2, 3, 1, 2, 3, \ \dots, 1, 2\}$ when $s \equiv 2(mod\ 3)$

$c_8(b_1, b_2, \dots, b_s) = \{3, 2, 1, 3, 2, 1, \ \dots, 3, 2, 1\}$ when $s \equiv 0(mod\ 3)$

$c_8(b_1, b_2, \ldots, b_s) = \{3, 2, 1, 3, 2, 1, \ \ldots, 3, 2\}$ when $s \equiv 2(mod\ 3)$

$c_8(b_0) = \begin{cases} 2, when\ s \equiv 0(mod\ 3) \\ 1, when\ s \equiv 2(mod\ 3) \end{cases}$

Hence $\chi_{r=1,2}(Pc_s) \leq 3$ and therefore $\chi_{r=1,2}(Pc_s) = 3$ when $s \equiv 0,2(mod\ 3)$.

Subcase 2: When $r = 1\ and\ s \equiv 1(mod\ 3)$.

By Lemma 4.9 we have the lower bound $\chi_{r=1}(Pc_s) \geq 3$. Consider the map $c_8: V(Pc_s) \rightarrow \{1,2,3\}$.

$c_8(a_1, a_2, \ldots, a_s) = \{1, 2, 1, 2, \ \ldots, 1, 2\}$ when $s\ is\ even$

$c_8(a_1, a_2, \ldots, a_s) = \{1, 2, 1, 2, \ \ldots, 1\}$ when $s\ is\ odd$

$c_8(b_1, b_2, \ldots, b_s) = \{2, 1, 2, 1, \ \ldots, 2, 3\}$ when $s\ is\ even$

$c_8(b_1, b_2, \ldots, b_s) = \{\ 2, 1, 2, 1, \ \ldots, 2,1,3\}$ when $s\ is\ odd$

$c_8(a_0) = 3$ and $c_8(b_0) = \begin{cases} 1, when\ s\ is\ even \\ 2, when\ s\ is\ odd \end{cases}$

Hence $\chi_{r=1}(Pc_s) \leq 3$ and therefore $\chi_{r=1}(Pc_s) = 3$ when $s \equiv 1(mod\ 3)$.

Case 2: When $r = 2\ and\ s \equiv 1(mod\ 3)$.

By Lemma 4.9 we have the lower bound $\chi_{r=2}(Pc_s) \geq 3$. But we need an extra color when $s \equiv 1(mod\ 3)$ to satisfy *r*-adjacency condition. So, the lower bound transforms to $\chi_{r=2}(Pc_s) \geq 4$.

For upper bound assign the following coloring provided with the mapping $c_9: V(Pc_s) \rightarrow \{1,2,3,4\}$.

$c_9(a_0) = 3$ and $c_9(b_0) = 4$

$c_9(a_1, a_2, \ldots, a_s) = \{1, 2, 3, 1, 2, 3, \ \ldots\}$

$c_9(b_1, b_2, \ldots, b_s) = \{2, 3, 1, 2, 3, 1, \ \ldots\}$

Hence $\chi_{r=2}(Pc_s) \leq 4$. Therefore, $\chi_{r=2}(Pc_s) = 4$ when $s \equiv 1(mod\ 3)$.

Case 3: When $r = 3\ and\ s \equiv 0(mod\ 4)$.

By Lemma 4.9 we have the lower bound $\chi_{r=3}(Pc_s) \geq r + 1 = 4$. But inorder to *r*-adjacency condition we need an extra color and hence the lower bound transforms to $\chi_{r=3}(Pc_s) \geq 5$.

Assign the coloring by the map $c_{10}: V(Pc_s) \rightarrow \{1,2,\ldots,5\}$.

For $1 \leq q \leq s$, $c_{10}(a_q) = \begin{cases} 1, when\ q \equiv 1(mod\ 4) \\ 4, when\ q \equiv 2(mod\ 4) \\ 3, when\ q \equiv 3(mod\ 4) \\ 2, when\ q \equiv 0(mod\ 4) \end{cases}$

$c_{10}(b_q) = \begin{cases} 2, when\ q \equiv 1(mod\ 4) \\ 5, when\ q \equiv 2(mod\ 4) \\ 1, when\ q \equiv 3(mod\ 4) \\ 4, when\ q \equiv 0(mod\ 4) \end{cases}$

$c_{10}(a_0) = 3$ and $c_{10}(b_0) = 5$.

Thus, the upper bound is $\chi_{r=3}(Pc_s) \leq 5$. Therefore, $\chi_{r=3}(Pc_s) = 5$.

Case 4: When $r = 3\ and\ otherwise$.

By Lemma 4.9 we have the lower bound $\chi_{r=3}(Pc_s) \geq 4$. But inorder to *r*-adjacency condition we are forced to introduce two new colors and hence the lower bound $\chi_{r=3}(Pc_s) \geq 6$. Assign the coloring by the map $c_{11}: V(Pc_s) \rightarrow \{1,2,\ldots,6\}$.

$c_{11}(a_1, a_2, \ldots, a_s) = \{1, 4, 2, 5, 1, 4, 2, 5, \ldots, 1\}$ when $s \equiv 1(mod\ 4)$

$c_{11}(a_1, a_2, \ldots, a_s) = \{1, 4, 2, 5, 1, 4, 2, 5, \ldots, 1, 4\}$ when $s \equiv 2(mod\ 4)$

$c_{11}(a_1, a_2, \ldots, a_s) = \{1, 4, 2, 5, 1, 4, 2, 5, \ldots, 1, 4, 2\}$ when $s \equiv 3(mod\ 4)$

$c_{11}(b_1, b_2, \ldots, b_s) = \{2, 5, 1, 4, 2, 5, 1, 4, \ldots, 2\}$ when $s \equiv 1(mod\ 4)$

$c_{11}(b_1, b_2, \ldots, b_s) = \{2, 5, 1, 4, 2, 5, 1, 4, \ldots, 2, 5\}$ when $s \equiv 2(mod\ 4)$

$c_{11}(b_1, b_2, \ldots, b_s) = \{2, 5, 1, 4, 2, 5, 1, 4, \ldots, 2, 5, 1\}$ when $s \equiv 3(mod\ 4)$

$c_{11}(a_0) = 3$ and $c_{11}(b_0) = 6$.

Thus, the upper bound is $\chi_{r=3}(Pc_s) \leq 6$.

Therefore, $\chi_{r=3}(Pc_s) = 6$, otherwise i.e., when $s \equiv 1,2,3(mod\ 4)$.

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