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Logo Description : It symbolizes an elephant within an ecological frame of peace and harmony moving towards prosperity and posterity. **Cover photo** (Anticlockwise from top) ; 1. Peafowl conservation at Khandadhar, Odisha 2. *Nostochopsis radians,* the cyanobacteria of Simlipal 3. Skunkvine *(Paederia foetida),* the Medicinal plant, Bhermpur, Orissa 4. Anthrax (*Bacillus anthracis*) leison in humanbeing 5. Lake view, Tawang, Arunachal Pradesh. **Cover background photo** : *Eulophia Spectabilis* at Koraput, Odisha, (By Mr. Prasad).

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EDITORIAL

Global warming is a topic, mostly talked about in recent years. Sun light reaches the earth as visible radiation of wave length 0.58 to 0.69 μm. A portion of it gets absorbed in the atmosphere and earth's surface and the remaining gets radiated back from the earth's surface. The reflected radiation has got longer wave length i.e. 10 μm. Gases like Carbon dioxide (CO_2), Chloro fluorocarbon (CFC), Methane (CH_4) and Nitrous oxide (N_2O) present in the atmosphere along with water vapour absorbs these radiations and do not allow that to escape. As a consequence of which the atmosphere gets warmed up, as in case of a ''Green House''. This phenomenon ensures the required temperature for the life to exist. When the release of green house gases exceeds the critical level, the equilibrium gets tilted and the global temperature starts increasing. Global temperature has been increasing at the rate of 0.3 to 0.6^oC over the last 140 years. But it is going to be raised by 2.5^oC by the next century. Rise of global temperature just by 2^oC would be catastrophic.

CO_2 is considered to occupy the top position among the green house gases as it is released to the atmosphere in quantities beyond imagination. In fact green house gases remain in atmosphere only for approximately 12.5 years. Each single methane (CH_4) molecule traps the heat about 30 times more effectively than a CO_2 molecule and causes about 15% as much global warming as CO_2 does. Its concentration has increased from 0.80 ppmv (pre industrial area) to 1.72 ppmv at present at an annual growth rate of 1%. Annual CH_4 input into the atmosphere is about 500 tonnes/year. Earlier it was believed that the main source of CH_4 gas is emission due to organic decomposition from flooded fields, marshy lands etc. But now it is believed that more of CH_4 comes from agricultural practices.

India's economy mainly depends upon agriculture. Recently hot debates are going on over the emission of methane from cattle and its impact on global warming followed by the consequences of methane effect on environment. Agriculture is the largest source of methane emissions accounted for approximately 50% of global anthropogenic methane. Livestock system contributes about 42% of the total green house gas production from agriculture. Green house gases emitted from manure are mainly methane (CH_4) and nitrous oxide (N_2O). Methane is produced in anaerobic conditions and is the main green house gas emitted from liquid manure. Intensity of production depends mainly on manure organic matter and on temperature and duration of storage. This means that systems with long term storage of liquid manure indoors or outdoors at high ambient temperature will result in much higher CH_4 emission. Among animals ruminants are primary source for emittance of methane. The vets working in this area have suggested that it can be minimized by various feeding strategies such as adding feed additives, dietary fat, antibiotics, organic acids, change in amount and type of feed etc. Even the type of animal (indigenous / upgraded animal), feeding habit (stall feeding / pasture feeding) and size of animals can also influence production of the harmful methane. Further research and suggestion from the relevant fields are invited for reducing methane load to check global warming singnificantly.

(Dr. R.K.Samantaray)

Editor-in-Chief

DIVERSITY OF CYANOBACTERIA FROM FRESHWATER BODIES OF SIMILIPAL BIOSPHERE RESERVE, ORISSA, INDIA

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ABSTRACT

The cyanobacterial diversity from 10 freshwater streams, 8 reservoirs and 4 waterfalls of Similipal Biosphere Reserve was estimated during the survey conducted from 2003 to 2008. In total 65 species under 3 orders, 7 families and 24 genera are reported for the first time from the Biosphere Reserve. Morphological descriptions, common habitats and distribution pattern in India for each species are presented systematically. Among the dominant genera, *Oscillatoria* accounts for the maximum number of species followed by *Anabaena*, *Calothrix*, *Nostoc* and *Scytonema*. *Hapalosiphon* and *Chroococcus* were the least dominant genera. After detailed literature review and specimen examination, 15 species were found to be new distributional record for fresh water bodies of India and 11 species are reported to be new record for fresh water bodies of Odisha.

Keywords : Cyanobacteria, freshwater streams, Similipal biosphere, algal flora

INTRODUCTION

Cyanobacteria (earlier known as blue-green algae) are morphologically diverse group of Gram-negative photosynthetic prokaryotes, which are unique in their cosmopolitan distribution ranging from Arctic to Antarctic habitats (Saha *et al.*, 2007). Cyanobacteria occupy a central position in global nutrient cycling especially due to their inherent capacity to fix atmospheric CO_2 and N_2 through Rubisco and nitrogenase enzymes respectively (Sinha *et al.*, 1995; 1997). Until past few decades of research cyanobacteria were of academic interest only and were mostly ignored as nuisance but, now are proved as potential candidates for much biotechnological utilization (Richmond, 1990; Sundararaman and Sekar, 2001; Thajuddin and Subramanian, 2005). Therefore, for several reasons, estimation and conservation of cyanobacterial biodiversity from yet unexplored/under explored habitats become very important, which need to be initiated with systematic survey followed by establishment of pure culture collection and their characterization. Cyanobacterial diversity studied by many researchers in Orissa (Mohanty and Padhi, 1984; Mohanty, 1984; Tripathy *et al.*, 1991; Sahu, 2000; Adhikary, 2002; Prasanna and Nayak, 2007; Saxena *et al.*, 2007; Dey and Bastia, 2008; Dey *et al.*, 2010; Ghadai *et al.*, 2010), had focused on rice fields in different agro climatic zones within the state. A very little work has been carried out on fresh water cyanobacterial diversity in Odisha. Bastia and Dey, 2007 studied algal flora in some selected water bodies of Similipal and its periphery. There is no further comprehensive studies available in fresh water algal flora of Similipal in general and cyanobacteria in particular. Similipal Biosphere Reserve lies between 21º 28' to 22º08'N latitude and 86º 04' to 86 º 37' E longitudes (Fig 1) which is considered as the biodiversity treasure of Odisha harbouring 1107 indigenous species of vascular plants, which includes 713 species of dicotyledons under 123 families, 328 species of monocotyledons under 24 families, 3 species of gymnosperms and 61 species of pteridophytes under 27 families.

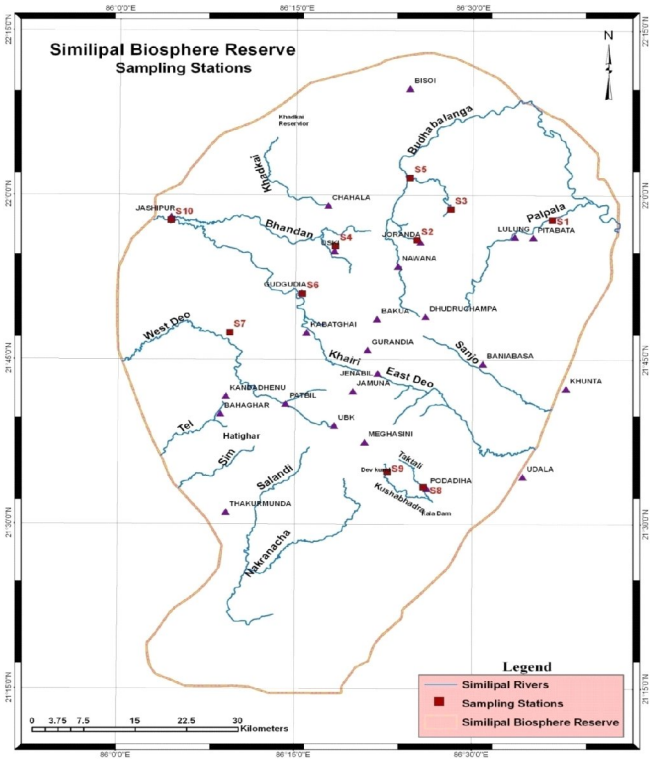

Fig. 1 : Map locating sampling stations within Similipal Biosphere Reserve.

Given the continual increase of pollution pressure on aquatic biota, it is increasingly urgent to study intact, natural ecosystems prior to their disturbance. It is the richest water-shed in the state of Odisha giving rise to many perennial rivers like Budhabalanga, Kharkhai, Khairi, Bhandan, West Deo, Salandi, Sanja and Palpala which provides ample opportunity to study the fresh water algal diversity and their contribution to the productivity of the aquatic ecosystem. The ecological and taxonomic status of algal flora in general and cyanobacteria in particular are lacking in many parts of the state, so also in Similipal. Keeping in view of the above facts the present study was under taken to estimate the cyanobacterial diversity in 10 perennial streams of the Biosphere Reserve.

MATERIALS AND METHODS

Extensive field surveys were made in Similipal during the period from May 2004 to May 2008 to collect the algal samples in core, buffer and transition zones of the Biosphere Reserve. The study sites include 10 perennial streams such as Palpala at Pithabata (S1), Khadkai at Bhajam (S2), Panasia dam (S3), Budhabalanga at Nawana (S4), Uski at Lingeswar (S5), West Deo at Tato-Dudhiani (S6), Joranda pond (S7), Khari at Kaliani (S8), Bhandan at Tulsibani (S9) and Bhandan and Khairi at Ramatirtha (S10), numerous hill streams, rice fields, ponds, rivulets and nalas within Similipal (Table- 1). Similarly, random sampling was conducted at 12 other fresh water bodies like Hadgarh reservoir, Hatimundi dam, Khadkai reservoir, Sunei dam, Deo dam, Kala dam, Nawana nala, Chahala nala, Joronda fall, Barheipani fall, Uski fall and Sitakund fall. For collection of fresh water algae 10 perennial streams were selected as study sites and collection were made on seasonal basis by random sampling method. Filamentous cyanobacteria were collected from mass growth by hand whereas less conspicuous forms found as films on submerged grasses and stems of aquatic plants in plastic vials. Scrapping from moist walls of hill streams, waterfalls and squeezing from aquatic weeds yielded a good number of cyanobacteria. For planktonic cyanobacteria, water samples were collected by filtering 20 liter of water with both 10 µm mesh size plankton net. If live materials were not observed within 48 hours of sampling, the samples were fixed in common fixative Lugol's solution (2g Potasium Iodide + 1g Iodine + 300 ml distilled water) and stored in dark bottle at room temperature. The free floating, epiphytic, epilithic and epizoic algal forms were collected in screw cap bottles and fixed with Lugol's solution on the spot. Simultaneously, 10 liters of water was filtered through a plankton net (10 µ mesh) and then fixed with Lugol's iodine on the spot and brought to the laboratory for planktonic cyanobacterial culture. After the preliminary observation, a set of sample was transferred to BG 11 complete medium (Rippka *et al.*, 1979) while; another set was transferred to BG 11 medium devoid of combined nitrogen to enhance the growth of heterocytous forms. These were subsequently cultured in the laboratory to get the monoculture. Camera Lucida diagrams and micrographs of each species were done in the laboratory (Meiji trinocular research microscope fitted with Nikon F10 camera). All samples were incubated at 25 ± 2 ^{o}C, 14/10 hour light/dark cycle, with illumination of 20 µE m^{-2} sec^{-1} by cool white fluorescent tubes (Philips, India). After two to three weeks of growth, samples were re-observed with the aid of microscope (Meiji trinocular research microscope fitted with Nikon F10 camera) under light field, dark field and phase contrast conditions to characterize them morphologically.–,

The taxonomic publications of Anagnostidis and Komarek (1985, 1988, 1990), and Komarek and Anagnostidis (1986, 1989) were mainly used to identify cyanobacteria with modern approach of classification system. However, the keys used in "Geitlerian" system (Geitler, 1932; Desikachary, 1959 and Starmach, 1966) were used for species identification whenever found essential. Other taxonomic papers such as, Komarek *et al.* (1999), Kovalenko *et al.* (1999) were also referred.

Table 1 : Sampling stations selected for the research work within Similipal Biosphere Reserve.

Sl. No.	Place	River/Dam	Status	Co-ordinates
S-1	Pithabata	Palpala (Balidiha dam)	Stagnant	86 36'44.20"E, 21 57'44.51"N
S-2	Joronda	Panasia Dam, Palpala	Stagnant	86 25' 7.80"E, 21 55' 57.37"N
S-3	Bhajam	Khadkai	Flowing	86 28' 4.13"E 21 58' 47.00"N
S-4	Lingeswar	Uski, Bhandan	Flowing	86 18' 14.55"E, 21 55'21.66"N
S-5	Jambu bridge	Budhabalanga	Flowing	86 24'34.32"E, 22 1'41.10"N
S-6	Gudgudia	Khairi	Flowing	86 15' 25.24"E, 21 51' 0.51"N
S-7	Tato	West Deo	Flowing	86 9'16.95"E, 21 47'26.23"N
S-8	Podadiha	Sanjo	Flowing	86 25'45.75"E, 21 33' 22.52"N
S-9	Devkund	Salandi	Flowing	86 22' 40.49"E, 21 34'45.11"N
S-10	Ramatirtha	Bhandan	Flowing	86 4'17.86"E, 21 57'42.27"N

RESULTS AND DISCUSSION

The Cyanobacterian flora of the reserve includes 65 species under 3 orders, 7 families and 24 genera (Fig 2) *Oscillatoria* is the dominant genera with 10 species followed by *Anabaena* and *Calothrix* with 8 species each, *Nostoc* and *Scytonema* with 4 species each; *Hapalosiphon* and *Chroococcus* with 3 species are the other dominant genera (Table 2).

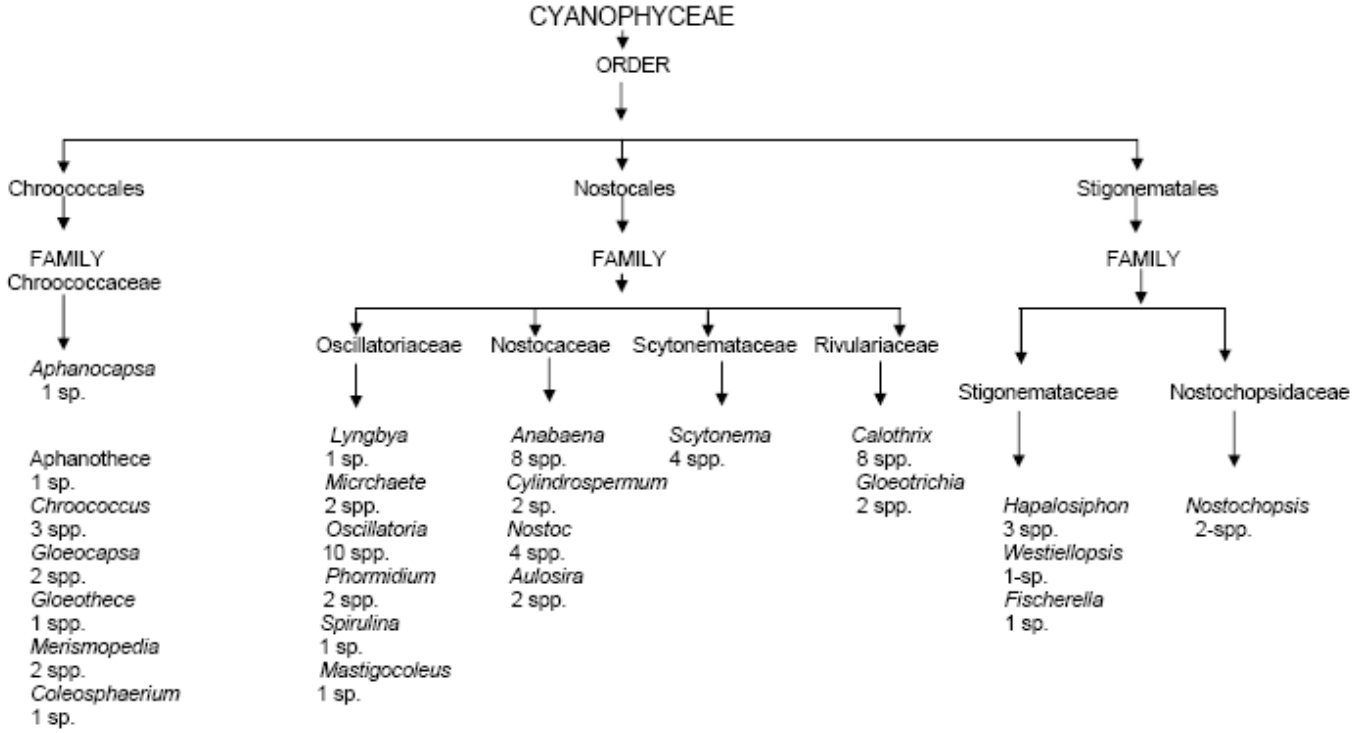

Fig. 2 : Classification of Cyanophyceae of Similipal Biosphere Reserve.

Fifteen species such as, *Anabaena macrospora* Klebahn, *Calothrix linearis* Gardner, *Calothrix parietina* Nag. ex Kutz., *Chroococcuss limneticus* Lemm., *Gleotheceae rupestris* (Lyngb) Bornet., *Hapalosiphon hiberinicus* West and West, *Hapalosiphon welwitschi* West and West, *Merismopedia punctata* var *punctata* Meyen, *Microchaete tenera* Thuretex Born.et Flah, *Nostochopsis lobatus* Wood em. Geitler, *Nostochopsis radians* Bharadwja, *Oscillatoria agardhi* Gomont, *Oscillatoria rubescens* De Candolle, *Scytonema mirabile* (Dillwyn) Agardh were probably new distributional record for fresh water bodies in India. Similarly 11 species such as, *Anabaena sphaerica* Born. et Flahault, *Aphanocapsa grevillei* (Hass.) Rab., *Calothrix javanica* de Wilde, *Chroococcus turgidus* (Kutz.) Nag., *Coleosphaerium kuetzingianum* Nag, *Cylindrospermum muscicola* Kutz., *Gloeocapsa nigrescens* Kutz., *Gloeotrichia natans* Rab and Flahault ex Bornet., *Lyngbya birgei* Smith, *Oscillatoria nigroviridis* Thwaites, *Phormidium unicinatum*, *Scytonema millei* Born. are reported to be new record for fresh water bodies of Odisha. The detail enumeration of each species along with its microscopic characterstics and distributional pattern in India is enlisted in Table 2. Out of the 65 species of Canobacteria, 5 species such as *Anabaena variabilis*, *Aulosira bombayensis*, *Calothrix clavatoides*, *Nostochopsis lobatus* and *Oscillatoria subrevis* were encountered in all the 10 stations. Almost all Cyanophycean members occurred as epiphytes or epilithic in rivers and streams except *Gloeotrichia ghosei* which occurred forming characterstic balls in ponds as well as stagnant water of the streams and rivers. *Scytonema hofmani* and *Westiellopsis prolific* occurred predominantly adhering to the rocks forming copious slime in Barheipani and Joronda falls. *Oscillatoria subervis* and *Nostochopsis lobatus* are occurring in all the 10 sampling station showing their adaptation to varied ecological factors within the Biosphere Reserve. Thirty one species are reported from S2 followed by 23 species each from S1 and S10. Both S1 and S10 are receiving different kinds of anthropogenic load due to its location in the periphery and near human habitation. At the same time S2 is located in the core area of the reserve and mostly received run offs from the nearby hills along with the excreta of wild animals. The distribution of cyanobacterian flora of the reserve in different sampling stations is given in Fig 3.

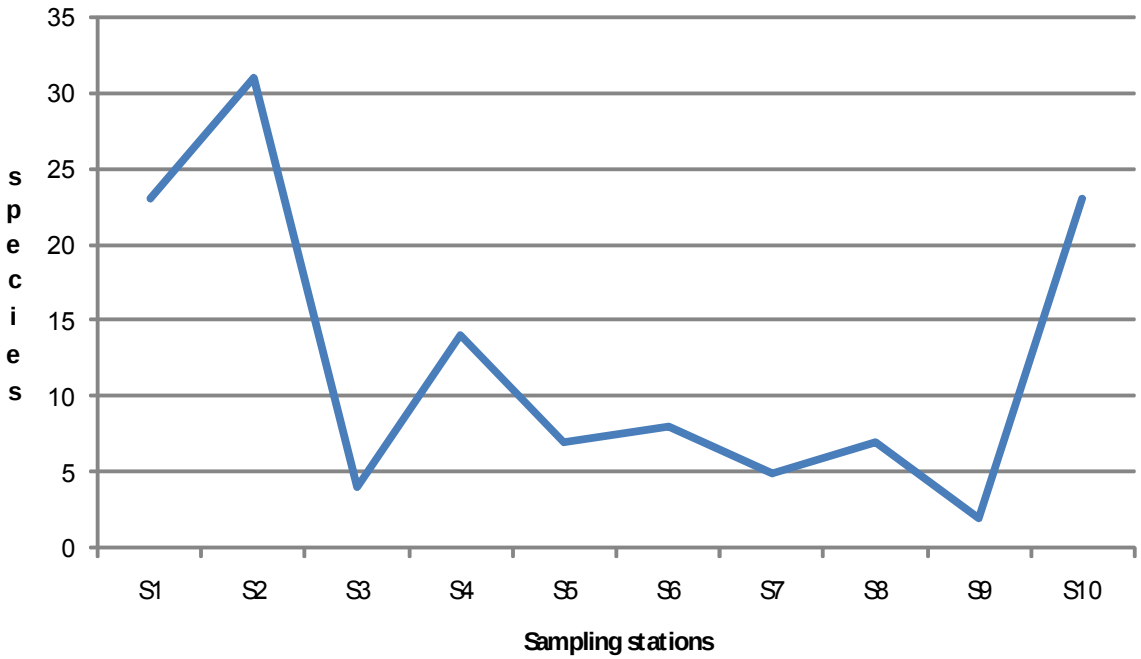

Fig. 3 : Distribution of cyanobacteria in different sampling stations

Table 2 : Systematic enumeration of Cyanobacteria flora of Similipal Biosphere Reserve

Family	Genus	Species	Description	Places of collection	Distribution in India
Nostocaceae	*Anabaena*	*Anabaena azollae* Stras	Cells 2.5-3.0 µm broad; heterocyst 3.5-4.0 µm broad,5.5-6.0 µm long	Hatimundidam,	Tamil Nadu (Perumal and Anand, 2008)
		Anabaena fertilissima Rao	Cells 4-4.6 µm broad and 4-5 µm long; heterocyst ellipsoidal, 5.4-6 µm broad and 6.8-7.2 µm long	S1, S2, S4, S8 and S10	Assam, Tamil Nadu and Uttar Pradesh (Perumal and Anand, 2008)
		Anabaena iyengarii Bharadwaja var. *unispora* Singh	Trichomes 3.2-4 µm broad, cells 4-4.8 µm long; heterocyst 4.8-7.3 µm broad and 8.8-12.8 µm long, akinetes13.6-17.6 µm broad and 26.4-44 µm long	Sunei dam, Barheipani fall and Kala dam	This species is reported for the first time from fresh water bodies of Orissa
		Anabaena macrospora Klebahn	Cells 6-7 µm broad, heterocyst 7-8 µm broad	Khadkai reservoir	This species is reported for the first time from fresh water bodies of Orissa
		Anabaena orientalis Dixit	Cells 3-4 µm broad; heterocyst 4.9-6.6 µm broad	Nawana nala	Assam and Tamil Nadu (Perumal and Anand, 2008)
		Anabaena oscillarioides Bory	Trichomes 3-6 µm broad; cells 4-6 µm long, 3-5 µm broad; heterocyst 5-8 µm broad, 6-14 µm long; spores 5-11 µm broad and 6-24 µm long	S2, S4 and S10	Assam and Tamil Nadu (Perumal and Anand, 2008)
		Anabaena sphaerica Born. et Flahault	Cells 6-8 µm broad, heterocyst 6-10 µm broad, 10-12 µm long; spores 10-13 µm long, 8-11 µm broad	S10 and Sunei dam	Karnataka, Kerala, Madhya Pradesh, Maharashtra, Orissa, Rajasthan, Tamil Nadu, Uttar Pradesh and West Bengal
		Anabaena variabilis Kutz.	Trichomes 4.5-6.0 µm broad; broad, cells, 3.0-5.5 µm heterocyst 4-5 µm broad	S1 to S10	Arunchal Pradesh, Assam, Bihar, Karnataka Kerala, Maharashtra, Rajasthan, Tamil Nadu, Uttar Pradesh, West Bengal and Tamil Nadu (Perumal and Anand, 2008)
Chroococcaceae	*Aphanocapsa*	*Aphanocapsa grevillei* (Hass.) Rab	Cells 4.0-5.8 µm in diameter	S1, Khadkai reservoir	Assam, Karnataka, Madhya Pradesh, Tamil Nadu and Uttar Pradesh (Perumal and Anand, 2008)
Chroococcaceae	*Aphanothece*	*Aphanothece conferta* Richter	Cells 2.5-3.0 µm in diameter	Hatimundi dam	Tamil Nadu (Perumal and Anand, 2008)
Nostocaceae	*Aulosira*	*Aulosira bombayensis* Gonzalves	Trichomes 4.5 µm broad; cells 2.6-3.2 µm broad and 3.2-6.8 µm long; heterocyst 3.6-5 µm broad and 7-13.6 µm long	S1 to S10	This species is reported for the first time from fresh water bodies of Orissa

Family	Genus	Species	Description	Places of collection	Distribution in India
		Aulosira fertilissima Ghose	Cells 3.8-4.4 µm broad and 3.8-4.8 µm long; heterocyst 5.2-5.8 µm long	S1 and S2	This species is reported for the first time from fresh water bodies of Orissa
Rivulariaceae	*Calothrix*	*Calothrix braunii* (A.Br.) Bornet et Flahault	Cells 4.5-12.5 µm broad, in the middle zone of trichomes, 5–7 long 2.5–5 µm broad; heterocytes 5–7 µm in diameter	S1, S2, Sunei dam and Khadkai reservoir	Tamil Nadu (Desikachary, 1959)
		Calothrix clavata West and West	Cells 3–7 µm long, .4-6 µm in diameter; heterocyst4-6 µm in diameter	Joronda fall	Jammu and Kashmir, Maharashtra, Tamil Nadu, West Bengal and Karnataka (Perumal and Anand, 2008)
		Calothrix clavatoides Ghose	Cells 3.4–9.4 µm long, 7.4 µm broad; heterocyst 8.0–9.4 µm in diameter	S1 to S10 and Joronda fall	This species is reported for the first time from fresh water bodies of Orissa
		Calothrix javanica de Wilde	Cells 4-8 µm broad; heterocyst 9 µm broad	S2 and Joronda fall	Andhra Pradesh, Assam, Chhattisgarh, Kerala, Orissa, Tamil Nadu and Rajasthan (Perumal and Anand, 2008)
		Clothrix linearis Gardner	Cells 14-18 µm long, cells 4-8 µm broad, heterocyst single 7-9 µm in diameter	S2 and Joronda fall.	Tamil Nadu (Perumal and Anand, 2008)
		Calothrix marchica Lemm. var. *intermedia* Rao	Cells 6-9 µm broad at the base, 3.5-5 µm broad and 5.4-8.1 µm long at the upper region; heterocyst single 6.8 µm broad	S2, Uski fall and Joronda fall	Madhya Pradesh, Orissa, Rajasthan, Tamil Nadu, and Uttar Pradesh (Perumal and Anand, 2008)
		Calothrix parietina (Nag.) Thuret	Cells 5–12 µm long 4.5–9 µm broad in the middle zone of trichome, 5.5–12.5 µm long and 5.5–10.5 µm broad at the base; heterocytes 7.5 –12.5 µm long and 7.5–10.5 µm broad	S2	Orissa and Tamil Nadu (Perumal and Anand, 2008)
		Calothrix brevissima West var. *moniliforme* Ghose	Thallus 8-10 µm broad; cells 4-8 µm broad; heterocyst 9 µm broad	Kala dam and Hadgarh reservoir	Himachal Pradesh and Tamil Nadu (Perumal and Anand, 2008)
Chroococcaceae	*Chroococcus*	*Chroococcus tenax* (Kirchn) Hieron	Cells without sheath 19 µm in diameter with sheath 29 µm in diameter	S6 and S10	This species is reported for the first time from fresh water bodies of Orissa
		Chroococcus turgidus (Kutz.) Nag	Cells without sheath 5-29 µm broad, with sheath 12.5-44 µm broad; colonies 10-24 µm long, 5-2 µm broad; cell diameter 20-20.5 µm	S5, S10 and Khadkai reservoir	Arunchal Pradesh, Kerala, Karnataka, Orissa, Rajasthan, Punjab, Tamil Nadu, Maharashtra, Jammu and Kashmir and Uttar Pradesh (Perumal and Anand, 2008)
		Chroococcuss limneticus Lemm.	Cells without sheath 10 µm in diameter, with sheath 14 µm in diameter	S7, S10 and Khadkai reservoir	Tamil Nadu (Perumal and Anand, 2008)

Family	Genus	Species	Description	Places of collection	Distribution in India
Chroococcaceae	*Coleosphaerium*	*Coleosphaerium kuetzingianum* Nag	Colony 100-110 µm in diameter, cells 2.5-6 µm in diameter	S2	Andhra Pradesh, Bihar, Haryana, Rajasthan, Jammu and Kashmir, Karnataka, West Bengal and Tamil Nadu (Perumal and Anand, 2008)
Nostocaceae	*Cylindrospermum*	*Cylindrospermum muscicola* Kutz. var *longispora* Dixit	Trichomes 4-4.4 µm broad; cells 6.4-10.4 µm long, heterocysts 5.2-7.2 µm broad and 8.8-11.2 µm long, akinetes 8-12 µm broad and 16-20 µm long	S2, S4 and S10	This species is reported for the first time from fresh water bodies of Orissa
		Cylindrospermum stagnale (Kutz.) Bornet et Flahault	Trichome 3-4 µm broad; cells 2.6-3 µm broad and 3-5 µm long; heterocyst 4-5 µm broad and 5-7 µm long; spore 6.8-8 µm broad and 10-14 µm long	S2, S4 and S7	Tamil Nadu (Perumal and Anand, 2008)
Stigonemataceae	*Frischerella*	*Fischerella muscicola* (Thuret) Gomount	Cells 8-10 µm broad and 10-12 µm long, heterocysts 12-14 in µm diameter	S2 and S3	This species is reported for the first time from fresh water bodies of Orissa
Chroococcaceae	*Gleocapsa*	*Gleocapsa gelatinosa* (Meyen.) Kutz.	Cells without sheath, 2.5 µm and with sheath 7.2 µm in diameter; colonies, 25 µm in diameter	Khadkai reservoir, Kala dam, Deo dam	Tamil Nadu and Karnataka (Perumal and Anand, 2008)
		Gloeocapsa nigrescens Kutz.	Cells without sheath, 3.4 µm and with sheath 8.2 µm in diameter; colonies, 35 µm in diameter	Chahala nala, Barhripani, Nawana nala.	Kerala, Karnataka, Rajasthan, Tamil Nadu and Uttar Pradesh (Perumal and Anand, 2008)
	Gleotheceae	*Gleotheceae rupestris* (Lyngb.) Bornet	Colony 28-35 µm in diameter, cells with envelope 8-11 µm in diameter, 10-15 long, without envelope 4.5-6 broad and 9-12 µm long	Sunei dam, Uski fall, sitakund fall	Assam, Karnataka, Jammu and Kashmir, Maharashtra, Orissa, Tamil Nadu and Uttar Pradesh (Perumal and Anand, 2008)
Rivulariaceae	*Gleotrichia*	*Gleotrichia ghosei* Singh	Trichome 6-10 µm broad at the base, 2.6-3 µm broad higher up; cells 3-7 µm long; heterocyst, 7-9 µm broad and 7-8.5 µm long; spores 9.5-13.2 µm broad and 25-50 µm long; sheath 2-4 µm	S1, S2, S10 and Joronda fall	Madhya Pradesh, Orissa, Tamil Nadu and Uttar Pradesh (Perumal and Anand, 2008)
		Gloeotrichia natans (Hedwig) Rab.	Trichomes 7.2-8 µm broad, cells 6.7–10.8 µm long; heterocyst 4.8-12 µm broad; akinetes with sheath 12-20 µm broad and 34.4-80 µm, without sheath up to 36 µm broad	S1, S2, S10, Joronda fall, Barheipani fall and Deo dam	This species is reported for the first time from fresh water bodies of Orissa
Stigonemataceae	*Hapalosiphon*	*Hapalosiphon hiberinicus* West and West	Cells 18-36 µm long, 8 µm broad; heterocysts 4-7 µm long and 3.2-5.3 µm broad	S1, S2 and S10	Tamil Nadu (Perumal and Anand, 2008)

Family	Genus	Species	Description	Places of collection	Distribution in India
		Hapalosiphon luteolus West and West	Cells 4.5–5.4 µm broad, 5.5–6.9 µm long; heterocysts 5-8 µm long and 3-5 µm broad	S1, S2 and S10	Arunachal Pradesh, Rajasthan, Uttar Pradesh and Tamil Nadu (Perumal and Anand, 2008)
		Hapalosiphon welwitschi West and West	Trichome, 5-10 long, vegetative cells, 4-6 µm in diameter, 6-8 µm long, cells 3.75-6.2 µm in diam, heterocysts 4.8-8.8 µm long and 3.5-6.5 µm broad	S1, S2 and S10	Arunchal Pradesh, Assam, Jammu and Kashmir, Karnataka, Himachal Pradesh, Karnataka, Madhya Pradesh, Tamil Nadu, Uttar Pradesh, Uttar Pradesh and Orissa (Perumal and Anand, 2008)
Oscillatoriaceae	*Lyngbya*	*Lyngbya birgei* Smith	Filaments 20-22 µm broad, cells 2.0-2.5 µm long	S4, S5, S6, S7, S8 and S10	Assam, Kerala, Karnataka, Gujarat, Jammu and Kashmir, Maharashtra, Rajasthan, Tamil Nadu, Uttar Pradesh and West Bengal (Perumal and Anand, 2008)
	Mastigocoleus	*Mastigocoleus testarum* Lagerheim.	Cells 24-28µm broad, 4- 8 µm long; heterocyst 8 – 10.6 µm broad and 4 – 10.6 um in long	Hatimundi dam	This species is reported for the first time from fresh water bodies of Orissa
Chroococcaceae	*Merismopedia*	*Merismopedia elegans* Braunow	Colonies 4-8-16 celled 7.2-9.2 µm long, 4.2-6.2 µm broad	S1 and S8	Orissa and Tamil Nadu (Perumal and Anand, 2008)
		Merismopedia punctata var. *punctata* Meyen	Colonies 6-64 cells, 2.6-3.4 µm in diameter	S1 and S8	Andhra Pradesh, Bihar, Gujarat, Haryana, Jammu and Kashmir, Karnataka, Maharashtra, Punjab, Rajasthan, Tamil Nadu, Utter Pradesh and West Bengal (Perumal and Anand, 2008)
Oscillatoriaceae	*Microchaete*	*Microchaete aequalis* (Fremy) Desikachary	Basal cells 5-6 µm broad, cells 8-9 µm broad, heterocyst 10-17 µm long, 4-5 µm broad	S2, Deo dam and Kala dam	This species is reported for the first time from fresh water bodies of Orissa
		Microchaete tenera Thuretex Born. et Flahault	Basal cells 7-8 µm broad, cells 8.7-9.8 µm broad, heterocyst 12-19 µm long, 4.6-5.8 µm broad	S2, Deo dam and Kala dam	This species is reported for the first time from fresh water bodies of Orissa
Nostocaceae	*Nostoc*	*Nostoc sphaericum* Vauch.	Cells 3-4.6 µm broad; heterocyst spherical, 6-6.5 µm broad	S2	This species is reported for the first time from fresh water bodies of Orissa
		Nostoc commune (L.) Vaucher ex Bornet and Flahault	Trichomes 4.5-6 µm broad, cells 3.2-5.6 µm long, 2.4-2.8 µm broad; heterocysts 4.8-6.4 µm long, 4.0-4.8 µm broad	S2	Tamil Nadu (Perumal and Anand, 2008)

Family	Genus	Species	Description	Places of collection	Distribution in India
		Nostoc muscorum Kutz.	Cells 5–8 µm long, heterocysts 5–7 µm in diameter	S4 and S5	Assam, Orissa, Rajasthan and Tamil Nadu (Perumal and Anand, 2008)
		Nostoc paludosum Kutz.	Trichome 5.8-7.2 µm broad; heterocyst 1.8-2.9 µm in diameter; akinates 8-10 µm broad, 12-15 µm long, 2-3 in series	S1 and S2	This species is reported for the first time from fresh water bodies of Orissa
Nostochopsidaceae	*Nostochopsis*	*Nostochopsis lobatus* (Wood) Geitler	Colonies up to 2 cm in diameter, Cells of the main axis 4-8 µm broad, 8-18µm long, heterocyst 7-9 µm broad	S1 to S10	This species is reported for the first time from fresh water bodies of Orissa
		Nostochopsis radians Bharadwja	Cells 2.6-5.2 µm broad, up to 3.9-6.6 µm long, terminal heterocyst 3.9-6.6 µm broad, 5.5-10.2 µm long, intercalary heterocyst 3.9-7.2 µm broad	S1, S3, S4, S5 and S6	This species is reported for the first time from fresh water bodies of Orissa
Oscillatoriaceae	*Oscillatoria*	*Oscillatoria agardhi* Gomont	Cells 2.0-6.6 µm broad, 1-3 µm long; calyptra 2.5 broad, 1.8 µm long	S2, S4, Nawana nala and Chahala nala	Assam and Tamil Nadu (Perumal and Anand, 2008)
		Oscillatoria formosa Bory	Trichomes 3.6-6.4 µm broad, cells 2.4- 5.6 µm long	S1, S2 and S10	Andhra Pradesh, Assam, Bihar, Gujarat, Himachal Pradesh, Kerala, Orissa, Tamil Nadu Uttar Pradesh and West Bengal (Perumal and Anand, 2008)
		Oscillatoria limosa Agardhi	Trichomes 10.5-13.5 µm broad, 4.5-6.5 µm long, granulated	S4 and S5	Andhra Pradesh, Bihar, Jammu and Kashmir, Kerala, Orissa, Tamil Nadu and Uttar Pradesh (Perumal and Anand, 2008)
		Oscillatoria margaritifera Kutz	Trichomes 20-24 µm broad, cells 4-6 µm long	Hatimundi dam, Khadkai reservoir, sunei dam	Assam, Kerala and Tamil Nadu (Perumal and Anand, 2008)
		Oscillatoria nigroviridis Thwaites	Trichomes 2.3-4.5 µm broad, cells 1.5-2 µm long	S2 and S8	Kerala, Maharashtra, Tamil Nadu and Uttar Pradesh (Perumal and Anand, 2008)
		Oscillatoria princeps Vaucher ex Gomount	Trichomes 27-29 µm broad; cells 2.3-7.0 µm long, 12- 28 µm broad	S6 and s10	Andhra Pradesh, Assam, Jammu and Kashmir, Karnataka, Kerala, Madhya Pradesh, Madras Presidency, Maharashtra, Orissa, Rajasthan, Tamil Nadu, Uttar Pradesh, Kerala, West Bengal and Tamil Nadu (Perumal and Anand, 2008)
		Oscillatoria rubescens De Candolle	Trichomes 6-7 µm broad, cells 2-3 µm long	S1, S6 and S10	Assam, Karnataka, Uttar Pradesh and (Perumal and Anand, 2008)

Family	Genus	Species	Description	Places of collection	Distribution in India
		Oscillatoria simplissima Gomont	Trichomes 18-22 µm broad; cells 2-5 µm long, 10- 18 µm broad	S6 and S10	Tamil Nadu (Perumal and Anand, 2008)
		Oscillatoria subbrevis Schmidle	Trichomes up to 77 µm long, 4.5- 10 µm broad, cells 1.5-3.5 µm long, 4.5-8.2 µm broad	S1 to S10 and Deo dam	Arunanchal Pradesh, Assam, Bihar, Karnataka, Kerala, Maharashtra, Rajasthan, Tamil Nadu, Jammu and Kashmir, Uttar Pradesh and West Bengal (Perumal and Anand, 2008)
		Oscillatoria tenuis Agardh	Trichomes 3-11 µm broad, cells 1.5-6.0 µm 4-5 µm broad	S2, S4, Nawana nala, Kala dam and Khadkai reservoir	Andhra Pradesh, Assam, Jammu and Kashmir, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Tamil Nadu and West Bengal (Perumal and Anand, 2008)
	Phormedium	*Phormedium ambiguum* Gomount	Trichomes 3-5 µm broad, cells 1.5-2 µm long	S1 and S2	Arunachal Pradesh, Assam, Bihar, Jammu and Kashmir, Kerala, Maharashtra, Tamil Nadu, Uttar Pradesh, Kusumlata, West Bengal (Perumal and Anand, 2008)
		Phormidium unicinatum Gomount	Trichomes 6-7 µm broad, cells 2.3-3.5 µm long	S1 and S2	Tamil Nadu (Perumal and Anand, 2008)
Scytonemataceae	*Scytonema*	*Scytonema sp*	Trichomes 10.8–12.0 µm; cells 2–6 µm long, 6–8 µm broad; heterocyst 16.8 µm long, 10 µm broad	S2	This species is reported for the first time from fresh water bodies of Orissa
		Scytonema hofmani Agardh ex. Bornet. Et Flahault	Filament 5.5-11.5 µm broad; cells 1-2.4 µm broad; heterocyst oblong, 5.5-11.5 µm long, 4.8-8.5 µm broad	S1, S2, S4, S8 and S10	This species is reported for the first time from fresh water bodies of Orissa
		Scytonema mirabile (Dillwyn) Agardh	Trichomes 20 -24 µm in diameter, cells 8 µm in diameter; heterocyst 20 µm long, 10 µm in diameter	S2	Tamil Nadu (Perumal and Anand, 2008)
		Scytonema millei Born.	Trichomes 17.5-20 µm in diameter, vegetative cells 5-7.5 µm long	S2	Tamil Nadu (Perumal and Anand, 2008)
Oscillatoriaceae	*Spirulina*	*Spirulina major* Kutz.	Trichomes without sheath 0.85-1.2 µm in diameter, helix 3.75 (2.5-4) µm wide, distance between regular spirals 4-5 µm	Joronda fall, Kala dam, Deo dam	Rajasthan, Tamil Nadu and West Bengal (Perumal and Anand, 2008)
Stigonemataceae	*Westiellopsis*	*Westiellopsis prolifica* Janet	Cells 6-7 µm broad and 11-15.5 µm long	S1 and S2	Andhra Pradesh, Bihar, Jammu and Kashmir, Karnataka, Kerala, Madras Presidency, Maharashtra, Orissa, Rajasthan, Tamil Nadu, Uttar Pradesh, West Bengal (Perumal and Anand, 2008)

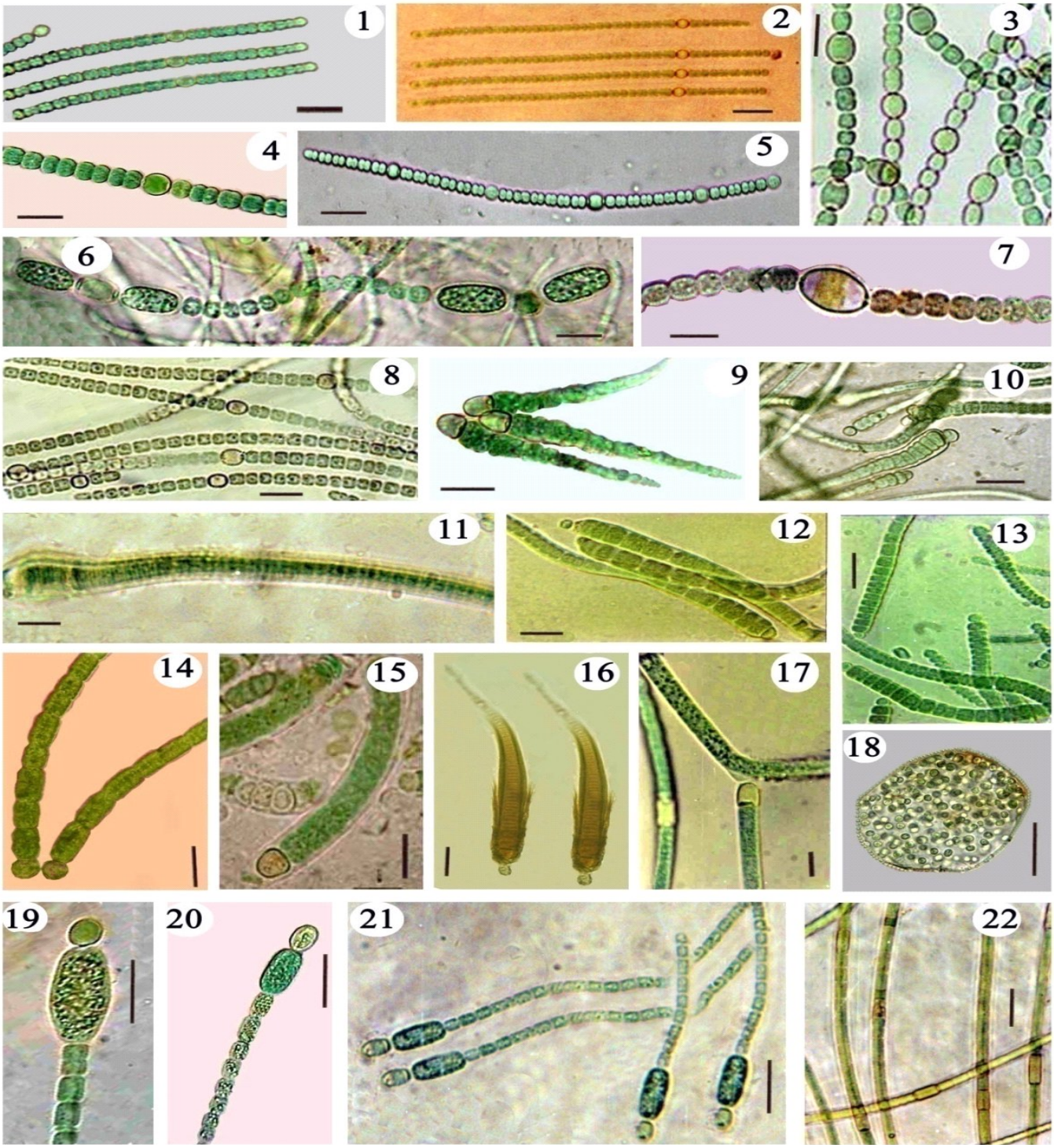

Fig.1 : *Anabaena fertilissima,* Fig.2 : *Anabaena orientalis*, Fig.3 : *Anabaena azollae* , Fig.4 : *Anabaena sphaerica*, Fig.5 : *Anabaena oscillarioides*,Fig.6: *Anabaena iyengarii* Fig.7 : *Anabaena macrospora*, Fig.8 : *Anabaena variabilis*, Fig.9 : *Calothrix braunii* (A.Br.), Fig.10 : *Calothrix brevissima*,Fig.11 : *Calothrix clavatoides*, Fig.12 : *Clothrix linearis*, Fig.13 : *Calothrix javanica*,Fig.14 : *Calothrix marchica*, Fig.15 : *Calothrix clavata*, Fig.16 : *Calothrix parietina*, Fig.17 : *Aulosira fertilissima*, Fig.18 : *Aphanocapsa grevillei* Fig.19: *Cylindrospermum muscicola*, Fig. 20 and 21 : *Cylindrospermum stagnale*, Fig. 22 : *Aulosira bombayensis*

Scale bars: 10 µm **:** Fig.18, 5 µm : Fig.1-17, Fig.19-22

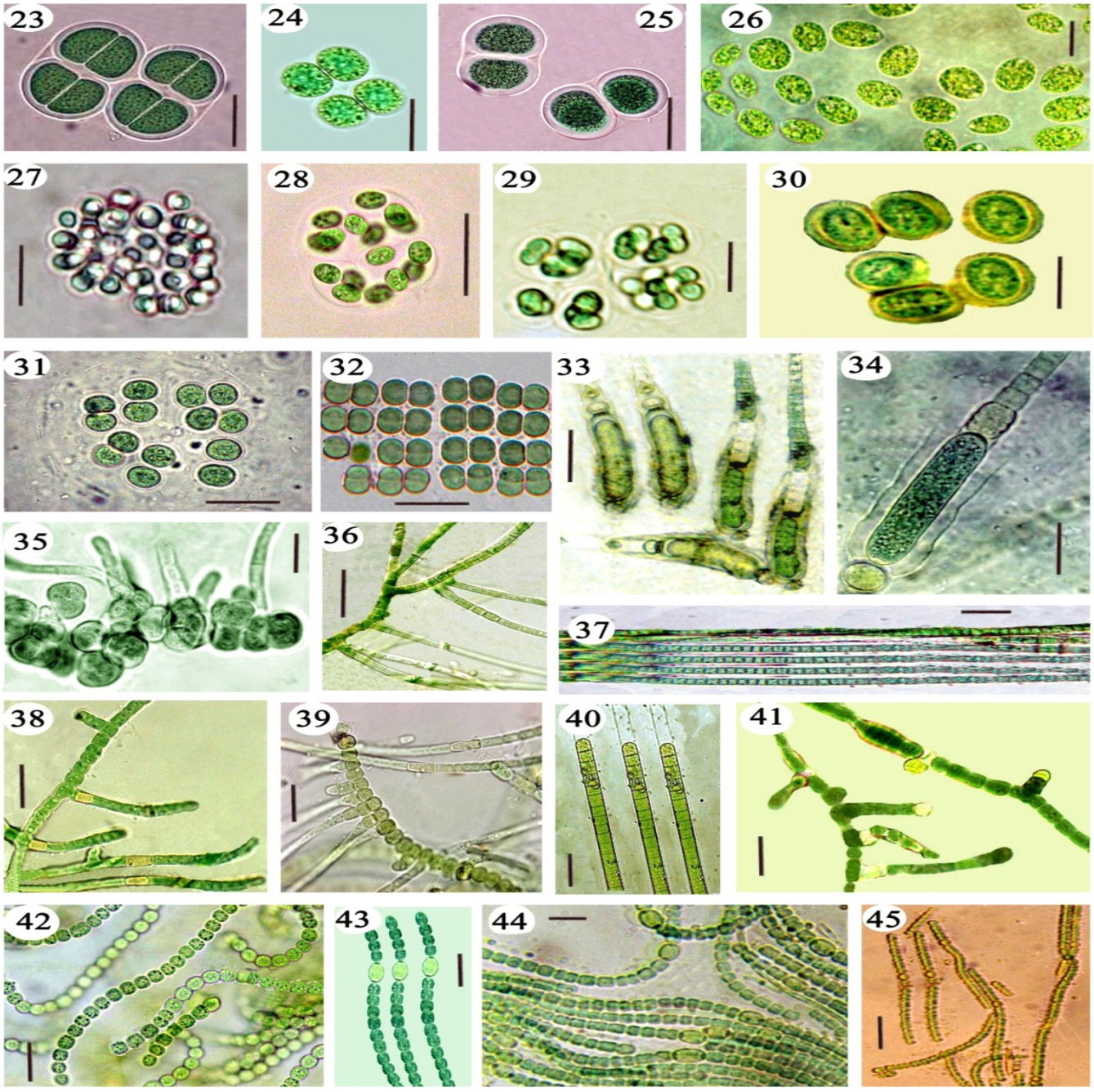

Fig. 23 : *Chroococcus tenax*, Fig. 24 : *Chroococcus turgidus,* Fig. 25 : *Chroococcuss limneticus,* Fig. 26 : *Aphanothece conferta,* Fig. 27 : *Coleosphaerium kuetzingianum*, Fig. 28 : *Gleocapsa gelatinosa*, Fig.29 : *Gloeocapsa nigrescens,* Fig. 30 : *Gleotheceae rupestris*, Fig. 31 : *Merismopedia punctata,* Fig. 32 : *Merismopedia elegans,* Fig. 33 : *Gleotrichia ghosei*, Fig. 34 : *Gloeotrichia natans*, Fig. 35 : *Fischerella muscicola*, Fig. 36 : *Hapalosiphon hiberinicus*, Fig. 37 : *Oscillatoria agardhi,* Fig. 38 : *Hapalosiphon luteolus,* Fig. 39 : *Hapalosiphon welwitschi*, Fig. 40 : *Lyngbya birgei,* Fig. 41 : *Mastigocoleus testarum,* Fig. 42 : *Nostoc commune,* Fig. 43 : *Nostoc muscorum,* Fig. 44 : *Nostoc phaericum,* Fig. 45 : *Nostoc paludosum.*

Scale bars : 50 µm : Fig.27, 30 µm : Fig.28, 29, 31, 32 and 41, 20 µm : Fig.23-25, Fig.33, 34 & 36, 10 µm: Fig.30 and 35, 5 µm: Fig.26- 40, Fig. 42-45.

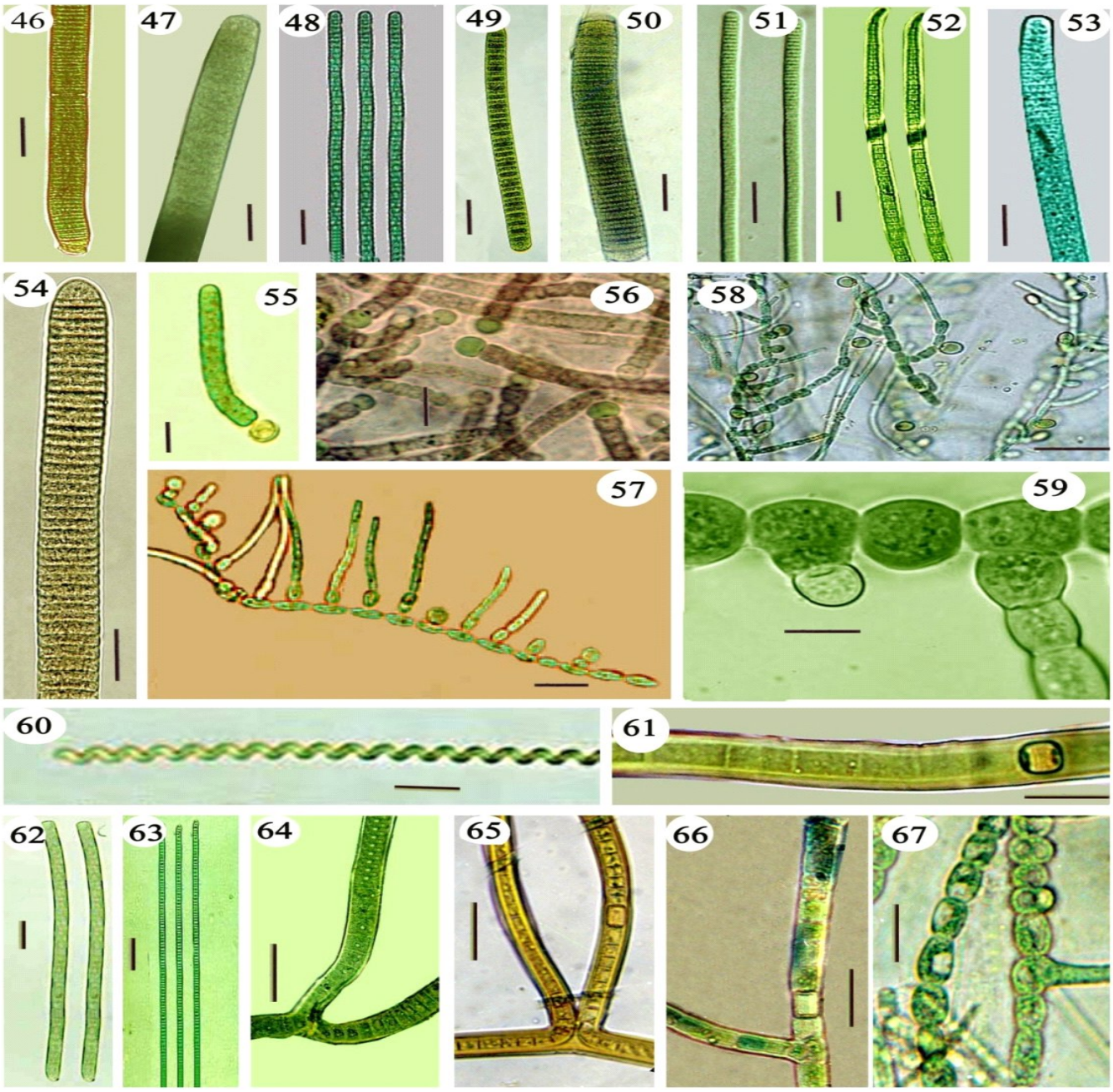

Fig. 46 : *Oscillatoria formosa*, Fig. 47 : *Oscillatoria limosa,* Fig. 48 : *Oscillatoria margaritifera*, Fig. 49 : *Oscillatoria nigroviridis*, Fig. 50 : *Oscillatoria princeps,* Fig. 51 : *Oscillatoria rubescens,* Fig. 52 : *Oscillatoria simplissima,* Fig. 53 : *Oscillatoria subbrevis,* Fig. 54 : *Oscillatoria tenuis,* Fig. 55 : *Microchaete aequalis,* Fig. 56 : *Microchaete tenera,* Fig. 57 : *Nostochopsis radians,* Fig. 58 and 59 : *Nostochopsis lobatus,* Fig. 60 : *Spirulina major*, Fig. 61 : *Scytonema mirabile,* Fig.62 : *Phormedium ambiguum,* Fig. 63 : *Phormidium unicinatum,* Fig. 64 : *Scytonema millei*, Fig. 65 : *Scytonema hofmani,* Fig. 66 : *Scytonema sp.*, Fig. 67 : *Westiellopsis prolifica*

Scale bars: 10 µm: Fig.61 and Fig.64- 67, 5 µm: Fig.46-60, Fig.62 and 63

The waters of the Similipal are ideal examples of natural systems less affected by anthropogenic pollution. The reserve is largely ecologically intact and remote from the industrial and agricultural centers of the state. As such, it presents unparalleled opportunities for research into fully functional, unperturbed aquatic ecosystems in the country. The present study can be used as a baseline status on

the cyanobacterial flora which could be used to monitor the aquatic environment in future. In conclusion, the present study documented a remarkable biodiversity of freshwater cyanobacteria that are distributed in various habitats suggesting their wide adaptability or varied genotype, which could be explored through future research.

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HYDROBIOLOGICAL DYNAMICS OF BIOFILTER IN RECIRCULATING AQUACULTURE SYSTEM WITH SPECIAL REFERENCES TO SILO

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ABSTRACT

Experiments were conducted by rearing rohu, *Labeo rohita* (Hamilton) in silo, the hi-tech recirculatory aquaculture system at CIFA, Bhubaneswar. Three sets of duplicate silo tanks of 3, 6 and 9 m^3 water holding capacities attached to a down-flow biofilter with locally available media were used in the study. Rohu fish (30, 20 and 10 kg) were reared for 30 days and 100% survival was obtained in all the experimental silos. The growth of fish was insignificant in all the experiments. Ammonia and nitrite levels were significantly lower (Pd"0.05) in the bio-filtered water than the water before biofiltration. Nitrate level of biofiltered water showed significantly higher (Pd"0.05) values 0.022-0.935 (0.36 ± 0.34) mg/l than the combined flux water from silos 0.013-0.311 (0.13 ± 0.11) mg/l. The studies suggest that the silos fish rearing system with biofilter will help to maintain better water quality.

Key words: Recirculatory aquaculture system, biofilter, silo, rohu, water quality

INTRODUCTION

Live fish marketing in India is growing rapidly. People are more concerned about their health and the risk caused due to stale fish. Metropolis and star hotels may develop the system where they can store the live fish at least for a period of 1 month, which can be served after processing to a number of visitors/ tourists. In order to have a low space requirement and high efficiency of the recirculatory water rearing system having tire system named as silos, have been designed and developed at Central Institute of Freshwater Aquaculture, Bhubaneswar (Mohapatra *et al.,* 2003). The concept of silos was initially introduced in agriculture, which is a cylindrical tower system used for storing of materials in bulk (Tadele *et al*., 2011). The silos were also used in military installations for ballistic missiles storing and firing (Altman, 2005). The concept of silos was taken from the source and used for marketable fish rearing, where people can get live fish for better taste and good health.

As water is a major constraint in big cities, CIFA developed a recirculatory system where the same water can be reused for an indefinite period maintaining the desired physico-chemical parameters. In recirculatory aquaculture system biological filter plays a pivotal role for reducing total ammonical nitrogen, nitrite, and dissolve organic matter and increases the nitrification rate. The concentration of the total ammonical nitrogen is the most important factor to consider the biofilter design and operation (Chen *et al.,* 2006). Various types of biofilters have been used by a number of workers in recirculatory aquaculture systems (Van Rijn, 1996; Skjolstrup *et al.,* 1997; Chen *et al.,* 2006; Eding *et al.,* 2006; Timmons *et al.,* 2006). Recirculatory aquaculture system using biological filter is highly accepted as it maintains a superior environment for fish growing, where water is frequently cleaned and reused. This type of system reduces the space and maintains desired water quality for maintenance of a particular fish species under various agro-climatic regions. There are worldwide popularization of this recirculatory aquaculture system for production of shrimps and other fishes, where water is of major concern. This will address the safety issue of the aquaculture and provide fresh fish for the human consumption and reduce various diseases.

In the present study we have developed a system, where biofilter is attached to the silos, in which healthy live fish can be maintained for consumption

purposes as well as fish can also be kept alive for a period of one month. We have also checked the efficiency of the biofilter in terms of physico-chemical parameters *i.e.,* variation of ammonia, nitrite, nitrate and dissolved organic matter (DOM).

MATERIALS AND METHODS

A 30 days (12 July - 10 August 2008) indoor experiment was carried out at Application of Plastics in Aquaculture (APA) Cooperating Experimental Center of All India Co-ordinated Research Project (AICRP) at Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar, India.

Experimental setup

The study was conducted in 3 sets of Fibre Reinforce Plastic (FRP) silos *i.e.,* large (S-1 and S-2), medium (S-3 and S-4) and small (S-5 and S-6). The S-1 and S-2 represent T-1; S-3 and S-4 represent T-2; and S-5 and S-6 represent T-3. A total of 123 fishes (avg. wt 0.451 kg) were stocked in S-1 & S-2 silos each having height of 2.7 m, diameter 2.15 m with water holding capacity of 9000 liter. Similarly 82 fishes (avg. wt 0.488 kg) were stocked in S-3 & S-4 silos each having the height of 1.8 m, diameter 1.5 m and water holding capacity of 6000 liter; and 53 fishes (avg. wt 0.465 kg) in the S-5 & S-6 silos tank each of height 0.9 m, diameter of 2.15 m water holding capacity of 3000 liter (Table 1).

Table 1 : Characteristics of the silos showing number of fish stocked and survival percentage

Silos characteristics	T-1		T-2		T-3	
Silos	S-1	S-2	S-3	S-4	S-5	S-6
Height (m)	2.7	2.7	1.8	1.8	0.9	0.9
Diameter(m)	2.15	2.15	2.15	2.15	2.15	2.15
Water holding (l)	9000	9000	6000	6000	3000	3000
Working volume(l)	8910	8910	5940	5940	2970	2970
Fish stocked	30	30	20	20	10	10
No of fish	66	67	43	39	22	21
Avg. initial wt (g)	454	447	465	512	454	476
Survival %	100	100	100	100	100	100

The effluent channels of all the silos were connected to the biofilter. The filtration media were kept in five layers in the biofilter. The bottom layer (1st layer) was fitted with fine net. The second layer was filled with pebbles of size 8-16 mm and height of the layer was 20 cm. The 3rd represented the charcoal layer (these were collected from leftovers of burnt firewood). Above to this a fine net of mesh size (0.02 mm) was placed to avoid clogging from the upper layer (sand layer). The top most layer was filled with very coarse sand of size 1-2 mm (Table 2). The effluent water from each silo was passed through the top layer, percolated down and stored in the filtered tank for recirculation with the help of 1HP water pump. The water of the filtered tank was pumped to the over head tank for recirculation through shower to maintain dissolved oxygen level in silo.

The physico-chemical quality of water was measured according to the protocols of APHA (1998) throughout the experimental period. The water samples from silos and filter tank were collected in clean plastic bottles in the morning hours before feeding to fishes. The water temperature, dissolved oxygen (DO) and pH were recorded *(in situ*) and the total alkalinity, total hardness, dissolve organic matter (DOM) and inorganic nutrients *viz.,* total ammonia nitrogen, nitrite-nitrogen, nitrate-nitrogen and phosphate-phosphorus were analyzed in laboratory, following standard methods of American Public Health Association (1998).

The analysis of temperature, pH, dissolved oxygen, total alkalinity and total hardness were done on daily basis and the other parameters like dissolve organic matter, total ammonia, nitrite and nitrate were analyzed on alternative days.

Table 2 : Characteristics of the biofilter showing various types of packing materials

Biofilter type	Down flow
Total Length of filtration unit (cm)	230
Total height of filtration unit (cm)	150
Total width of filtration unit (cm)	170
Total base area of filtration unit (cm^2)	39,100
Length of biofilter (cm)	170
Height of biofilter unit (cm)	100
Width of biofilter unit (cm)	170
Total volume of the filter (cm^3)	2890,000
Upper surface area of biofilter (cm^2)	28,900
Packing (Media) volume of biofilter (cm^3)	2312,000
Total surface area of the packing Media (cm^2)	112,200
Side area of biofilter (cm^2)	13,600
Storage capacity of the filtered water (l)	2975
Size of gravel (mm)	8-16
Height of the gravel layer (cm)	20
Volume of the Gravel (cm^3)	578,000
Size of the sand particle (mm)	0.25-0.5
Free space for flux water above the sand layer (cm)	20
Height of the sand layer (cm)	30
Volume of the sand (cm^3)	867,000
Size of the charcoal (mm)	8-28
Height of the charcoal layer (cm)	30
Volume of the charcoal (cm^3)	867,000
Mesh size of Fine net liner (mm)	0.02

RESULTS AND DISCUSSION

The details of the height, diameter, water holding capacity, working volume of silo, number of fish stocked with average initial weight and percentage survival after 30 days of experiment are presented in the table 1.

The important water quality parameters for a period of 30 days are presented in table 3. The physico-chemical parameters such as temperature, pH, DO remained within the acceptable limits in the silos during the entire rearing period. The concentrations of total ammonical nitrogen in three different silos are represented in the Fig. 1. In all the three treatment groups, ammonical nitrogen increased from day 1 to day 30. The average ammonical nitrogen concentration was higher in treatment 1, where 30 kg of fish were reared. The effluents of these three groups of treatment water were collected to a common point (flux water) and filtered through the biofilter. There was significant reduction of ammonical nitrogen (Fig. 2) and the reduction was 37% on day 3 which reached up to 81% on day 30. This reduction of ammonical nitrogen was very high and maintained in the silos, and daily increase rate was checked. The variations of the nitrite nitrogen in the three sets of the treatments are depicted in the Fig. 3 and the percentage reduction of nitrite nitrogen in comparison to flux and biofilter water is shown in Fig. 4. The nitrite nitrogen was stable for first 15 days, and then increased up to 27 days there by increasing the rate of nitrite nitrogen in treatment 2. When nitrite nitrogen was recycled through the biofilter, it was noticed that there was 90% reduction in 30 days of rearing. This indicated that the present biofilter used in the silos had the power to reduce both ammonical nitrogen and nitrite nitrogen to a great extent *i.e.,* up to 90%.

Table 3: Physico-chemical parameters of the silo water (data on the parenthesis represents the mean ± SE)

Parameters	T-1	T-2	T-3	Flux	Filtered
Temp (°C)	23.5-24.6 (24.34 ± 0.32)	23.5-24.6 (24.29 ± 0.42)	23.5-24.6 (24.28 ± 0.44)	23.5-24.5 (24.28 ± 0.36)	23.1-24.2 (23.94 ± 0.44)
pH	6.8-7.4 (6.95 ± 0.23)	7.1-7.4 (7.18 ± 0.11)	7.1-7.4 (7.22 ± 0.09)	6.9-7.4 (7.18 ± 0.16)	6.9-7.4 (7.23± 0.09)
DO (mg/l)	3.7-5.0 (4.04 ±0.35)	3.85-5.0 (4.2 ± 0.29)	2.55-5.4 (4.82 ± 0.81)	5-5.8 (5.53 ± 0.23)	5.8-6.3 (6.01 ± 0.11)
Alkalinity (mg/l)	95-110 (101.75 ± 6.46)	100-110 (106 ± 5.1)	100-112.5 (106.25 ± 5.43)	100-110 (105.5 ± 4.97)	110-110 (110 ± 0.0)
Hardness (mg/l)	130-145 (135.5 ± 5.5)	130-145 (137 ± 5.8)	125-140 (130.5 ± 4.9)	120-140 (129 ± 8.7)	120-140 (129 ± 8.7)
DOM (mg/l)	24.0-30.5 (27.85 ± 2.13)	19.0-27.5 (21.35 ± 2.67)	23.5-33 (26 ± 3.03)	30.0-38.0 (33.4 ± 2.27)	11.0-19.0 (13.8 ± 2.52)
Ammonia (mg/l)	0.011-0.457 (0.231 ± 0.17)	0.016-0.345 (0.215 ± 0.11)	0.015-0.357 (0.216 ± 0.14)	0.017-0.432 (0.264 ± 0.14)	0.010-0.077 (0.050 ± 0.02)
Nitrite (mg/l)	0.004-.022 (0.073 ± 0.07)	0.006-0.256 (0.093 ± 0.09)	0.001-0.249 (0.096 ± 0.09)	0.011-0.285 (0.135 ± 0.12)	0.011-0.017 (0.01 ±0.00)
Nitrate (mg/l)	0.006-0.313 (0.13 ± 0.12)	0.007-0.269 (0.14 ± 0.09)	0.009-0.411 (0.22 ± 0.15)	0.013-0.311 (0.13 ± 0.11)	0.022-0.935 (0.36 ± 0.34)

T-1 (Avg. of S-1 and S-2), T-2 (Avg. of S-3 and S-4) and T-3 (Avg. of S-5 and S-6)

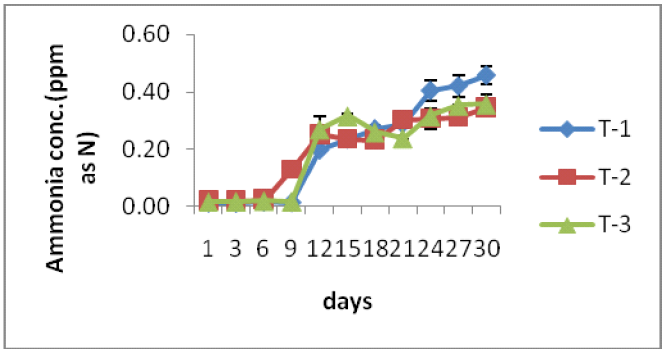

Fig. 1 : Variation of ammonical-nitrogen in three treatments for a period of 30 days

In addition to ammonical nitrogen and nitrite nitrogen, nitrification rate was increased from day 3 to day 30 (Fig. 5). In treatment 3, nitrification rate was maximum in comparison to treatment 2 and 1. When the effluent water was filtered through biofilter, contrast to nitrite and ammonia, the quantum of nitrate- nitrogen increased up to 47% from day 1 to day 3, then reduced to 20% & 37% on day 6 and day 9 respectively (Fig. 6).

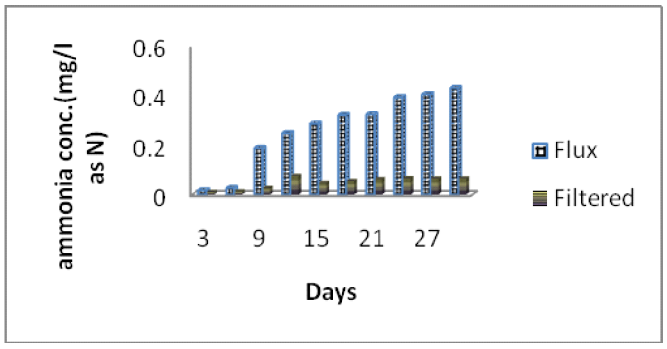

Fig. 2 : Percentage reduction of ammonical-nitrogen both in biofilter and flux water for a period of 30 days

The variations of dissolve organic matter over the period of 30 days are given in Fig. 7. The percentage reduction of the DOM was from 30 to 38% with an average reduction of 33% from day 3 to day 30 (Fig. 8).

The results of statistical analysis for finding the level of significance for DOM, ammonia, nitrite and nitrate are presented in tables 4, 5, 6 and 7 respectively.

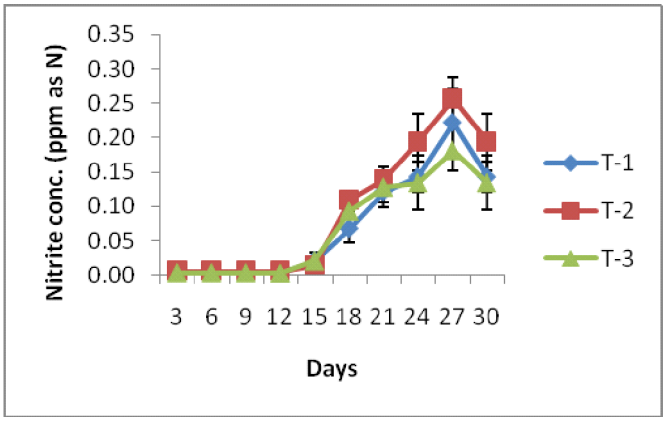

Fig. 3 : Variation of nitrite-nitrogen in three treatments for a period of 30 day

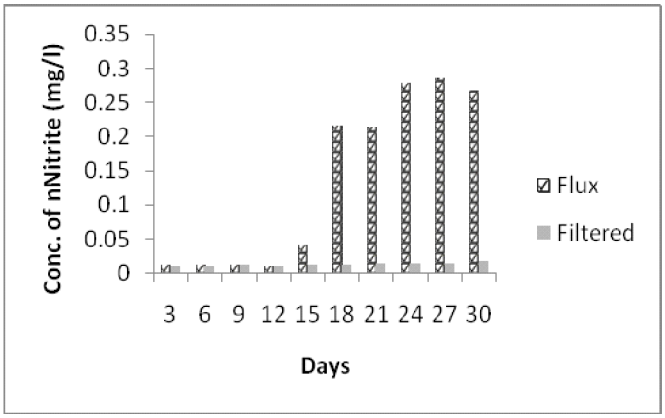

Fig. 4 : Percentage reduction of Nitrite-nitrogen both in biofilter and flux water for a period of 30 days

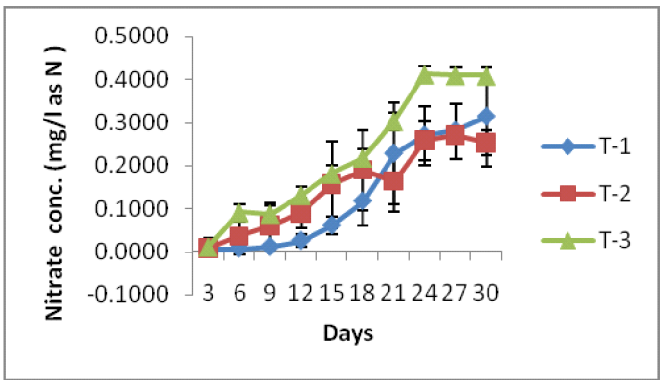

Fig. 5 : Variation of nitrate-nitrogen in three treatments for a period of 30 days

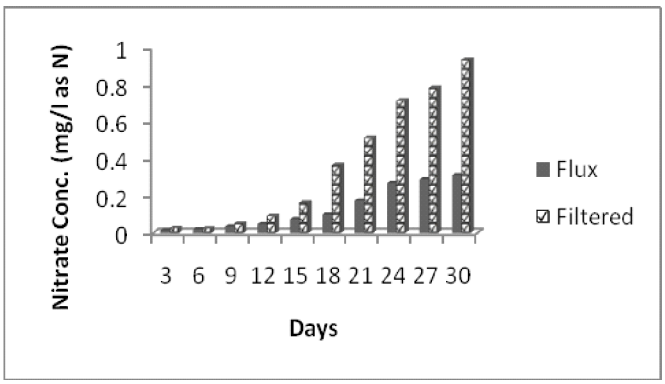

Fig. 6 : Percentage reduction of nitrate-nitrogen both in biofilter and flux water for a period of 30 days

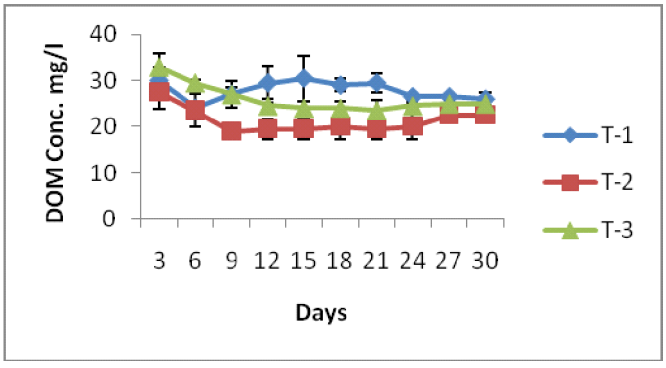

Fig. 7 : Variation of DOM in three treatments for a period of 30 days

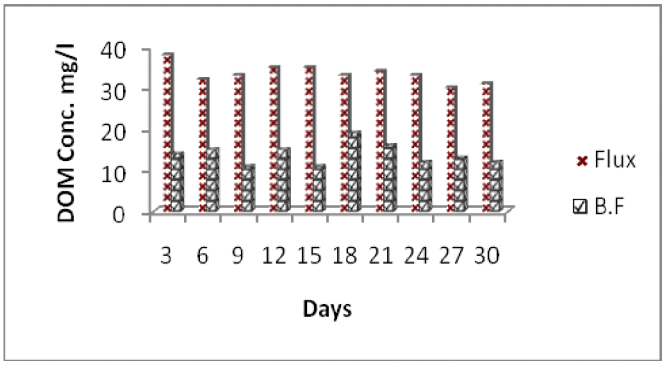

Fig. 8 : Percentage reduction of DOM both in biofilter and flux water for a period of 30 days

Table 4 : Variation of DOM (mg/l) in the silos from fish stocking to one month period (Data represents mean ± SE of duplicate sets; Parameters in each column with same superscript are not significantly differ at 5% level).

Treatment	Days									
	3	6	9	12	15	18	21	24	27	30
T-1	30.0 ± 2.8 ^{a}	24.0 ±1.0 ^{a}	27.0 ±2.0^{b}	29.0 ±2.5 ^{a}	30.5 ±3.5 ^{a}	29.0 ± 1.4^{b}	29.5 ± 1.5 ^{b}	26.5 ±0.5 ^{a}	26.5 ±1.0 ^{a}	26.0 ±0.5 ^{a}
T-2	27.5 ± 3.53^{a}	23.5 ±2.5 ^{a}	19.0 ±1.0^{a}	19.5 ±1.5 ^{a}	19.5 ±1.5 ^{a}	20.0 ± 2.8^{a}	19.5 ±1.5 ^{a}	20.0 ±2 ^{a}	22.5 ±0.5 ^{a}	22 ±0.5 ^{a}
T-3	33.0 ±2.82 ^{a}	29.5 ±0.5 ^{a}	27.0 ±1.0 ^{b}	24.5 ±0.5 ^{a}	24.5 ±1.0 ^{a}	24.0 ±1.4^{ab}	23.5 ±1.5 ^{ab}	24.5 ±0.5 ^{a}	25.0 ±1.4 ^{a}	25 ±0.0 ^{a}

Table 5 : Variation of ammonia (mg/l) in the silos from fish stocking to one month period (Data represents mean ± SE of duplicate sets; Parameters in each column with same superscript are not significantly differ at 5% level).

Treatment	Days									
	3	6	9	12	15	18	21	24	27	30
T-1	0.011 ± 0.00^{a}	0.015 ± 0.003^{a}	0.01 ± 0.00^{a}	0.198 ±0.002^{a}	0.236 ±0.013^{a}	0.271 ± 0.006^{a}	0.289 ± 0.33^{a}	0.404 ± 0.067^{a}	0.420 ± 0.050^{a}	0.457 ± 0.026^{a}
T-2	0.016 ± 0.003^{a}	0.022 ± 0.008^{a}	0.128 ± 0.16^{a}	0.251 ±0.005^{b}	0.235 ±0.028^{a}	0.232 ± 0.021^{a}	0.302 ± 0.034^{a}	0.306 ± 0.012^{a}	0.311 ± 0.009^{a}	0.345 ± 0.007^{a}
T-3	0.016 ± 0.002^{a}	0.018 ± 0.001^{a}	0.017 ± 0.001^{a}	0.269 ±0.006^{c}	0.316 ±0.002^{b}	0.260 ± 0.007^{a}	0.239 ± 0.007^{a}	0.316 ± 0.002^{a}	0.352 ± 0.033^{a}	0.357 ± 0.041^{a}

Table 6 : Variation of nitrite (mg/l) in the silos from fish stocking to one month period (Data represents mean ± SE of duplicate sets; Parameters in each column with same superscript are not significantly differ at 5% level).

Treatment	Days									
	3	6	9	12	15	18	21	24	27	30
T-1	0.004 ± 0.001^{a}	0.004 ± 0.002^{a}	0.004 ± 0.001^{a}	0.004 ± 0.001^{a}	0.020 ± 0.012^{a}	0.0067 ±0.020^{a}	0.121 ± 0.014^{a}	0.142 ± 0.022^{a}	0.222 ± 0.049^{a}	0.142 ± 0.22^{a}
T-2	0.006 ± 0.005^{a}	0.006 ± 0.005^{a}	0.006 ± 0.005^{a}	0.006 ± 0.005^{a}	0.014 ± 0.002^{a}	0.109 ±0.00^{ab}	0.139 ± .010^{a}	0.193 ± 0.041^{a}	0.256 ± 0.031^{a}	0.193 ± 0.04^{a}
T-3	0.001 ± 0.00^{a}	0.002 ± 0.002^{a}	0.002 ± 0.001^{a}	0.001 ± 0.00^{a}	0.030 ± 0.00^{a}	0.140 ±0.00^{b}	0.170 ± 0.029^{a}	0.180 ± 0.071^{a}	0.249 ± 0.055^{a}	0.180 ± 0.71^{a}

Table 7 : Variation of nitrate (mg/l) in the silos from fish stocking to one month period (Data represents mean ± SE of duplicate sets; Parameters in each column with same superscript are not significantly differ at 5% level).

Treatment	Days									
	3	6	9	12	15	18	21	24	27	30
T-1	0.0064 ± 0.002^{a}	0.007 ± 0.0055^{a}	0.011 ± 0.007^{a}	0.025 ± 0.013^{a}	0.060 ± 0.021^{a}	0.177 ± 0.057^{a}	0.227 ± 0.118^{a}	0.269 ± 0.068^{a}	0.280 ±0.063^{a}	0.313 ± 0.115^{a}
T-2	0.0077 ± 0.001^{a}	0.035 ± 0.042^{a}	0.059 ± 0.054^{a}	0.090 ± 0.035^{a}	0.155 ± 0.100^{a}	0.189 ± 0.092^{a}	0.163 ± 0.069^{a}	0.257 ± 0.044^{a}	0.269 ± 0.019^{a}	0.252 ± 0.029^{a}
T-3	0.0099 ± 0.003^{a}	0.090 ± 0.105^{a}	0.087 ± 0.043^{a}	0.130 ± 0.078^{a}	0.180 ± 0.077^{a}	0.217 ± 0.094^{a}	0.303 ± 0.026^{a}	0.411 ± 0.104^{a}	0.408 ±0.062^{a}	0.408 ± 0.071^{a}

Based on the review provided by Sharma and Ahlert (1977), the optimal pH for the growth of nitrifying bacteria varies widely. But, poor agreement existed on how much, and at what point, pH begins to affect nitrification rates (Biesterfeld *et al*., 2001). Optimum pH for nitrification can range from 7.0 to 9.0 and from 7.2 to 8.8 for *Nitrosomonas* and 7.2 to 9.0 for *Nitrobacter* respectively. In the present study the pH was slightly alkaline which represented better nitrification rate and increased nitrate nitrogen.

Dissolved oxygen is the most critical water quality variable and depends on water temperature, stocking and feeding rates of fishes and the effectiveness of the aeration installed within the recirculation system. Dissolved oxygen concentration should be kept above 60% saturation (or around 5 ppm) to ensure the survival and growth of the culture species. A minimum DO concentration of 5 ppm is required for proper functioning of recirculating system (Greiner and Timmons, 1998). Amount of higher oxygen levels in the biological system might have also ensured better mineralization process (Avnimelech *et al*., 1992; Avnimelech, 1999). In the present study it was found that the DO level in all the three treatments remained from 2.5 – 5.4 mg/l and in the biofilter increased from 5.2-5.8 mg/l. As DO was mostly higher than 5 mg/l in biofilter, this might have triggered the nitrification and other mineralization processes.

The components of alkalinity *i.e*., carbonate and bicarbonate, are suitable for nitrifying bacteria and they provide the buffering capacity for the change in pH due to acid production in the nitrification process. So there is an interrelated link among the pH, alkalinity and nitrification rate. The nitrification efficiency showed a linear increase of 13% per unit pH increases from pH 5.0 to 8.5 (Villaverde *et al*., 1997). They also observed and reported a linear correlation between the alkalinity and pH, with a stoichiometry coefficient of 7.1 mg $CaCO_3$ consumed/ mg NH_4^+–N oxidized. In biofiltration system there was an increase in the nitrification rate which indicated that there might be growth of nitrifying bacteria, though it was not isolated and quantified the nitrifying bacteria. The alkalinity remained at 110 mg/l. This level of alkalinity might have attributed for the better nitrification rate as reflected in the experiment, both in silos water as well as after filtration.

The dissolved organic matter contents in treatment tanks indicated the level of degradation of organic matter applied, which was much lower in filtered water. Feed application resulted high dissolved organic matter contents in silos, but filter media chosen for biofilters showed a high impact on the removal of Dissolve Organic Matter (DOM). In the present study it was noticed that the average DOM reduced up to 58% from day 3 to day 30, which indicated that the biofilter efficiency was high for reducing the DOM content, there by sustaining the fish in the silos in different stocking densities using a limited volume of water through recirculation.

In this study, filter media exhibited increased nitrification rate as revealed by the percentage increase in the nitrification rate value from day 3 to day 30. This is probably due to better establishment of the bacterial colony as reported by Ridha and Cruz (2001). The nitrification rate also increased in three treatment tanks and the level of increase was within the acceptable range. Midlen and Redding (1998) stated that at level of more than 100 mg/l Nitrate-nitrogen become toxic to fish. It was observed from the experiment, that the nitrification rate was influenced by buildup nitrifying bacterial community, but the nitrification rate was not the highest, indicating the process was also influenced mainly by environmental factor such as pH and oxygen concentration (Wild *et al.,* 1971).

The mean ammonia concentration in the three treatments remained more or less same for the first 12 days and subsequently increased. However, the ammonia- nitrogen concentration after biofiltration was very low which indicated the establishment of the nitrifying bacteria in the biofilter surface and reduced the toxic ammonia excreted from fishes. There was also addition of feed in the silos, the undigested food materials and fish excreta might have attributed to increase in ammonical nitrogen in three treatments. The ammonical – nitrogen content in silos was lower than 0.6 mg/l and also similar value is reported by Leclercq and Hopkins (1985) using an airlift recycling tank system. The level was much less than the lethal level (48h-LC50) of 2.4 mg/l reported by Redner and Stickney (1979) for *O. aureus*. As ammonical nitrogen value was less in the recirculatory silos, the stress on the fish due to ammonia was presumed to be less.

Otte and Rosenthal (1979) reported monthly growth of 30% with no mortality at higher nitrite concentration of 1.0 mg/l. In the silos, the nitrite concentration remained same up to 15 days, but increased up to 0.256 mg/l by 30 days of rearing of fish in all the three treatments indicating no adverse

effect on the growth and survival of the stocked rohu. The biofilter media might have the ability in initial days of the experiment to absorb the toxic nitrite as the nitrifying colony takes longer time to establish on the filter bed. Subsequently it was seen that filtered water had low nitrite content and the percentage reduction was very high indicating establishment of bacterial colonies.

Fish was kept in silos for a period of one month. There was no mortality in the three treatments at various stocking densities, indicating the safe environment for rearing of fishes.

From the above study it was concluded that small, medium or large silos based on the requirement of the consumer, entrepreneur or the hotelier adapting a recirculatory system could be established for live fish rearing for a short period. Further it is concluded that biofilter with locally available media such as sand, gravel and charcoal having high growth of nitrification bacteria on them can be fitted to the silos for water cleaning and hygiene

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EFFECT OF IMMUNOSTIMULANT ON ENHANCEMENT OF THE IMMUNE RESPONSE OF KURUMA SHRIMP *Marsupenaeus japonicus* AND ITS RESISTANCE AGAINST WHITE SPOT SYNDROME VIRUS (WSSV)

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ABSTRACT

An experiment of 45 days duration to test the efficacy of the *Vibrio alginolyticus* as an immunostimulant was conducted in *Marsupenaeus japonicus* against white spot syndrome virus (WSSV) infection, considered as one of the serious pathogens of shrimps. The shrimps were fed with the experimental diets coated with different concentrations of bacteria of 17 ml / kg feed and 10 % of animal body wt. They were challenged with WSSV to assess the increase in survival rate and growth if any due to immune enhancement. Another group of shrimp was used for drawing the haemolymph to estimate the increase in the level of prophenoloxidase activity. Samplings for the above said analysis were carried out at 15 days interval for a period of 45 days. The highest prophenoloxidase activity (39.78 with sand substrate-T1) and the highest survival rate (66.66 % with sand substrate-T1) and highest growth rate (7.04 % in 17ml/kg- with sand substrate-T1) were recorded in experimental diet (17 ml / kg feed). Hence the 17 ml / kg feed of *Vibrio alginolyticus* formalin killed added to the diet could be an eco-friendly and economically viable immunostimulant for penaeid shrimps.

Key words : Kuruma Shrimp, *Marsupenaeus japonicus,* Prophenoloxidase activity, *Vibrio alginolyticus,* immunostimulant and WSSV Challenge

INTRODUCTION

Shrimp culture has undergone rapid development in most south-east Asian countries, but successful production is increasingly hampered especially by diseases in addition to environmental pollution and poor management practices. Of all the infectious diseases, bacterial infections cause serious diseases like vibriosis, ulcer disease, tail rot, necrosis etc. Vibriosis, especially luminescent disease caused by *Vibrio harveyi* leads to mass mortality in the shrimp hatcheries (De la Pena *et al*., 1993).

At present Shrimp culture in India has high susceptibility to a plethora of diseases which resulted severe loss. Traditionally, treatment of shrimp diseases was carried out by the use of chemical and antibiotic products. Chemical treatment is intricate mainly due to the huge requirement of chemicals needed to produce the desired therapeutic effect and the after effects of hither to unknown consequences of such treatment to the ecosystem. The fear of development of plasmid mediated resistant strains of bacteria to antibiotics (Gabriel AG and Felipe AV, 2000) and accumulation in the shrimp, restricts continuous use of the antibiotics. However, this indiscriminate and unabated usage of chemicals and antibiotics has led a serious debate amongst environmentalist and governmental agencies for complete ban of these products. These reasons paved way for the development of wide array of strategies in sustaining the shrimp aquaculture. Thus, the concept of biological disease control, particularly using non-pathogenic bacterial strains for disease prevention has received wide spread attention during the last decade (Gram, *et al.*, 1999).

Effective vaccination of penaeid shrimps with a formalin – killed *Vibrio* spp. vaccine has been reported by Kou *et al*. (1989) and Itami *et al*. (1989). However, study on the development of *V.harveyi*

vaccine and its effect on vibriosis in *Fenneropenaeus indicus* was found scarce.In the present study, a more effective way of protecting cultivable penaeid shrimps against vibriosis, by using formalin - killed *V.alginolyticus* as immunostimulant was developed and the relative percent survival (RPS) of immunostimulated against control *Marsupenaeus japonicus* and growth was also investigated.

MATERIALS AND METHODS

Experimental Design

The experiment was conducted at Muttukkadu Experimental Station of Central Institute of Brackishwater Aquaculture, CIBA, Chennai. for a period of 45 days in six distinct experimental groups namely C1, T1, T2 (with sand substrate) and C2, T3, T4 (without sand substrate) with each group having three replicates including controls. Uniform size Fiberglass Reinforced Polypropylene (FRP) tanks of 100 L capacity each were cleaned and disinfected with bleaching powder before starting the experimental trial. The tanks were filled with 70 L of filtered seawater and continuous aeration was provided throughout the experimental period. The juvenile shrimp average weight 4 ± 2 g with 2 nos/m^2 density were stocked in100 L tank capacity. The juveniles of *Marsupenaeus japonicus* from single broodstock were obtained from CIBA hatchery. PCR test was done before the shrimps were used for the experimental trials.

Immunostimulant Preparation

Innoculum of pure *Vibrio spp., viz., V. alginolyticus,* were cultured individually in pre-sterilized alkaline peptone water broth (15g / l, pH 7.4, 1% NaCl), at room temperature for 48 hrs. After 48 hrs, the bacterial preparation was harvested by centrifugation at 8000 rpm for 5 min at 4°C. The supernatant was collected and the pellet was resuspended in sterile Phosphate Buffer Solution (PBS) .The cultures were inactivated by formalin method: Adding 0.5% Formalin to 1 L of bacterial culture (Akhlaghi, 1999). The sterility test of killed bacteria was conducted by inoculating the preparation on TCBS plates and incubating at 37°C for 24 hours. The formalin killed bacterial cultures were diluted individually in PBS to give a series of concentration of 10^5 – 10^9 cells / ml. The solutions were stored in glass bottles at 4°C till further use. However, during the suspension of desired concentration was prepared fresh, mixed with the shrimp feed and sun dried for 2-3 hours before feeding.

The experimental animals were fed with commercial shrimp feed (2mm size). Two experimental diets using commercial feed were mixed with T1 (17 ml (1 x 10^8 cells)/kg), T2 (10% of animal body wt.) (with sand substrate) and T3, T4 (without sand substrate) and each of with formalin killed *Vibrio alginolyticus* bacterial suspension and dried for 1 hour before feeding. The control feed C1, C2 (with sand substrate) and C3, C4 (without sand substrate) were devoid of any bacterial suspension. The juveniles were fed thrice a day at 08.00 hrs, 14.00 hrs and 18.00 hrs at the rate of 8% of the body weight per day. The daily allowance was divided into three equal portions. The rate of feeding was adjusted daily depending on the feed consumptions. Complete (100%) water exchange was carried out in all the tanks everyday. Daily, before water exchange, abiotic parameters – salinity, pH and temperature were recorded. Before water exchange, samples were collected twice a week and estimated for ammonia and nitrite using standard methods.

Parameters for evaluating efficacy of immunostimulant :

Growth Rate

In order to assess the growth performance in the tank, sampling was conducted in the tank regularly with the Ricker, W. E. (1979) methodology. The final weight of the shrimps was taken at the end of the study (45 days); growth percentage and survival percentage (Sung *et al.*, 1994) were calculated using the formulae given below.

$$\text{Growth\%} = \frac{(\text{Final weight} - \text{Initial weight}) \text{ of shrimps}}{\text{Initial weight of shrimps}} \times 100$$

$$\text{Survival\%} = \frac{(\text{Final number} - \text{Initial number}) \text{ of shrimps}}{\text{Initial number of shrimps}} \times 100$$

Prophenoloxidase Activity Assay

Prophenolxidase activity (proPO) in haemocytes of shrimp's haemolymph was measured by the formation of dopachrome from L-Dihydroxy-phenylalanine (DOPA) (Soderhall and Unestam, 1979) on a Multiscan ELISA reader. Prophenol-oxidase activity (proPO) in the haemolymph of *M japonicus* fed with or without immunostimulant was estimated. 500µl of haemolymph was drawn from the ventral sinus (5th pleiopod) of shrimp in a 1000 µl syringe pre-chilled with 0.5 ml of cold anticoagulant solution. The haemolymph was collected in pre-cooled Eppendoff's vials (2 ml). The samples were thawed and immediately analyzed for proPO activity. The haemolymph collected and centrifuged at 5000 rpm for 5 min and the supernatant fluid i.e., haemocyte lysate supernatant (HLS) was separated from plasma protein for determination. The cell suspension was incubated with Cacodylate buffer for 30 min at room temperature. At the end of incubation, the mixture was centrifuged for 3 min at 6000 rpm. The supernatant was collected and estimated for proPO activity.

ELISA Reading

Triplicates of 50µl of haemolymph sample were taken and to which 50µl of trypsin was added and incubated at room temperature. After 20 min of incubation, 50µl of L-DOPA was added and the optical density was measured at 490 nm on an ELISA reader every one minute interval for 5 minutes. Protein concentrations of haemolymph were determined by Lowry *et al.,* (1951), method. To 1 ml of diluted protein standard (BSA) and haemolymph sample, 5 ml of alkaline copper sulphate was added and mixed thoroughly. The solution was put under incubation. After 30 minutes, the blue colour so developed was measured at 640 nm using UV-Spectrophotometer (Shimadzu). Using the standard curve, protein concentration of unknown samples were determined by plotting concentration (mg / ml) of BSA against standard 640 nm . One enzyme activity unit was equivalent to an increase of 0.001 in absorbance. Prophenoloxidase (unit/min/mg of protein) was calculated using the formula.

$$\text{Change in Absorbance} = \frac{2^{nd}-1^{st}}{1}+\frac{3^{rd}-2^{nd}}{1}+\frac{4^{th}-3^{rd}}{1}+\frac{5^{th}-4^{th}}{1}\times 2$$

$$\text{Prophenoloxidase level} = \frac{\text{Change in absorbance}}{\text{Pr otein value of plasma}}$$

Challenge studies

The tank cultured shrimps from both treatment and control tanks were challenged with *V.alginolyticus* as well as White Spot Syndrome Virus (WSSV). The harvested live shrimps from both treatment and control were maintained in troughs (15 l) and acclimatized for 24 hours prior to the challenge test. The infected WSSV shrimp were cut into small pieces and fed at the rate of 3% of the body weight of the shrimps to both groups-treatment and control groups which were maintained separately. After 4 hours of feeding the residual feed and water was removed and replaced with fresh sea water. The shrimps were fed with commercial feed till the conclusion of the experiment. On forty-five days of post vaccination, the Juveniles were challenged with *V. harveyi* ($6.1x10^5$ cfu/ml- challenge dose) and the mortality of the shrimps was recorded for a period of 5 days (Itami *et al.,* 1989). Mortality number and time of death was monitored for 7 days and clinical signs were verified by re-isolation of WSSV from freshly challenged dead shrimp or survivors through PCR tests. Cumulative mortality was registered and percent survival was calculated.

Polymerase chain reaction (PCR) screening for WSSV :

WSSV Isolation and Preparation

Virus used in this study originated from an infected shrimp farm located near Muthukadu, Chennai. The presence of WSSV in infected shrimp was confirmed by PCR analysis of DNA isolated. The WSSV stock

solution was prepared following the method of Tsai *et al*. (2000). Infected shrimp were homogenized in TNE and then centrifuged at 1700xg for 10 min at 4°C. The supernatant was filtered through a 0.45 ìm membrane and was stored at -80°C until used for challenging.

Detection of WSSV

Different parts of shrimp body such as pleopods, gills and tail were cut using a sterilized scissor. The parts were immediately digested using the solubilising buffer (NaOH -100 µl of 0.05 N, Sodium dodecylsulphate solution-0.025%). The contents were thoroughly homogenized with a Teflon homogenizer and the same was placed in water bath at 100°C for 5 min and then centrifuged at 6000 rpm for 5 min. 5 µl of the collected supernatant were transferred to PCR tubes. PCR cocktail reagents were added and the contents of the PCR vials were mixed gently by tapping with the fingers. The amplification of DNA molecules was performed using the PCR program specific to the kit (WSSV detection kit, Genei, Bangalore and CIBA, Chennai) was used.

Statistical Analysis :

The data collected on the different parameters of the experimental study was subjected to statistical analysis by one way analysis of variance ANOVA (Snedecor and Cochran, 1968), the statistical level of significance was tested at 1% and 5% levels.

RESULTS AND DISCUSSION

Effect of immunostimulant on growth percentage

An insignificantly higher ($P \leq 0.05$) growth percentage was observed irrespective of the treatments compared to control groups. The highest growth percentage was recorded in treatment group (T1) compared to other groups over the period of 45 days with the sand substrate (Table 1). There is no significant difference between the control and treatment groups (C1, T1 and C1, T2). Similarly there is no significant difference ($P \geq 0.05$) between the control and treatment groups (C2, T3 and C2, T4) in without sand substrate during the experimental period. The maximum average mean value of growth percentage was found to be 7.033 ± 0.274 in with sand substrate treatment group (T1) compared to other groups.

Table 1 : Growth percentages of Kuruma shrimp *(M. japonicus)* fed with immunostimulant reared with and without sand substrate in 45 days of experimental duration.

Serial Number	Treatment	Tank Nos.	Growth Percentage (Mean ± S.E.)
With sand substrate			
1.	Control	C1	6.149 ± 0.269^{a}
2.	17 ml / kg feed	T1	7.033 ± 0.274^{a}
3.	10 % of animal body wt.	T2	6.676 ± 0.271^{a}
Without sand substrate			
4.	Control	C2	6.472 ± 0.244^{a}
5.	17 ml / kg feed	T3	6.494 ± 0.234^{a}
6.	10 % of animal body wt.	T4	6.665 ± 0.169^{a}

Means not sharing a common superscript letter between column values for each indexes differ significantly ($P < 0.05$).

Effect of immunostimulant on Prophenoloxidase activity

Prophenoloxidase activity observed significant difference ($P \leq 0.05$) between control and treatment groups in with sand substrate (Table 2). The highest Prophenoloxidase activity was recorded in treatment group T1, 39.78 ± 0.365 compared to other groups over a period of 45 days with sand substrate. There is no significant difference ($P \geq 0.05$) between the treatment groups (T3 and T4) but there were significant difference between control and treatment groups (C2, T3 and C2, T4) in without sand substrate during the experimental period.

Table 2 : Prophenoloxidase -Activity in haemolymph of kuruma shrimp *M. japonicus* fed with immunostimulant reared with and without Sand substrate in 45 days period.

Serial Number	Treatment	Tank Nos.	Prophenoloxidase Activity(units / min / mg / protein) (Mean ± S.E.)
With sand substrate			
1.	Control	C1	29.98 ± 0.215[a]
2.	17 ml / kg feed	T1	39.78 ± 0.365[b]
3.	10 % of animal body wt.	T2	38.81 ± 0.317[c]
Without sand substrate			
4.	Control	C2	33.14 ± 0.384[a]
5.	17 ml / kg feed	T3	39.01 ± 1.163[b]
6.	10 % of animal body wt.	T4	39.62 ± 0.342[b]

Means not sharing a common superscript letter between column values for each indexes differ significantly (P < 0.05).

Table 3 : Survival percentages of Kuruma shrimp *(M. japonicus)* fed with immunostimulant reared with and without sand substrate for a period of 45 days.

Serial Number	Treatment	Tank Nos.	Survival Percentage (Mean ± S.E.)
With sand substrate			
1.	Control	C1	38.33 ± 1.123[a]
2.	17 ml / kg feed	T1	66.66 ± 2.842[b]
3.	10% of animal body wt.	T2	50 ± 3.015[c]
Without sand substrate			
4.	Control	C2	41.66 ± 4.578[a]
5.	17 ml / kg feed	T3	58.33 ± 3.859[b]
6.	10% of animal body wt.	T4	56.33 ± 3.859[b]

Means not sharing a common superscript letter between column values for each indexes differ significantly (P <0.05)

Effect of immunostimulant on survival percentage

Survivay percentage showed that shrimps reared with and without sand substrate at the end of 45 days of feeding with immunostimulant had complete mortality (100%) in the 4th day of post challenge in both control groups (C1 and C2) (Table 3). But in with sand substrate treatment groups (T1 and T2) were recorded in the survival of shrimps in the 7th day of post challenge The highest survival was found to be 66.66 ± 2.842 in with sand substrate treatment group (T1) compared to other treatment and control groups. A PCR test was conducted randomly to check whether shrimps have positive or negative WSSV in all the experimental tanks. It was found that all treatment groups showed negative results except without sand substrate control group which showed positive result of WSSV (Fig. 1 and 2).

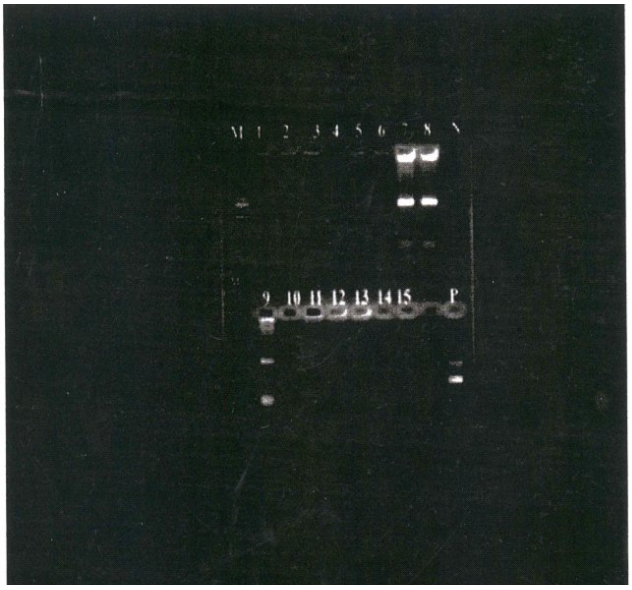

Fig. 1 : Agarose Gel before the Challenge Test from shrimp *Marsupenaeus japonicus* fed with immunostimulant for the period of 45 days.

Lane M-Marker, Lane 1 to 2 -C1, C2, Lane 3 to 6 -T1, T2, T3, T4, Lane 7 to 8-C3, C4, Lane 9 to 11-T5, T6, T7, T8, Lane N-Negative, Lane P-Positive

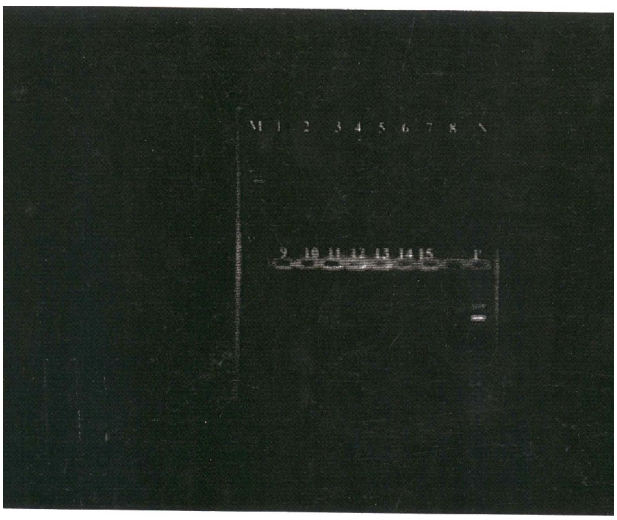

Fig. 2 : Agarose Gel after the Challenge Test from shrimp *Marsupenaeus japonicus* fed with commercial feed for the period of 45 days.

Lane M-Marker, Lane 1 to 2-C1& C2, Lane 3 to 6-T1, T2, T3, T4, Lane 7 to 8-C3, C4

Lane 9 to 11-T5, T6, T7, T8, Lane N-Negative, Lane P-Positive

Different approaches to prevent shrimp diseases have normally involved antibiotics, vaccination against specific pathogens and even different immunostimulants that protect shrimps nonspecifically. Till some years ago scientists believed that shrimps do not possess acquired immunity but recent studies showed that shrimps have acquired immunity and even memory cells that make the vaccinations efficient in these groups of animals (Van Hulten, *et al.*, 2009). In present study we compared two immunostimulants i.e. (a) 17ml / kg feed (b) 10% of animal body wt. in two types of substrates (a) with sand substrate, (b) without sand substrate in Kuruma shrimp, *Marsupenaeus japonicus* looking at its commercial importance. Physiological concentration and the efficacy of immunostimulants were also tested.

The growth percentage was found to be higher in all the treatment groups than the control group. The highest growth percentage was observed in 17ml/ kg feed treatment group T1 (7.033 $\pm$ 0.274) and the lowest growth percentage was recorded in 10% of animal body wt. treatment groups T2 (6.676 $\pm$ 0.271) fed with immunostimulants. In without sand substrate experimental groups, percentage of growth was found to be higher in all the treatment groups than the control group, C2 was 6.472 $\pm$ 0.244 for a period of 45 days. This result is in accordance with study by Montero-Rocha *et al*. (2006); that showed an increase in body weight during 15 days of using Ergosan in intermoult stage shrimp. Similarly, (Sang HM and Fotedar R, 2004) recorded the highest growth of western king prawn, *Penaeus latisulcatus* reared at 34 ppt followed by 22 ppt and 46 ppt. The result obtained in the present study is concurrent with the results obtained by (Muthu MS, 1980) and (Liao IC, 1988). The enhanced survival and growth with immunostimulation suggests that immuno-stimulant elevates the physiological condition and possibly it has an indirect role in the osmoregulation process.

Prophenoloxidase is the key enzyme in the syntheses of melanin. It occurs in haemolymph as an inactive pro-enzyme prophenoloxidase (proPO) and is activated when it reacts with zymosan (carbohydrates for yeast cell walls), bacterial lipopolysaccharide (LPS), urea, calcium ions, trypsin, or heat and converts to active form as proPO. This active proPO is one of the terminal components of a cascade of proteolytic reactions that accompany the recognition of foreign materials in numerous crustaceans and insects (Soderhall K *et.al.,* 1990), (Johansson M W and Soderhall K, 1992) (Nappi A and Vass E, 1993a) and (Nappi AJ and Sugumaran M, 1993b). Results from several experiments of the putative proPO activating system, stimulate several cellular defence reactions, including phagocytosis, module formation, encapsulation and haemocyte locomotion (Soderhall K and Smith VJ, 1986).

At the end of 45 days the prophenoloxidase activity was estimated from the haemolymph in the treatment and control group. All the treatment groups showed higher prophenoloxidase activity than the control groups with sand substrate and without sand substrate. With sand substrate group, highest mean prophenoloxidase activity was recorded in 17 ml / kg feed treatment group T1 (39.78 $\pm$ 0.365) than 10

% of animal body wt. treatment group T2 (38.81 ± 0.317). But the case of without sand substrate experimental groups showed highest prophenol-oxidase activity in 17 ml / kg feed treatment group T3 (39.01 ± 1.163) than 10% of animal body wt. treatment group T4 (39.62 ± 0.342) and followed by the control group C2 (33.14 ± 0.384).

The prophenoloxidase factor as a measure of immune enhancement in penaeid shrimps had been demonstrated in number of studies (Soderhall K, Cerenius L and Johansson MW, 1996), (Vargas-Albores F, Jimenez-Vega F and Soderhall K, 1996), (Perazzolo LM and Barracco MA, 1997), (Devaraja *et al.*, 1998) ;(Karunasagar I and Karunasagar I, 1999a) and (Karunasagar I and Karunasagar I, 1999b), (Felix *et al.*, 2004) (Citarasu *et al.*, 2006) observed that prophenoloxidase value increased significantly in the shrimps fed with immunostimulant, while in immunostimulant deficient control groups, it decreased. In the present study the prophenal-oxidase was high in with sand substrate treatment tanks compared to without sand substrate treatment groups, in 17 ml/ kg feed immunostimulant. But it was noticed that prophenoloxidase activity was less in both the control groups. The prophenoloxidase (proPO) activity of haemolymph, an important enzyme linked mediator of crustacean immunity has been demonstrated to be an effective tool that can be used independently or along with challenge studies for confirmation of immune enhancement pattern in penaeid shrimps. The effectiveness of the Vibrio vaccine as an immunostimulant in penaeid shrimps is comparable to those of other immunostimulants (LPS, chitin and â-Glucan) tested with proPO effects (Felix S and Sivakumar, 2003).

The survival percentage was found to be higher in all the treatment groups than the control group, C1 was 38.33 ± 1.123. The highest survival was recorded in 17 ml/ kg feed treatment group T1 was 66.66 ± 2.842. The lowest survival was recorded in 10% of animal body wt. treatment groups T2 was 50 ± 3.015. The highest survival was observed in 17 ml / kg feed of immunostimulant concentration compared to 10 % of animal body wt. of immuno-stimulant concentration.

Without sand substrate experimental group's survival percentage found to be higher in all the treatment groups than the control group, C2 was 41.66 ± 4.578. The highest survival in 17ml/kg feed treatment group recorded in T3 was 58.33 ± 3.859 and followed by lowest survival in the 10% of animal body wt. treatment group T4 was 56.33 ± 3.859. Compared to with sand substrate the survival was low in without sand substrate. The different parameters of the present work revealed that the immunostimunats are very effective against WSSV infection and the outcome of the results will serve long way towards the sustainable shrimp culture which at present struggling under the impact of disease threat.

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WATER BALANCE STUDY OF TANK CUM WELL SYSTEM IN HIGH RAINFALL REGIONS OF EASTERN INDIA

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ABSTRACT

The tank cum open dug well system is a proven technology for providing reliable irrigation to croplands in the plateau region of eastern India. The system comprises of a series of tanks with open dug wells in the recharge zone of the tank that reharvest back the seepage water. Thus, the rainwater remaining in the tank as well as partial seeped water is used for providing round the year full irrigation. This system was initially evaluated in field in Keonjhar district of Odisha of eastern India with six tanks and five wells in two drainage lines. In the current study the water balance components of the tank cum well system has been evaluated. The water balance analysis showed that there is effective seepage into the tank in the standard week no. 28 to 49. The analysis of piezometric water level data showed higher specific subsurface discharge in the region between the tank and the well indicating that the tank is able to recharge to the downstream well. The pumping test analysis in the open well showed that the yield of the open well varies from 8.74 m^3/day in the month of October to 3.72 m^3/day in the month of April.

Key words: Recirculatory aquaculture system, biofilter, silo, rohu, water quality

INTRODUCTION

Irrigation is a catalyst for improving and stabilizing agricultural productivity which in turn alleviates poverty. Hence there is a strong justification for investing in water resource development for small-scale agriculture. Small holder irrigation is generally accepted to include farmers who irrigate areas up to fraction of a hectare to 10 hectares of land (Albinson and Perry, 2002). In high rainfall regions of eastern India, rainwater management has a potential of being an irrigation water resource which can provide full irrigation in conjunction with rainfall. Several researchers have designed small systems for irrigation management and water resource development based on runoff recycling (Palmer *et al*., 1982; Helweg and Sharma, 1983; Verma and Sarma, 1990). But most of them are for supplementary irrigation and hence their impact on total livelihood scenario is not significant enough to bring them out of vicious circle of poverty. Bhatnagar *et al*. (1996), Srivastava (1996) and Srivastava (2001) have shown that reliable irrigation system through runoff recycling can be designed in sub-humid high rainfall areas of India. The technology was further refined and a micro level water resources development system through rainwater management comprising of tanks and wells was developed. This system was initially evaluated in field in Keonjhar district of Odisha of eastern India with six tanks and five wells in two drainage lines. (Srivastava *et al,* 2009).

In this tank cum well system, the tank is constructed to store runoff water. This tank is able to provide irrigation to transplanted rice in kharif and low duty crops in full command or heavy duty rabi crops in partial command. The seepage loss from this tank as well as percolation loss from the paddy fields is reharvested back from an open dug well downstream the pond. This well serves for providing water for nursery and irrigate additional area during kharif, rabi as well as summer. However, there is a need to study the water balance components of the tank cum well system to completely understand the behavior of the system. In order to carry out the study, the experiment was carried out in Directorate of Water Management research farm by constructing a tank cum well system in a 3.2 ha micro-watershed area.

MATERIALS AND METHODS

A 3.2 ha micro-watershed wasteland area was selected in the Directorate of Water Management research farm at Deras, Bhubaneswar. A 2500 m^3 capacity tank was constructed on the drainage line of the micro-watershed. Inlet to the tank was provided by a 165 mm dia PVC pipe and outlet of the tank was provided in

terms of a drop spillway. An open dug well of 8 m depth and 6 m outer diameter was constructed at about 70 m downstream of the tank. The irrigation facility in the area was developed by construction of underground pipeline system. Four storage reservoirs were constructed at four different elevated locations of the micro-watershed so that majority of area can be covered by irrigation using them. The storage reservoirs were used for initially storing the water and then irrigating the plants by gravity fed irrigation. The water was pumped from the tank and the well, and stored in the storage reservoir through underground pipeline system.

Twelve hundred acacia mensium plants and 150 eucalyptus plants were planted in the waterlogged portion of the land. Four hundred mango plants, 90 guava plants, 2000 pineapple suckers and 3000 lemongrass slips were also planted in the area. The guava, pineapple, and lemon grass were planted as intercrops to the mango plants. The irrigation was done by flexible PVC pipe drawing water from the storage reservoirs by gravity.

Water balance analysis of the tank

The water balance of the tank was studied using the mass balance equation where difference between inflow and outflow is equal to change in storage. The following equation was used for the study.

$$(I + P) – (E + Q + O + S) = \Delta S \text{ ... . (1)}$$

Where, I = surface water inflow into the tank, P = precipitation, E = evaporation, Q = pumping of water from the tank for irrigation, O = surface outflow from the tank, S = seepage losses from the tank and ΔS is change of storage in the tank.

The rainfall and evaporation data were available in the meteorological observatory of the research farm. The surface water inlet to the pond through the pipe and the water outlet from the tank through the weir was monitored regularly. Similarly the water withdrawal from the tank for irrigation in different days was recorded. The change in storage in the tank was found out from the water level in the tanks. The seepage loss from the tank was obtained from the water balance equation as all other components were measured and thus were having a known value.

Groundwater monitoring and analysis

In order to monitor the groundwater level, six piezometers were installed in the study area, two in the upstream side of the tank, two between the tank and the well and two in the downstream side of the well. Periodic monitoring of groundwater level was done in six piezometers. In order to estimate the specific subsurface discharge, hydraulic conductivity was measured at upper, middle and lower reaches. The average hydraulic conductivity was 1.68, 1.65 and 1.68 cm/hour in upper, middle and lower reach. The specific subsurface discharge in three sections was calculated using Darcy's law

$$v = K.i \text{ ..... (2)}$$

Where, v = specific subsurface discharge, K= hydraulic conductivity, i= hydraulic gradient=$\Delta h / \Delta l$.

Pumping tests in open wells

In order to study the monthly variation of well yield, pumping tests were conducted in the open well in different months. After pumping the wells for a sufficient duration, the recuperation of the open well with time was measured. The recuperation rate and cumulative recuperation for each pumping test were analyzed. The well yield in different months were obtained and compared.

RESULTS AND DISCUSSION

Water balance of the tank

The water balance analysis in the year 2008-09 showed that there is an effective seepage into the tank in the monsoon season i.e. from standard week no. 28 to 49 (Fig. 1). It implies that the effects of monsoon existed till the 49th week, upto which the seepage into the tank exceeded the seepage from the tank. From 50th week to 27th week there is a seepage loss from the tank. Maximum effective seepage of 6.17 mm/day into the tank was observed in week no. 45 and maximum effective seepage loss of 6.40 mm/day from the tank was observed in week no. 5. The surface water inlet into the tank started in the week no. 25 at 17 lit/min, reached a maximum of 300 lit/min in week no. 38 and then gradually reduced and became zero from week no. 7 onwards (Figure 2). Generally the surface water inflow into the tank increased with rainfall.

The tank water level along with well water level and rainfall has been plotted and shown in Figure 3. The water level in the tank and well were arrived at considering the reference water level of 25 m of the tank weir. The tank water level varied from a minimum of 24.32 m in week no 24 to a maximum of 25.03 m in week no. 38. The maximum water level in the tank was restricted by a spillway in the form of a weir at the outlet of the tank. The well water level varied from a minimum of 22.18 m in week no. 24 to a maximum of 23.55 m in week no. 38. There was a similarity in trends between the tank water level and well water level and there is an increase in both the water levels in the monsoon season. There was a sudden rise in water level with the start of the rainy season in the week no. 24 to 26. Due to surface water inflow into the tank, there was an immediate rise in water level in the tank in comparison to well.

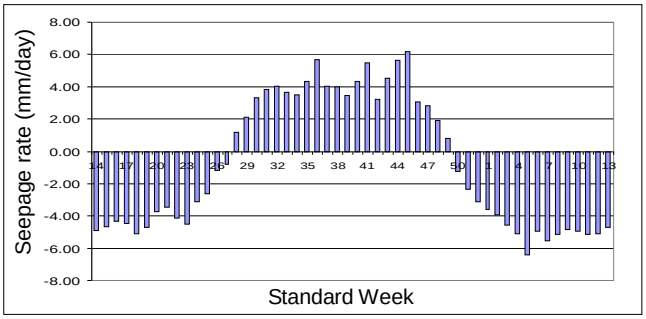

Fig. 1 : Seepage losses from the tank in different standard weeks.

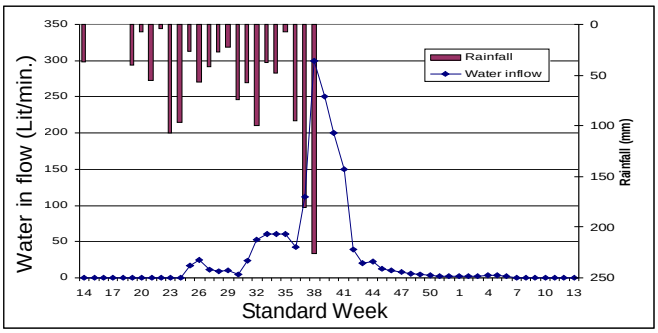

Fig. 2 : Surface water inflow into the pond with rainfall data

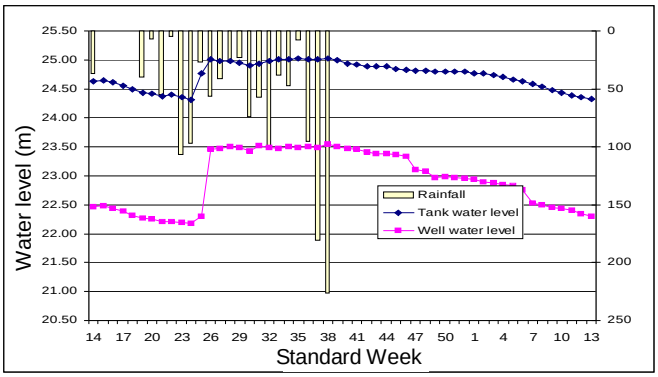

Fig.3 : Water level in the tank and well along with rainfall data

Groundwater level and specific subsurface discharge variation

Figure 4 shows groundwater level in six piezometers in different weeks. Piezometers 1 and 2 lie in the upper reach of the study area, piezometers 3 and 4 lie in the middle reach of the study area and piezometers 5 and 6 lie in the lower reach of the study area. There was lesser variation of groundwater level in the upper reach as this area remains waterlogged due to seepage from upstream dam and canal. As there was a water flow from the upstream side to downstream side, groundwater levels are higher in the upstream side followed by middle reach and lower reach respectively. The groundwater level in piezometer 1 varied from a minimum of 25.51 m to a maximum of 26.14 m, the groundwater level in piezometer 2 varied from a minimum of 25.11 m to a maximum of 25.93 m, the groundwater level of piezometer 3 varied from a minimum of 23.29 m to a maximum of 24.46 m, the groundwater level in piezometer 4 varied from a minimum of 22.75 m to a maximum of 23.80 m, the groundwater level in piezometer 5 varied from a minimum of 21.74 m to a maximum of 23.05 m and the groundwater level in piezometer 6 varies from a minimum of 21.65 m to a maximum of 23.05 m.

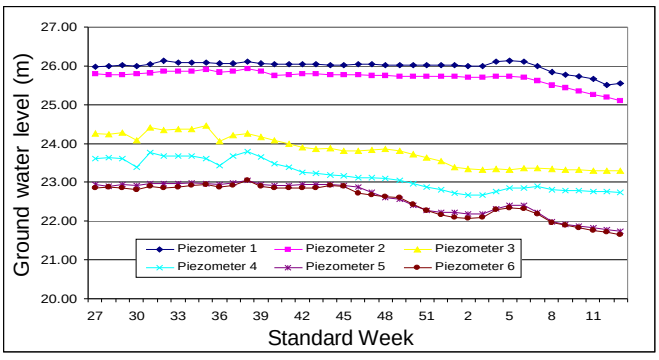

Fig. 4 : Variation of groundwater levels in piezometers

The specific subsurface discharge (v) at three sections in different weeks is shown in Figure 5. The average values of v in upper reach, middle reach and lower reach are 5.82, 10.64 and 1.88 mm/day respectively. The higher specific subsurface dis-

charge in the middle reach indicates that the tank is able to recharge to the downstream well.

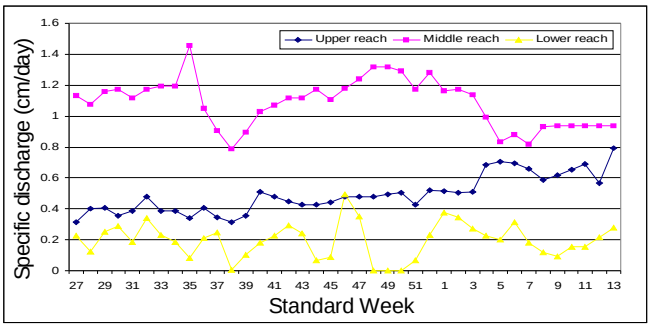

Fig. 5 : Specific subsurface discharge at different sections.

Yield of open well

The variation of yield of the wells in different months is shown in Figure 6. The yield of the open well was 8.74 m^3/day in the month of October. After this it slowly decreased to 5.76 m^3/day in the month of November, 4.7 m^3/day in the month of December, 4.4 m^3/day in the month of January, 4.15 m^3/day in the month of February, 3.92 m^3/day in the month of March and 3.72 m^3/day in the month of April. The yield of the open well has been observed to be on a lower side due to relatively low hydraulic conductivity in the soil profile.

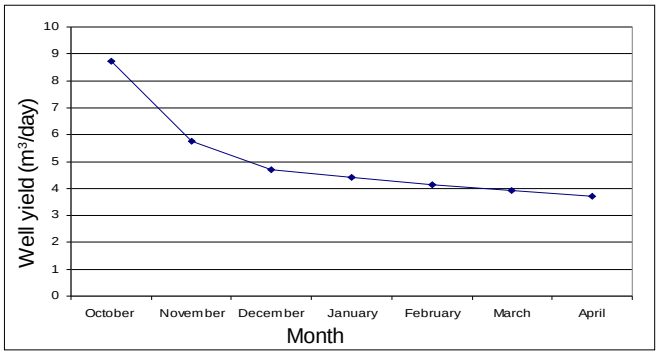

Fig. 6 : Variation of yield of open well in different months

CONCLUSION

The water balance components of the tank system has been analyzed. There is an effective seepage into the tank in the monsoon season i.e. from week no. 28 to 49 and from 50th week to 27th week there is a seepage loss from the tank. The specific subsurface discharge was highest in the region between the tank and the well which indicates that the tank is able to recharge to the downstream well. The pumping tests showed that yield of the open well varies from 8.74 m^3/day in the month of October to 3.72 m^3/day in the month of April.

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SEED PRODUCTION OF ROHU (*Labeo rohita* H.*)* IN FRP HATCHERY IN NUAGAON BLOCK, NAYAGARH DISTRICT, ORISSA

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ABSTRACT

One unit of Fibreglass Reinforced Plastic (FRP) carp hatchery with one breeding pool, three hatching pools, one egg/ spawn collection tank and one cemented overhead tank of capacity 12 cu.m were installed and operated at Nuagaon, Nayagarh District, Odisha in July-August 2010. Two times induced carp breeding were conducted taking Indian major carp rohu, *Labeo rohita* (Hamilton) as the test organism. Breeders were collected from Kuanria Reservoir, Daspalla, Nayagarh District, 22 km from Nuagaon and acclimatized for 15 days in brood pond. During breeding of rohu, 0.8-0.925 lakh eggs/ kg of female, 75-95% fertilization of eggs and 0.46-0.65 lakh spawn/ kg of female were produced from the hatchery operation. The water quality was found suitable with total alkalinity 100-140 mg/l; total hardness 90-120 mg/l; and pH 7.5-8.0. The temperature of 29.2-33.3 ^{0}C during the breeding was within the limit of hatchery operation. Stocking 4.5 lakh spawn that was produced from hatchery operation in 4 earthen ponds of size 0.02 ha each (@ 5.6 million spawn/ha), 1.8 lakh fry was harvested in 15-20 days for restocking in rearing ponds for fingerling production. 0.72 lakh fingerlings were produced after 90 days of rearing from fry stage. The survivability was calculated to be 40% during the rearing period.

Key Word : Carp, FRP carp hatchery, induced breeding, seed production

INTRODUCTION

The technology of induced breeding through hypophysation has helped in mass production of quality carp seed under controlled condition. It has reduced the dependence on the natural seed collection. Quality seed production can be achieved through scientific brood stock management, establishment of hatcheries, refinement of induced breeding techniques and rearing of seed across the country. The long way contribution of several researchers from hapa breeding to cemented eco-hatchery, and then to portable carp hatchery has made easy in availability of stocking material for aquafarming. For easy accessibility and timely production of quality seed, "ICAR-All India Coordinated Research Project on Application of Plastics in Agriculture" Center at CIFA has designed and developed fibreglass reinforced plastic (FRP) carp hatchery, which can be transported from place to place for the stated purpose (Mohapatra *et al.,* 2003, 2004, 2005, 2007, 2008; CIFA, 2004; ICAR, 2005; Sarangi *et al.,* 2008). The innovation of portable FRP carp hatchery has added a feather to the blue revolution in the country by producing fish seed at the farmers' field. The unit consists of four major parts *i.e.,* breeding-cum-spawning pool, hatching-cum-incubation pool, egg-cum-spawn collection chamber and overhead water storage tank. The system is so designed that it creates an environment suitable for fish breeding in the field conditions for 10-12 kg of carps in one operation. In one run, up to 1.0-1.2 million spawn can be produced from the system which is sufficient to stock water area of about 30 hectares. The "FRP hatchery for carp seed production" has been released as a technology package by CIFA in 2004 and ICAR in 2005 and 2006.The transportation of carp seeds from different far off places to the farm sites involving substantial cost is getting reduced by introduction of this hatchery. The hatchery unit has some benefits *viz*., it is portable, easy to install and operate, consumes less quantity of water during fish breeding and seed production, needs less space for installation and the

product is durable for about fifteen years (Mohapatra *et al.,* 2005). One complete run of the hatchery requires minimum water quantity of 90 cu.m. (Mohanty *et al.,* 2009). In lean season, the system can be used for ornamental fish rearing in which village women may take part in leisure time. The unit can be operated by pre-trained unemployed youth, progressive and resource-rich farmers, persons of Gram Panchayat and Cooperative societies on self-operational or rental basis.

MATERIALS AND METHODS

Brief description of FRP carp hatchery installed at Nuagaon, Nayagarh District :

One unit of FRP carp hatchery was installed at Nuagaon, Nayagarh District, Orissa in July 2010. It consists of four parts *i.e.,* breeding pool (1 no.), hatching pool (3 nos), egg collection tank (1 no.) and cemented overhead tank (1 no.) of capacity 12 cu.m.

Breeding pool

The breeding pool is cylindro-vertical in shape with 2.15 m diameter, 0.9 m height, 1:22 bottom slope and 3409 l water holding capacity (operation capacity: 2950 l). The wall thickness varies between 4.2 and -6.0 mm. Water supply to the pool comes from the overhead tank placed at a minimum height of 3.3 m from the hatchery floor. To provide water circulation inside the breeding pool, 5 numbers of 15 mm diameter rigid PVC elbows, carrying nipples fitted in the same direction. A single point water inlet of 25 mm diameter is also fitted at the sidewall of the pool bottom. All the water inlet pipes are inter connected and fitted with individual full-way valves to regulate the flow of water. One shower is provided at the top of the pool for better aeration. The flow rate during egg collection was maintained 1-1.5 l/sec. The installed system is suitable for breeding of 10-12 kg of carps per day in single operation.

Hatching pool

The hatching or incubation pool is of 1.4 m diameter, 0.98 m height, 1400 l total volume and 1200 l net egg incubation volume with a FRP inner chamber (0.4 m diameter and 90 cm height covered with nylon bolting cloth of 0.25 mm mesh to filter the excess water to the drain), water supply system through six numbers of 15 mm diameter duck-mouths fitted at the bottom of the hatchery at 45^{o} angle. It also has drainage outlets fitted at the center and also at the outer chamber of the pool. It has the capacity of hatching 1.0-1.2 million eggs per operation. The flow rate in the pool during operation was maintained at 0.3-0.4 l/sec.

Egg / spawn collection tank

The egg / spawn collection tank is rectangular with size of 1.0 x 0.5 x 0.5 m and water holding capacity of 250 l. The water level in the tank during operation was maintained at a height of 0.45 m (net water volume 225 l) by fixing the drainpipe of 63 mm diameter at a distance of 38.7 cm from the bottom. Cotton inner hapa of the tank size was fixed inside it to collect egg/ spawn from breeding/ incubation pool, respectively.

Water storage tank

Cemented rectangular water storage tank of size 3 x 3 x 2 m is installed to supply water to the hatchery unit. Breeding pool and hatching pool are connected to water storage tank together in the same water line. One 1.0 HP pump set is used to fill the storage tank periodically to supply water to hatchery continuously.

RESULTS AND DISCUSSION

On-site operation and demonstration of FRP carp hatchery at Nuagaon

To develop the socio-economic conditions of some selected Schedule Caste / Schedule Tribe population, a Department of Biotechnology (Government of India)- sponsored project has been implemented through CIFA in Nayagarh District of Odisha since July 2010. A total of 19 fish farmers have been adopted under the project at Nuagaon area. Pond area of 6.13 ha is adopted under the project in Nuagaon for brood fish culture and seed rearing demonstration. The main objective of the study is to produce locally and supply quality carp seed to the aquaculturists of the area. This hatchery for carp seed production is the first of its kind in the district.

Indian major carp, rohu (*Labeo rohita*) breeding trials were undertaken for the first time in Nuagaon Block of Nayagarh District for production and supply of seed to the adopted farmers. The hatchery was operated by a Self Help Group (SHG), named Pitabali SHG, Harijansahi, Nuagaon and the carp breeding started on 28 July 2010.

Fifteen days prior to the breeding programme, 30 kg brooders were collected from Kuanria Reservoir, Daspalla, Nayagarh District which is 22 km from Nuagaon. Brooders were transported in water drums and stocked in a pond covering water area of 0.2 ha near the hatchery unit. Approximately twenty percent of the pond area was having more than 1.0 m water depth. Prior to breeding, the fishes were fed with locally available groundnut oil cake and rice bran (1:1) @ 2-3% body weight in the reared pond.

1st Hatchery operation

On 28 July 2010, male and female brooders of *L. rohita* were caught from the brood pond early in the morning, transported to the hatchery in hammock and conditioned for one hour in the breeding pool prior to administration of inducing agent, Ovaprim @ 0.5 ml/ kg body weight to female and 0.2 ml/ kg body weight to male. Five females and equal number of males with average weight of 1000g and 1060g respectively were injected with hormone and released into the breeding pool. Normal hatchery operation practice was followed for production of carp seed (Mohapatra *et al.,* 2005, 2007, 2008). After 355 minutes of injection, 4 lakh fertilized eggs were collected in the hapa of the collection tank and were released into the hatching pool for incubation. Water temperature of the hatching pool varied from 30.4-33.3 ^{0}C and pH 7.2-7.6 during entire operation period. After 10 hours from egg release, hatchlings passing through the twitching stage were first observed in the hatching pool. Water circulation was maintained as per recommendation (Mohanty *et al.,* 2009) in the pool for the next three days and the pool was disinfected by sprinkling 5 ppm potassium permanganate solution at an interval of every two hours. After 72 hours of egg hatching, 3.25 lakh spawn were collected from the hatching pool through the spawn collection tank. The collected spawn was released into pre-prepared nursery ponds for further rearing (Table 1).

Table.1: FRP carp hatchery operation data from Nuagaon, Nayagarh District

Parameters	1st rohu breeding	2nd rohu breeding
Number of male brooders	5	3
Number of female brooderd	5	3
Total weight of male brooder (kg)	5.0	3.5
Total weight of female brooder (kg)	5.3	2.7
Dose of inducing agent given to male brooder (ml/ kg body weight)	0.2	0.2
Dose of inducing agent given to female brooder (ml/ kg body weight)	0.5	0.5
First egg released (latency period) (A) (min)	305	265
Egg release ended/ended (B) (min)	355	335
Effective spawning period (B-A) (min)	50	70
Egg collected (lakh)	4.25	2.5
Fertilization rate (%)	90-95	75-80
Spawning fecundity (egg release per kg female body weight) (lakh)	0.80	0.925
First Hatchling observed (hr)	10	11
Time of yolk absorption from hatching (hr)	65	68
Spawn recovered (lakh)	3.25	1.25
Spawn production per kg body weight (lakh)	0.65	0.46

2nd Hatchery operation

The hatchery was operated for the second time on the next day of 1st breeding operation, *i.e.*, 29 July 2010 to demonstrate the technology to the local farmers through a training programme. 38 persons including 18 ladies attended the programme. Three male and three female brooders of *L. rohita* with an average weight of 1165g and 900g respectively were injected with Ovaprim. Egg release was started after 265 minutes from the time of injection to brooders and completed by 335 minutes. A total of 2.25 lakh eggs were collected for incubation. Fertilized eggs were transferred to the hatching pool and hatchlings were first observed after 11 hours of fertilization. Water temperature of the hatching pool varied from 29.2-32.6 ^{0}C which was less than the 1st breeding operation, but pH ranged between 7.1 and 7.8. On fourth day of hatchery operation 1.25 million spawn were collected and released in the nursery ponds for rearing and fry production.

One complete cycle of fish seed rearing practice was demonstrated with hatchery-produced rohu spawn at Nuagaon, Nayagarh District during July-August 2010. 4.5 lakh spawn was stocked in 4 earthen ponds of size 0.02 ha each (@ 5.6 million spawn/ ha) for harvest of fry in 15-20 days. 1.8 lakh fry was harvested for restocking in rearing ponds for fingerling production. 0.72 lakh fingerlings were produced after 90 days of rearing and stocked for yearling production in the same farm. The survivability was calculated to be 40% during the rearing period. Normal seed rearing practices, an aquaculture technology of Indian Council of Agricultural Research were followed for production of the end product.

Odisha Watershed Development Mission (OWDM) conducted fish breeding operations in FRP carp hatcheries under the project "Western Odisha Rural Livelihood Project (WORLP)" at Nuapada and Bargarh Districts (Sudhin, 2007; Alan Casebow, 2008). In its first year of operation in 2005, the hatchery supported 38 SHGs to nurse 5.5 million fish seeds, which in turn led 153 SHGs to take up grow-out culture of fish in 530 ha of pond area in Western Odisha (Sudhin, 2007). Due to brooder shortage in the area, in the present study, the hatchery could not be operated to its fullest capacity. But, the present hatchery operation revealed that, breeding of carps can be undertaken successfully in that area. The water quality and temperature regime were within the limits of hatchery operation. Breeder transport from a reservoir 22 km away from the hatchery, rearing them in a less deep pond prior to breeding produced only 0.8-0.925 lakh eggs/ kg of female, 75-95% fertilization of eggs and 0.46-0.65 lakh spawn/ kg of female during the hatchery operation. According to Sarangi *et al.* (2008) at Tanar village, Odisha the FRP hatchery could produce similar results in rohu. This carp breeding technology through FRP carp hatchery installation and demonstration has been transferred in 25 states of the country.

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INFLUENCE OF ENVIRONMENT AND ECOLOGICAL FACTORS IN PRECIPITATING ZOONOTIC DISEASE WITH SPECIAL REFERENCE TO ANTHRAX IN THE STATE OF ODISHA

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ABSTRACT

An impact study of zoonotic diseases with reference to anthrax was undertaken to ascertain the impact on human and livestock health and the environmental and ecological factors precipitating such diseases and vice versa. The overall incidence of Anthrax in man and animal was found to be 2.437 to 6.78 per cent respectively. Environment, agro-climatic conditions, forest, pasture land etc. at times play a major role in precipitating out such diseases. The high rainfall, humidity and excessive dry heat during summer helps in propagation of vectors as a result the incidence of vector borne diseases is always high and seen in the state round the year.

Key Words : Anthrax, zoonotic disease, human and livestock health, environment, ecology

INTRODUCTION

Since time immemorial man has always depended on animals, as source of his basic needs viz. food, transport, animal power (labor), Bio-fertilizers, house and a faithful as well as useful companionship. However countless species of animals are sources of viral, bacterial and parasitic diseases transmitted in many ways, including direct contact from them or air/water/soil contamination to human beings. Thus, the science of zoonoses emerges as one of the most important subject of- medical science. Zoonoses are the infection of bacterial, viral, ricketsial, parasitic or any such pantheons that are naturally transmitted directly or indirectly between vertebrate animals/birds to human beings. Some agents cause diseases both in man and animal host and some others are commensal in animal host. An increase in emergence and re-emergence of infectious diseases is evident in many parts of the world (Weiss and McMichael, 2004). Many of the emerging diseases are zoonoses. Most new human pathogens reported in the last 25 years have zoonotic origin and the risk of zoonotic infection is predicated to continue to increase (Brownile et al., 2006). Over the years, these diseases, with their reservoirs in domestic and wild animal and birds in many countries have been imposing serious threat especially among the vast number of people living and working in rural areas. Apart from causing human suffering, morbidity and mortality, they hamper agricultural production, decrease availability of food and create barriers to international trade. Further, the leather industry, fertilizer industry, poultry industry and other related industries sustain a great loss.

Anthrax is a dreaded and rare infectious disease caused by the bacterium *Bacillus Anthracis*. Anthrax is occurring sporadically in some areas in domestic cloven-footed animals, especially cattle, buffalo, sheep, goats and pigs. Wild animals are also affected. It also occurs in human when they are exposed to the bacterium, usually through handling animals or animal hides and skin. It is most common in agricultural regions, where it occurs in animals. When anthrax affects humans, it is usually due to an occupational exposure to infected animals or their products. There is also possibility of infection of cattle by human beings.

Keeping the above broad heads of zoonotic disaster in the changing interface of environment, socio-economic dynamics and cultural habits of man coupled with global warming, diversified and ever changing agro-climatic conditions and metrological factors, an exhaustive study is undertaken to ascertain the influence of environment and ecological factors in precipitating zoonotic disease with special reference to anthrax in the state of Odisha.

MATERIALS AND METHODS

The study was undertaken over a period of 5 years from 2000 to 2005. During this period the data available at various Medical and Veterinary Institutions

were analyzed statistically. Besides, field survey was undertake, in endemic areas as well as reported out break area from field. Different morbid material such as blood, paired sera samples were collected and examined in the laboratory following the standard procedures of medical protocol. In animal for detection and diagnosis of anthrax the collected blood smear slides were examined with methylene stain where as differential diagnosis of other bacterial diseases were done with Grams Stain. Hemoglobin was estimated by Sahali Method. Wherever human beings or animal were encountered with the disease, they were treated systematically with specific drugs. Medical practitioners' help were taken when human patients were treated.

RESULTS AND DISCUSSION

Anthrax in Odisha is endemic to the state for most of the districts. The precipitating factors for cause of anthrax in Odisha are many. Altogether 112 independent variables comprising of 4 morning (medium and high cloud levels, dew point and relative humidity) one evening (wind capacity), 4 metrological parameters measured in 24 hours interval (rainfall, maximum temperature, cold, evaporation rate) along with one agro-ecological parameter (soil depth) and 2 population profile parameter (cattle and buffalo population) are having high significance in precipitating anthrax disease in addition to these, 13 independent variables comprising of 9 metrological, 3 agro-ecological and 1 population profile had low significance in precipitating anthrax disease. Six variables had no significance in precipitating the disease. This finding closely agrees with the observation of the findings of Rajsekhar, 1998.

Anthrax in Odisha is seen more commonly and most frequently in the districts namely Koraput, Rayagada, Sonepur, Kalahandi, Malkangir, Cuttack, Baragarh, Sambalpur, Bolangir, Nayagarh where as the occurrence of anthrax in other districts are recorded at long intervals. The diseases are seen affecting cattle, buffalo, sheep and goat most commonly and in pigs very rarely. Over a period of 10 years from 1995-96 to 2004-05 only 340 anthrax outbreaks were recorded from all the 30 districts with only 1260 attacks. During the year 1997-98 and 1999-2000, Anthrax outbreak was recorded from this district like Rayagada, Koraput, Malkangiri, Kalahandi and Sonepur killing many human lives as well as animal lives. The morbidity rate recorded in affected population was 2.437 % and within the population of the affected area was 6.78 %. The mortality rate recorded in affected population was 100% and in the population of the districts was 0.024%(Report of ADRI, Odisha 1997/2007). Season wise incidence was recorded to be 16.66 % in summer season, 40.00 % in rainy season and 58.33 % in winter season (Table-1). Similar is the observations of earlier workers Banjha 2008, Annual Report, Anon (2003 and 2008).

Although the state has some larger endemic pockets for anthrax, these are in the dry and southern and western district of Odisha other than the coastal districts. Out of the 46 variables of the state for livestock diseases which includes 8 metrological parameters comprising of low cloud levels, relative humidity, wind, station pressure and the total rain measurement in addition, the rain day parameter measured at 24 hrs interval have very high significance in precipitating this disease. Further, six metrological parameters comprising of morning parameters, wind capacity, wind speed, total rain, evening high cloud level, thunder and minimum temperature parameters measured at 24 hrs along with agro-ecological parameter, climate and having high significance in precipitating the disease. Similar observations were made by Rajsekhar 1998.

Anthrax in Odisha is a disease of man and animals. In the animal group cattle; buffalo, sheep, goat and pigs are most susceptible animals. The disease is seen in the state round the year in the aforesaid animals. The state is broadly divided in to four zones as per the intensity of attack viz. (i) high endemic zones (ii) medium endemic zones (iii) moderate endemic zones (iv) low endemic zones.

Table 1 : Season wise incidence of anthrax in affected districts of Odisha

Sl.No.	Season	Incidence of Anthrax in Percentage
1	Summer	16.66
2	Rainy	40.00
3	Winter	58.33

The morbidity rate due to anthrax in animals was 1.45% and the mortality rate was 12.56 % with case fatality rate of 30.20 %(Table-2). This is in close agreement with the findings of Animal Disease Research Institute, Phulnakhara, Cuttack 2008.

Indigenous cattle and goat population were found to be more affected. The mortality rate in different species of livestock was found to be 6.70%, 31.32%, 21.40%, 52.38%, 60.93%, 93.33%, 58.26% and 66.66% in indigenous cattle, crossbred cattle, exotic cattle, buffalo, sheep, goat and pigs, respectively (Table-3).

Table 2 : Number of out breaks and number of attacks in livestock of Odisha due to anthrax

Sl. No	Year	No. of out Breaks of Anthrax	No. of Attacks	No. of animals affected	No. of animals died
1	1997-1998	35	152	275	87
2	1998-1999	28	98	128	45
3	1999-2000	30	121	185	43
4	2000-2001	42	143	289	76
5	2001-2002	31	112	202	84
6	2002-2003	29	84	98	57
7	2003-2004	20	94	102	59
8	2004-2005	34	112	224	58
9	2005-2006	32	102	78	32
10	2006-2007	36	105	340	120
11	2008-2009	23	131	301	102
	TOTAL	340	1260	2172	656 (30.20%)

Table 3 : Different species of animals affected due to anthrax

Sl. No	Species of animals Affected	Number of animals Affected	No. of animals Died
1	Non descriptive Cattle	702	47 (6.70%)
2	Indigenous	249	78 (31.32%)
3	Cross Bred	598	128 (21.40%)
4	Exotic	21	11 (52.38%)
5	Buffalo	128	78 (60.93%)
6	Sheep	105	98 (93.33%)
7	Goat	357	208 (58.26%)
8	Pig	12	8 (66.66%)
	TOTAL	2172	656 (30.20%)

To evaluate the present findings less literatures were found as references. Further, no systematic and scientific epizootiological studies have been undertaken in the field of anthrax. But there are notable studies in the field of medical science where the observations made closely agree with the present findings. Thus taking both the fields under life science as a major subject the epidemiology of anthrax in man may best fit to the epizootiological of animals.

SIGNIFICANCE OF THE STUDY

The great changes of the last decade, especially the increasing urbanization, most of which is inadequately planned, large movements of populations, opening up of badly needed new areas for food production, the increasing trade in meat, milk and other products of animal origin, the vastly increasing number and speed of vehicles and even tourism have contributed to making the problem of zoonoses not only rural and characteristic of defined areas but regional and in some cases, global. Close association between human and animals, consumption of un-pasteurized milk and dairy products, intensification of animal production and increased trade of animals and animal products, the large number of stray dogs, illegal slaughtering and inappropriate waste disposal are some of the principal factors perpetuating infection in humans.

Besides, environmental factors have played an important role in the spread of zoonoses and the environment itself has suffered, through the alteration of ecological conditions. Increased incidence caue pollution of the environment, not only through biological factors due to their agents and dangerous vectors, but also through adoptation of many control measures, such as the widespread use of pesticides. It has got far-reaching consequences in so many biological cycles, and which have negatively affected human health and well-being. Environmental conditions and their impact on health are strongly linked to demographic and socio-economic development and these pressures have got significant impact over the environment. The health transition accompanying development and socio-economic changes has been described as a transition from traditional to modern rises (Smith, 1999; WHO, 1997; and Frank, 1991)

Environmental impact of diseases in order of magnitude between least developed and most developed regions is greater as exemplified by the disease burden in the sub-Saharan region which is known to be much higher than the mean values in the developing world. The rural/urban differences or the difference for high risk communities even within one nation are likely to be important. (Murray and Lopez, 1996; Smith, 1999; De Hollander, 1999)

Mc Michael *et al.* (1998, 1999) proposed scenario based forecasting of health impacts addressing global environmental changes such as climate change, the depletion of fresh water supplies or food

producing systems or the accumulation of pesticides. More generally, they recommend extension beyond proximal, individual level risk factors and application with large scale social ecological systems perspective.

So, it is a tree menace both for human and animal health planners as there are reports for loss of life in the affected areas as the disease has zoonotic importance. There is a lot of impact on the civil society during the infection and large scale casualties in human beings. Human health and the surrounding environment are intimately interrelated. Over past decades, environmental quality has declined significantly in many geographical regions, increasing both the severity and complexity of numerous human health problems (Foster, 1994). Environmental quality and human health will be greatly affected in the future by climate change.

Under the socio-economic bracket, many studies have dealt with the economics of climate change. The most important aspect of the economic debate is determining the costs of halting climate change for government and industry (Westell and Bowman, 2002; Shogren, 1999; Jaccard *et al.*, 2002). Little attention has been paid to the conscious and unconscious responses made by households and communities to the gradual changes in their environments and the costs that are incurred at these levels of analysis. To date, social scientists have been largely underrepresented in climate change debate and discussion.

A great deal of climate change research has focused on the potential and actual health impacts experienced by individuals, communities, regions and nations (Martens and McMichael, 2002; McMichael *et al.*, 2003). The health impacts of climate change will be influenced by interactions with other determinants of health, and by the coping and adaptation strategies adopted to reduce the range of health risks and threats (Anon 2001). Climate change affects health both directly (death and injury in floods and storms) and indirectly (changes in range of disease vectors). Climate change is one aspect of the physical environment influencing health as part of the web of causation influencing human well being.

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Fig. 1 : Closure view of an anthrax lesion of human affection

Fig. 4 : Anthrax lesions seen in fingers of a man in Rayagada district of Odisha

Fig. 2 : A tribal woman of Koraput suffering from anthrax

Fig. 5 : Severity of human affection - Typical anthrax lesions over fingers of a man at Koraput, Odisha

Fig. 3 : A man suffering from anthrax in Malkangiri district of Odisha

Fig. 6 : Cutaneous lesion in thumb of a man in Rayagada district of Odisha due to anthrax

INFLUENCE OF TEMPERATURE VARIATION ON SOME QUANTITATIVE TRAITS OF ECO-RACES OF *Philosamia ricini H.* (LEPIDOPTERA : SATURNIIDAE)

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ABSTRACT

During the last few years, seasonal temperatures have increased at a much faster rate and global climate models predict further increase in both frequency and intensity. Such rapid increase during the growth stages, particularly during spinning stages of Eri silkworm (*Philosamia ricini Hutt*) leads to reduced values in some quantitative traits like silk ratio, cocoon weight, pupal weight, shell weight, effective rate of rearing and total silk production. Field experiments and lab analyses were carried out using the commercially most exploited eco-races of *P.ricini,* i.e. Borduar, Titabar and Mendipathar to study the effect of a wide range of temperature variations in the year 2009- 2010. Studies conducted for a period of one year revealed that temperature plays a significant role on some commercially important quantitative traits. Correlation between temperature and quantitative traits is also established. Higher temperatures during summer had a significant negative effect on silk yield ($p < 0.01$) and total silk production ($p < 0.001$). Lower temperatures gave significantly better yield and quality ($p < 0.01$).

Keywords: Temperature, quantitative traits, *Philosamia ricini*, eco-races

INTRODUCTION

Erisilkworm *Philosamia ricini* Hutt. (Lepidoptera: Saturniidae) is a commercial silk producing insect which is believed to have originated in Brahmaputra valley (Jolly *et al.*, 1979). Eco-races of *P.ricini* that are commercially exploited are indigenously present in different regions in North-East India. They exhibit wide variations in morphological and quantitative characters such as larval weight, absolute silk content, cocoon weight, shell weight and silk ratio (Singh *et al.*, 2001). Out of eight eco-races identified, the three recommended eco-races for commercial rearing are Borduar, Titabar and Mendipathar (Chakravorty, 2005). Recently these eco-races are gaining importance due to their high silk yield and various literatures are reporting rearing performance of these eco-races in their respective states. The present investigation was undertaken with a view to understand the influence of temperature on important economic quantitative traits of this silkworm eco-races which may enable us to know their adaptability to the fluctuating climatic conditions of the state of Odisha.

MATERIALS AND METHODS

Eco-races of *Philosamia ricini* namely Borduar, Titabar and Mendipathar were collected from Central Sericultural Research and Training Institute, Central Silk Board, Berhampore, West Bengal. Rearing of these Eri worms was conducted through the year 2009-2010, covering five seasons i.e. summer (April-June), Rainy (July-August), autumn (September-October), winter (November- December) and spring (February- March) in the Ericulture lab, School of Life sciences, Sambalpur University and in the field in Sundergarh district of Odisha as per Sarkar *et al.*, (1980). The same procedure of rearing and grainage practices was applied in both field as well as in the laboratory. Rearing and grainage experiments were conducted with five replicates of 50 larvae selected randomly, each in separate bamboo trays to maintain purity of the eco-races. Cocoon harvesting was done after fifth and sixth day of spinning. Commercial traits like cocoon weight (g), shell weight (g), shell ratio (%), pupal weight (g) and Effective Rate of Rearing (ERR) (%) were recorded. Daily temperature was recorded in the laboratory. The data from field and laboratory were collected together and were recorded as mean of five independent replicates (± S.D.) and were subjected to correlation analyses. To test the

effectiveness of these eco-races for commercial rearing in the state the total silk production (g) or otherwise as the raw silk recovery was also calculated for purely theoretical purpose and its correlation with temperature variation was calculated.

RESULTS AND DISCUSSION

The data pertaining to the five quantitative characters in different seasons are given in Table 1. The biotic factors of the rearing period are given in Table 2. The correlation coefficient (r) for the relationship between different quantitative traits of the three eco-races is given in Table 3. The data reveals that all the quantitative values were highest in winter followed by that of spring, autumn, rainy and summer. Quantitative traits were highly significant and had negative correlation ($p < 0.01$) with temperature effects. At very high temperatures (beyond 45° C) there was increased mortality of the worms indicating their inadaptability to high temperatures. Mortality was also observed in rainy season due to sudden fluctuations of temperature experienced during this period. At very low temperature (< 14 ° C) during winters in Odisha, the cocoon performance of these eco-races was found to be poor which was due to low degree of feeding by the larvae, reduced rate of digestion and leaf to silk conversion efficiency. The performance of these eco-races was optimum around 21° C and expressed significant values for all the quantitative traits like cocoon weight, shell weight, pupal weight, silk ratio , effective rate of rearing (ERR%) and total silk production.(Figures 1-18).

It is a well established fact that phenotypic expressions of genotypes for various quantitative traits are not consistent in all the environments (Rao *et al.,* 2003). It was Harada (1961) who demonstrated a high degree of phenotypic traits with regard to various economic traits in the silkworm during different seasons of the year. Variable gene frequencies at several loci in different silkworm eco-races make them to respond differently to changing environmental conditions. The dynamic conditions prevailing in different seasons of the year bring profound changes in growth and development and expression of economic characters in different silkworm races (Kumar *et al.*, 2010). The winter season characterized by low temperature(average 21 °C) and relatively constant temperatures with little rainfall proved to be the best season for commercial rearing of these eco-races followed by spring and autumn seasons characterized by relatively low and constant temperatures. Rainy season characterized by sudden fluctuations in temperatures and high rainfall did not prove to be a good period for rearing of these eco-races in contrast to the results of Kumar *et al.,* (2010), who reported monsoon period to be a good period of rearing in the state of Uttar Pradesh. The summer season characterized by very high temperatures and relative scarcity of major food plants for these eco-races i.e. Castor (*Ricinus communis*) and showed minimum quantitative production and was associated with lower ERR as well as lower weight of cocoon and shell. From the present study, it can thus be inferred that higher temperatures during summer had a significant negative effect on silk yield ($p <0.01$) and total silk production ($p< 0.001$) and relatively lower temperatures (21° C) gave significantly better yield and quality ($p< 0.01$).

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Table 1 : Seasonal findings of commercial traits of three eco-races of *Philosamia ricini*

Eco-race	Seasons	Cocoon Wt (g)	Pupal Wt (g)	Shell Wt (g)	Shell Ratio (%)	ERR (%)	Total silk production(g)
Borduar	Summer	3.2±0.1	2.7±0.1	0.516±0.01	15.75±0.68	94.98±0.31	48.96±1.21
	Rainy	3.34±0.05	2.79±0.06	0.54±0.013	16.36±0.61	96.1±0.08	52.65±1.08
	Autumn	3.47±0.08	2.89±0.07	0.58±0.039	16.77±1.1	96.9±0.059	56.39±3.8
	Winter	3.79±0.01	3.06±0.08	0.73±0.06	19.26 ±1.0	97.88±0.13	71.64±3.97
	Spring	3.5±0.07	2.87±0.07	0.65±0.01	18.45±0.34	96.18±0.08	63.35±0.856
Titabar	Summer	3.11±0.02	2.6±0.02	0.428±0.01	13.72±0.85	94.0±0.032	42.3±1.55
	Rainy	3.26±0.06	2.8±0.15	0.46±0.02	14.092±0.536	95.12±0.01	46.64±2.41
	Autumn	3.36±0.17	2.85±0.07	0.51±0.01	15.31±0.81	96.27±0.01	50.06±0.7
	Winter	3.49±0.08	2.91±0.01	0.578±0.01	16.549±0.52	96.96±0.02	56.24±1.86
	Spring	3.41±0.06	2.86±0.06	0.54±0.008	16.07±0.55	95.76±0.64	53.04±0.9
Mendipathar	Summer	2.864±0.07	2.616±0.08	0.248±0.0083	8.6±1.1	94.28±0.39	23.36±3.14
	Rainy	3.03±0.11	2.7±0.126	0.332±0.0083	10.95±1.3	94.52±0.02	31.38±3.75
	Autumn	3.23±0.03	2.8±0.04	0.44±0.0083	13.71±1.5	95.26±0.03	42.27±5.12
	Winter	3.39±0.03	2.9±0.06	0.53±0.013	15.69±1.2	95.48±0.08	50.79±3.64
	Spring	3.27±0.05	2.82±0.036	0.48±0.011	14.62±1.34	95.45±0.01	45.79±4.86

Table 2 : Biotic factors of the study site during 2009-2010.

Study Parameters	Summer (Apr – Jun)	Rainy (Jul – Aug)	Autumn (Sep – Oct)	Winter (Nov – Jan)	Spring (Feb – Mar)
Average Temp(°C)	42.3	36.4	32.1	21.22	27.0
Relative Humidity (%)	70.1	81.3	78.4	73.5	71.9
Total Rainfall (mm)	264.18	512.26	491.17	76.33	21.14

Table 3 : Correlation coefficient (r) for the relationship between different economic quantitative traits of the three eco-races and temperature.

Quantitative trait	Borduar	Titabar	Mendipathar
Cocoon Wt.	-0.9607**	-0.9787**	-0.9737**
Shell Wt.	-0.9797**	-0.975**	-0.9761**
Shell Ratio (%)	-0.9758**	-0.976**	-0.9888***
ERR (%)	-0.9798**	-0.920**	-0.9702**
PupalWt.	-0.9195**	-0.9422**	-0.9295**
Totalsilk production	- 0.98422***	-0.9968***	-0.9768**

***, ** & * indicate correlations significant at 0.001, 0.01 and 0.05 probability levels respectively. Correlation analysis were based on 50 cocoons X 5 replicates (per eco-race) observation.

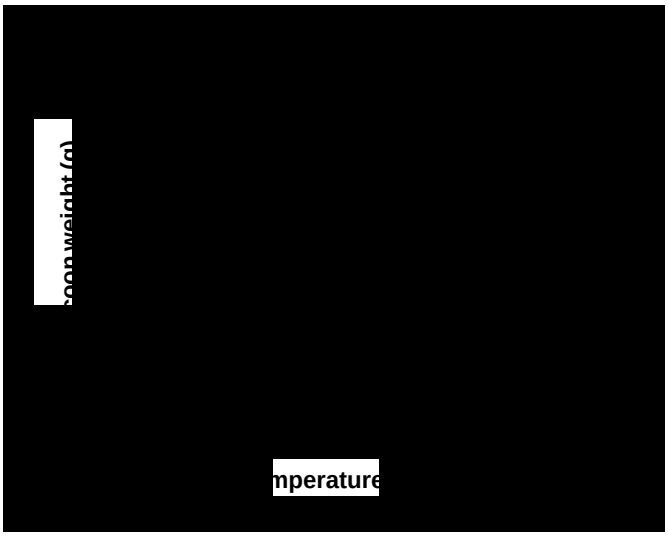


Figure 1

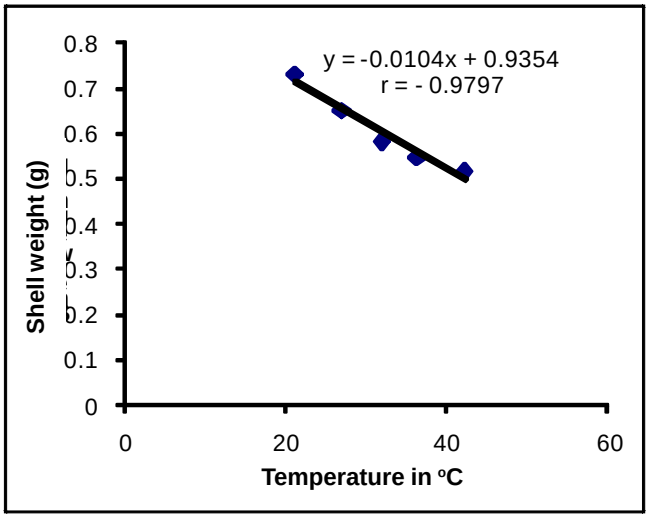


Figure 2

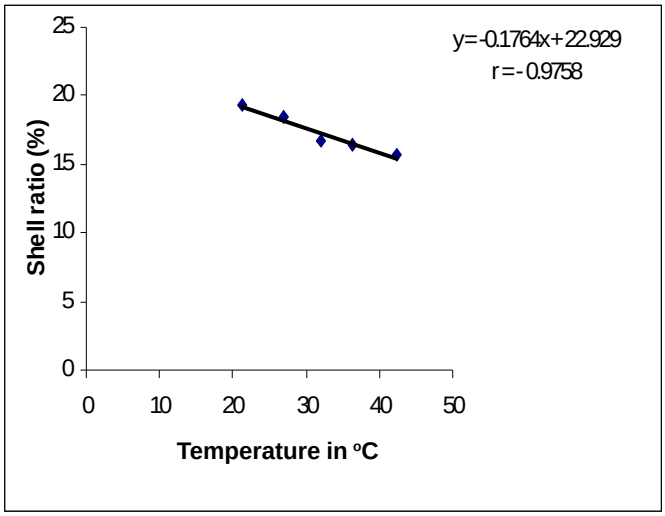
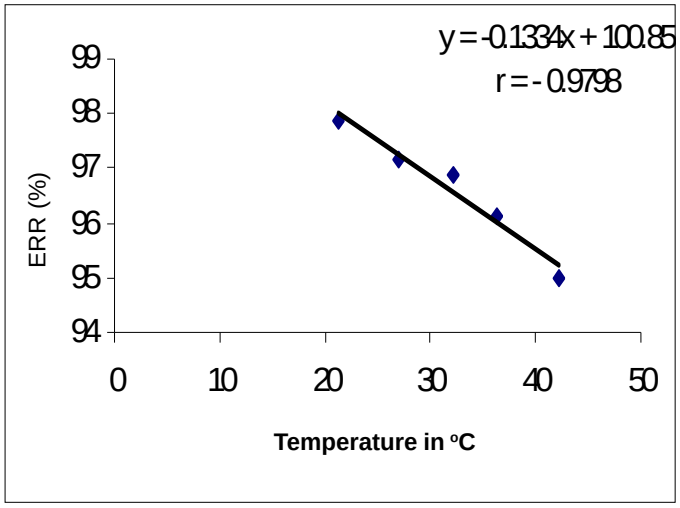


Figure 3



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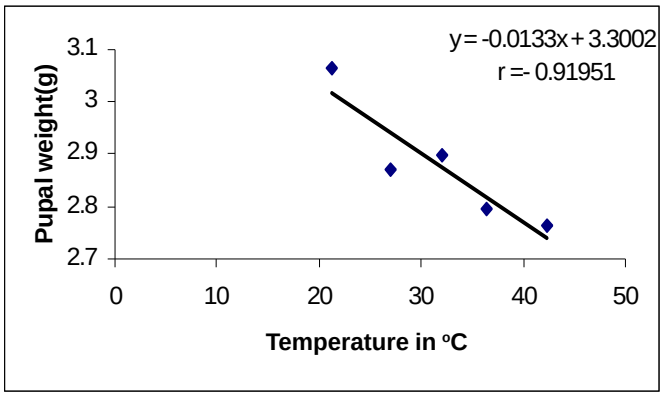

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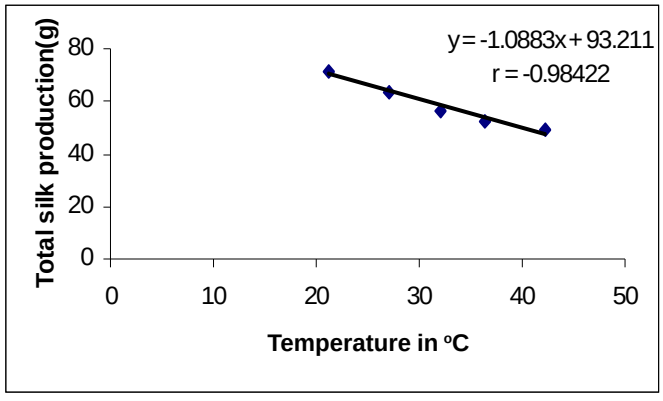

Figure 6

Fig. 1- 6 : Correlation charts of different quantitative traits of Borduar eco-race with temperature.

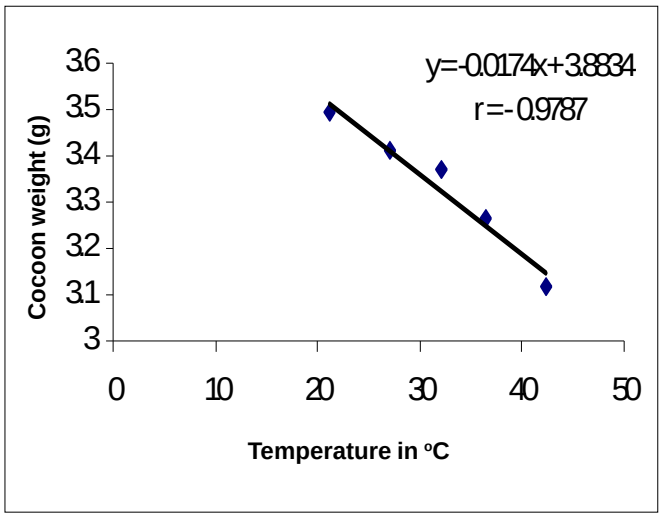

Figure 7

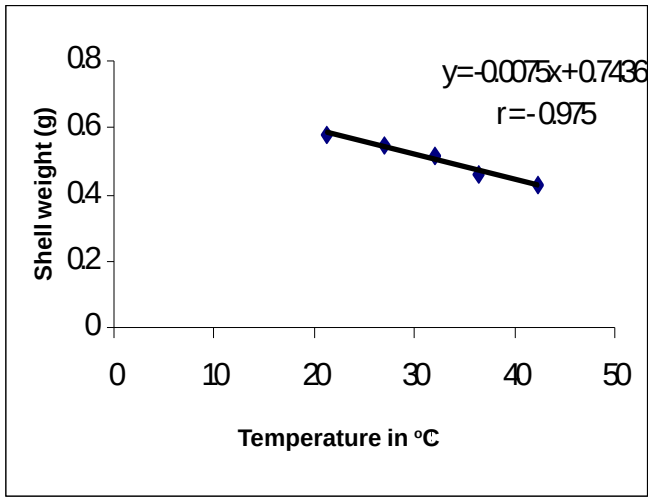

Figure 8

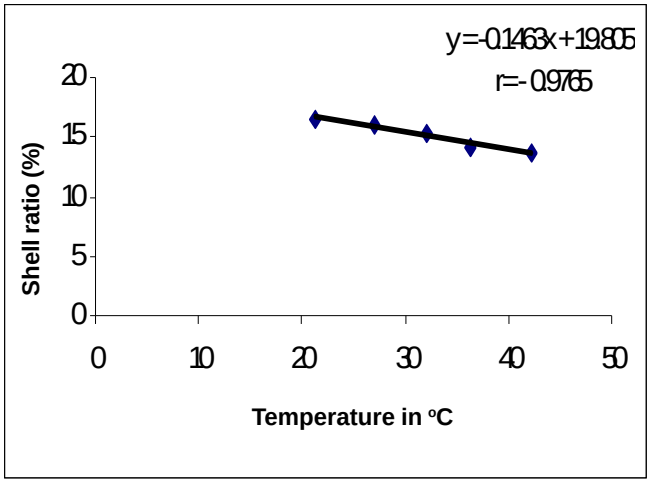

Figure 9

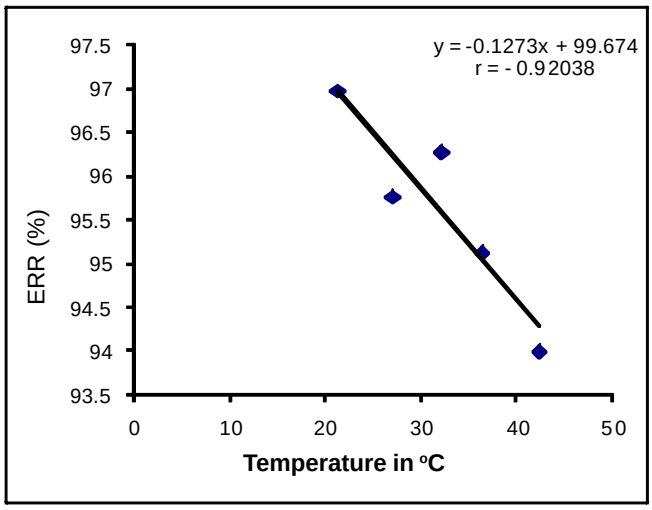

Figure 10

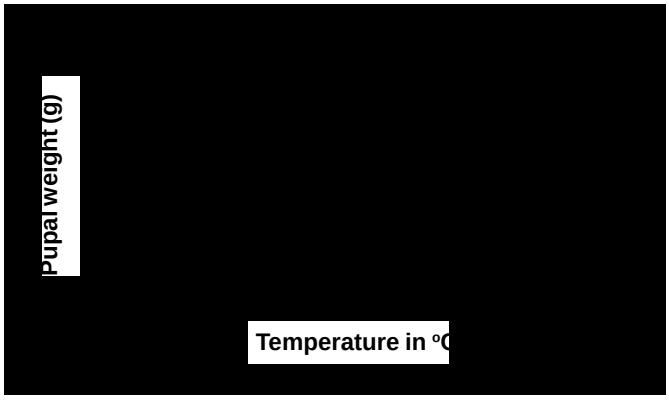

Figure 11

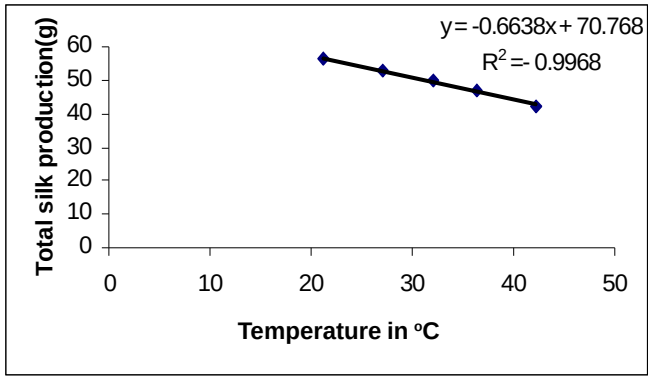

Figure 12

Fig. 7 - 12 : Correlation charts of different quantitative traits of Titabar eco-race with temperature

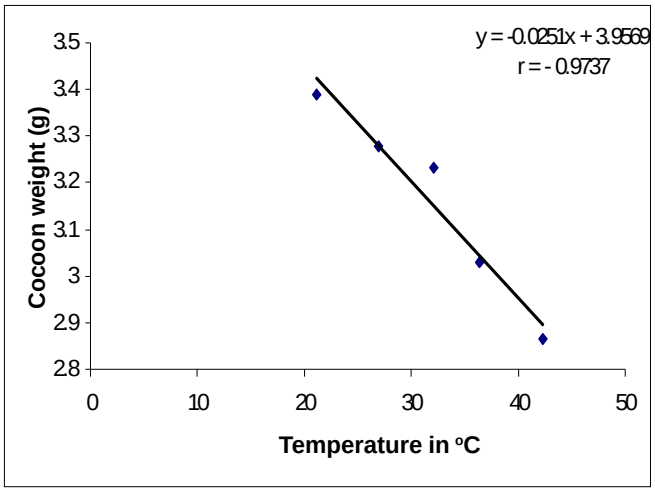

Figure 13

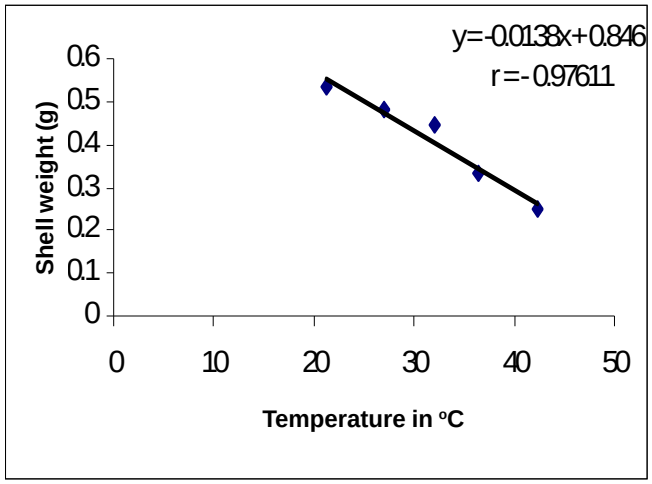

Figure 14

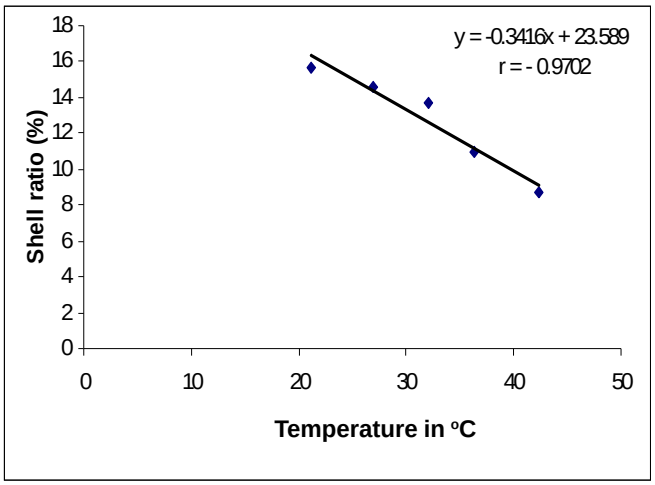

Figure 15

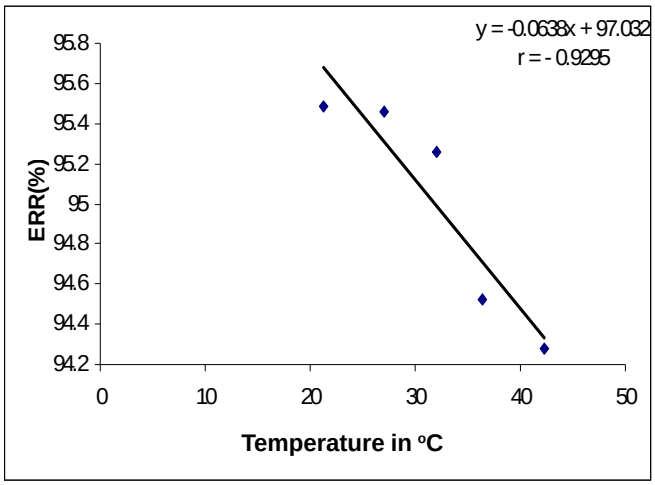

Figure 16

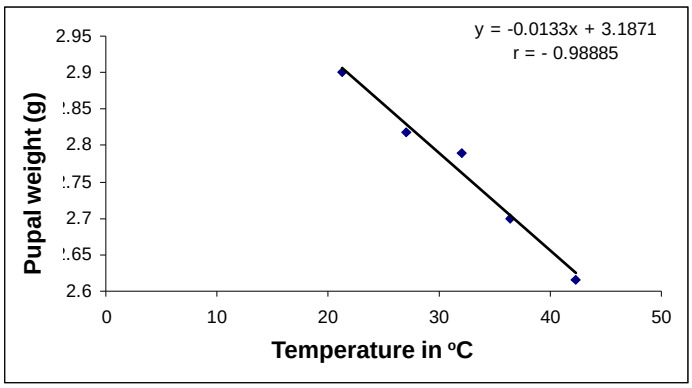

Figure 17

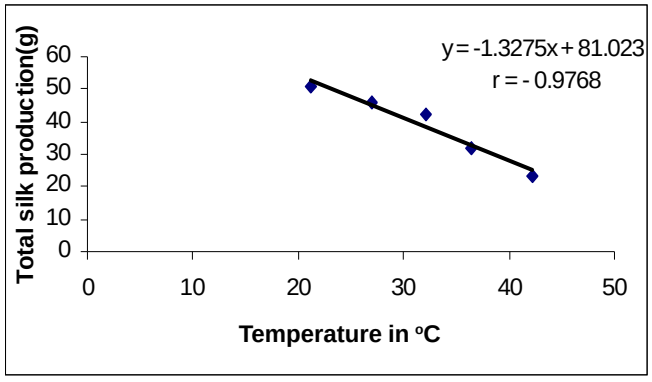

Figure 18

Fig. 13 - 18 : Correlation charts of different quantitative traits of Mendipathar eco-race with temperature

EFFECT OF DRIP IRRIGATION TECHNOLOGY WITH AND WITHOUT APPLICATION OF MULCH ON GROWTH AND YIELD PARAMETERS OF GUAVA (*Psidium guajava* L.)

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ABSTRACT

Water stress during the critical stages of fruit growth and development is one of the main reasons for low productivity of guava (Psidium guajava L.) that happens to be important fruit crop of India. For efficient water and weed management, drip irrigation along with mulching is the best option, which saves up to 25-30 per cent irrigation water. Thus, to determine the extent of improvement by drip irrigation, with and without mulching, the present investigation was designed at guava orchard of Precision Farming Development Centre, Department of Horticulture, IGKV, Raipur .The variety L-49 was used as experimental material with eight treatments having different volumes of water, with and without mulching and was replicated five times using randomized block design. The observations were recorded on various growth and yield parameters. Out of eight treatments, treatment T_3 (0.8 V of water) was found to be far superior to rest of the treatments as most of the parameters viz. plant canopy height, plant canopy spread, primary branches per plant, number of leaves per m^2, number of twigs per m^2, number of fruits per plant, fruit yield per plant and per hectare had showed better results than that of other treatments.

Keywords : Drip irrigation technology, mulch, Psidium guajava L.

INTRODUCTION

Guava (*Psidium guajava* L.) belonging to family "Myrtaceae" is an important and commercial fruit crop of tropical and subtropical regions of India. These fruits are fair source of vitamin A, vitamin C and contain appreciable quantities of thiamine, niacin and riboflavin. It also contains considerable amount of calcium, phosphorus and iron. The area under guava in India is 181.7 thousand ha and production is 1823.3 thousand metric tonnes with a productivity of 10.03 t/ha, whereas in Chhattisgarh, the area and production is 10814 ha and 85863 metric tonnes, respectively with a productivity of 8t/ha, which is not encouraging and is one of the lowest in the country (Anon., 2008).

Water stress during the critical stages of fruit growth and development is the main reason for low productivity. In such situation, water and weed management especially during the period between fruit set to maturity plays an important role in improving the yield. For efficient water and weed management under such situation, drip irrigation along with mulching is the best option which saves 25-30 per cent irrigation water. Insufficient soil moisture and fluctuation in temperature are the main limitation in achieving the maximum yield. Presently, with the adaptation of intensive agriculture, naturally-available inputs are becoming scare and synthetic materials such as, plastic films are replacing unconventional mulching. Plastic mulch is practically impervious to carbon dioxide, a gas that is of prime importance in photosynthesis and a very high level of CO_2 is build up under the plastic, because the film does not allow it to escape. It has to come out through the holes made in the plastic for the plants and a "Chimney effect" is created, resulting in localized concentrations of abundant CO_2 for the actively growing leaves responsible for luxuriant growth of plants**.** The beneficial effect of plastic mulch for guava production has been widely discussed by several workers such as Maji and Das (2008).

MATERIAL AND METHODS

The experiment was carried out at guava orchard of Precision Farming Development Centre, Department of Horticulture, IGKV, Raipur .The variety L-49 was used as experimental material and was planted at a spacing of 6mt. x 6mt.There were eight treatments consisting of different volumes of water, with and without mulching. The guava trees were mulched using black plastic mulch of 50 µ.The experiment was replicated five times in Randomized Block Design. The details of experiment were as follows:

T_1= 1.0V of water (irrigation by drip system)

T_2= 1.0V of water (irrigation by drip system) + Black plastic mulch (50 micron)

T_3= 0.8V of water

T_4= 0.8V of water + Black plastic mulch (50 micron)

T_5= 0.6V of water

T_6= 0.6V of water + Black plastic mulch (50 micron)

T_7= 1.0V of water (irrigation by flood system)

T_8= 1.0V of water (irrigation by flood system) + Black plastic mulch (50 micron)

The observations were recorded on various growth and yield parameters such as plant height (m), canopy height (m), canopy spread (m), plant girth (cm), number of primary branches per plant, number of leaves per m^2 , number of twigs per m^2 , number of fruits per plant, fruit yield per plant (kg), fruit yield per hectare (tonnes), days to fruit maturity and days to 50% flowering.

RESULTS AND DISCUSSION

The data recorded on various growth and yield parameters in response of different levels of irrigation with and without mulching were as follows and presented in Table 1 (a & b).

Plant height (m)

It is apparent from the table that significantly maximum plant height was found in T_3 (4.47 m) followed by T_7 (4.36 m), T_1 (4.32 m), T_8 (4.18 m), T_5 (4.02 m), T_2 (3.95 m), T_4 (3.51 m) and T_6 (3.50 m) respectively. While, significantly minimum plant height was obtained in T_6 (3.50 m). The treatment T_3, T_7 and T_1 were almost similar. In view of the above finding, similar results were obtained by Nath and Pathak (2006) in guava. The taller plant height with 80% water through drip irrigation with black plastic mulch might be due to the optimum availability of moisture which can be retained for longer duration, thus facilitating production of better root biomass, resulting better nutrient uptake from the soil.

Plant canopy height (m)

It is clear from the table that significantly maximum canopy height was found in T_4 (3.09 m) followed by T_2 (3.07 m), T_1 (2.90 m), T_8 (2.80 m), T_7 (2.67 m), T_3 (2.46 m), T_6 (2.20 m) and T_5 (2.15 m), respectively. Whereas, significantly minimum canopy height was observed in T_5 (2.15 m). The treatments T_4, T_2, T_1 and T_8 were statistically at par. Similar results have been reported by Patil and Patil (2001) in guava.

Plant canopy spread (m)

The plant canopy spread ranged from 6.66 m to 5.29 m and 6.52 m to 5.10 m for N-S and E-W directions, respectively. Looking to both N-S and E-W direction, T_4 (6.66 m N-S to 6.52 m E-W) had significantly larger canopy spread followed by T_2 (6.46 m N-S to 6.48 m E-W), T_1 (6.28 m N-S to 6.25 m E-W), T_8 (6.17 m N-S to 6.05 m E-W), T_7 (5.92 m N-S to 5.47 m E-W), T_3 (5.64 m N-S to 5.37m E-W), T_6 (5.40 m N-S to 5.24 m E-W) and T_5 (5.29 m N-S to 5.10 m E-W). The canopy spread from North-South in case of T_6, T_2 and T_3 were statistically similar and in case of E-W canopy spread, T_4 and T_6 were statistically at par. Significantly minimum canopy spread was observed in T_7 (5.29 m N-S to 5.10 m E-W). This was probably due to greater CO_2 concentration and greater soil temperature due to plastic mulch which enhanced the vegetative growth of plants leading to greater spreads. Obminskaya (1991) reported that black poly mulch improved soil temperature water and nutritional regimes and stimulated the plant growth. The above findings are in accordance with the report of present investigation.

Table-1(a) : Effect of different levels of irrigation and black polyethylene mulches on Growth and Yield parameters

Treatments	Plant Height(mt.)	Plant Canopy Height(mt.)	Plant Canopy Spread(mt.)		Plant Girth(cm) per Plant	Primary branches	No. of leaves per m^2
			NS	EW			
T_1= 1.0V of water (irrigation by drip system)	4.32	2.90	6.28	6.25	72.40	3.20	626
T_2= 1.0V of water (irrigation by drip system) + Blackplastic mulch (50 micron)	3.95	3.07	6.46	6.48	74.20	3.40	653
T_3= 0.8V of water	4.47	2.46	5.64	5.37	66.00	2.60	610.20
T_4= 0.8V of water + Black plastic mulch (50 micron)	3.51	3.09	6.66	6.52	66.00	4.00	779.20
T_5= 0.6V of water	4.02	2.15	5.29	5.10	63.40	2.20	542.00
T_6= 0.6V of water + Black plastic mulch (50 micron)	3.50	2.20	5.40	5.24	66.00	2.40	587.80
T_7= 1.0V of water (irrigation by flood system)	4.36	2.67	5.92	5.47	67.20	2.80	613.00
T_8= 1.0V of water (irrigation by flood system) + Blackplastic mulch (50 micron)	4.18	2.80	6.17	6.05	68.00	3.00	619.60
SEm	0.09	0.10	0.06	0.04	2.38	0.09	18.34
CD at 5% level	0.27	0.29	0.17	0.12	6.88	0.25	53.12

Table-1(b) : Effect of different levels of irrigation and black polyethylene mulches on Growth and Yield parameters

Treatments	No. of twigs/m	No of fruits/plant	Fruit yield per plant (kg.)	Fruit yield per hectare (t/ha.)	Days to 50% flowering	Days to fruit maturity
T_1= 1.0V of water (irrigation by drip system)	85.80	237.31	44.83	12.31	33.13	68.15
T_2= 1.0V of water (irrigation by drip system) + Black plastic mulch (50 micron)	91.40	246.44	46.13	12.78	32.93	67.94
T_3= 0.8V of water	76.60	200.10	39.37	10.49	36.21	74.76
T_4= 0.8V of water + Black plastic mulch (50 micron)	95.20	250.40	47.63	13.28	32.57	65.70
T_5= 0.6V of water	69.80	181.52	32.42	9.05	39.75	78.77
T_6= 0.6V of water + Black plastic mulch (50 micron)	71.20	195.12	35.93	9.90	37.75	77.65
T_7= 1.0V of water (irrigation by flood system)	76.60	215.28	37.59	11.04	35.47	71.76
T_8= 1.0V of water (irrigation by flood system) + Black plastic mulch (50 micron)	81.80	228.67	42.30	11.81	34.45	68.95
SEm	1.72	2.01	1.43	0.35	0.66	0.70
CD at 5% level	4.99	5.81	4.13	1.01	1.92	2.02

Plant girth (cm)

It is vivid from the table that maximum plant girth was found in T_2 (74.20 cm) followed by, T_1 (72.40 cm), T_8 (68.00 cm), T_7 (67.20 cm), T_3 (66.00 cm), T_4 (66.00 cm), T_6 (66.00 cm) and T_5 (63.40 cm) respectively; whereas minimum plant girth was recorded in T_5 (63.40 cm).

Number of primary branches per plant

It is apparent from the data that the number of primary branches were influenced significantly by irrigation levels and mulching. The maximum number of primary branches per plant was recorded in T_4 (4.00) followed by T_2 (3.40), T_1 (3.20), T_8 (3.00), T_7 (2.80), T_3 (2.60), T_6 (2.40) and T_5 (2.20) respectively while minimum number of primary branches per plant was obtained in T_5 (2.20). It might be due to the reason that, the better root system developed by sufficient moisture and CO_2 improves the vegetative growth of plants, producing more number of primary branches per plant.

Number of leaves per m²

It is evident from the data that significantly maximum number of leaves per m^2 was recorded in T_4 (779.20) followed by T_2 (653.00), T_1 (626.00), T_8 (619.60), T_7 (613.40), T_3 (610.20), T_6 (587.80) and T_5 (542.20) respectively while the minimum number of leaves per m^2 was found in T_5 (542.20). Drip irrigation with 60% water and mulching produced more leaves as compared to control. This might be due to availability of more photosynthetic area and better distribution of reflected light, which could help in more photosynthetic activity. Secondly, higher plant metabolism and more biosynthesis of auxins might be responsible for overall growth of the plant. The maximum number of leaves per plant provided large photosynthetic area which ultimately helped the plant to produce higher amount of carbohydrates, which will enhance overall growth of the plant. The lower canopy growth and number of leaves may be due to the result of short supply of water and higher evaporation rate.

Number of twigs per m²

It is obvious from the data that significantly maximum number of twigs per m^2 was recorded in T_4 (95.20) followed by T_2 (91.40), T_1 (85.80), T_8 (81.80), T_7 (76.60), T_3 (76.60), T_6 (71.20) and T_5 (69.80) respectively while significantly minimum number of leaves per m^2 was found in T_5 (69.80). This might be due to greater CO_2 concentration and improved soil temperature enhancing the vegetative growth of plants. This is in confirmity with the finding of Lareau *et al.* (1991) in strawberry crop.

Number of fruits per plant

A perusal of data clearly indicates that significantly maximum number of fruits were found in T_4 (250.40) followed by T_2 (246.44), T_1 (237.31), T_8 (228.67), T_7 (215.28), T_3 (200.10), T_6 (195.12) and T_5 (181.52), respectively. Significantly minimum number of fruits per plant was recorded in T_7 (181.52). The maximum number of fruit per plant might be due to the higher fruit set which increased the number of fruits per plant. The above results are in good agreement with the finding of Maji and Das (2008).

Fruit yield per plant (kg)

Data revealed that the total fruit yield ranged from 47.63 kg to 32.42 kg per plant. Significantly maximum number of fruit yield per plant was recorded in T_4 (47.63 kg) followed by T_2 (46.13 kg), T_1 (44.83 kg), T_8 (42.30 kg), T_3 (39.97 kg), T_7 (37.59 kg), T_6 (35.93 kg) and T_5 (32.42 kg), respectively. The treatment T_4, T_2, T_6 as well as T_8 and T_7 were statistically at par with the above findings. Significantly minimum fruit yield per plant was obtained in T_5 (32.42 kg). Drip irrigation provides appropriate moisture at field capacity, better root development in terms of number and spread of roots, which facilitated luxuriant growth of plant due to better nutrient uptake. Hence,nthere is better fruit growth and development, ultimately achieving higher yield. Higher yield with mulching could be ascribed due to difference in plant light environment among the treatments under the field condition. In confirmity of this, similar findings have been reported by Patil and Patil (2001) and Maji and Das (2008) in guava crop.

Fruit yield per hectare

It is apparent from the data that the total fruit yield ranged from 13.28 to 9.05 tonn per hectare. Significantly maximum fruit yield per hectare was found in T_4 (13.28 t/ha) followed by T_2 (12.78 t/ha), T_1 (12.31 t/ha), T_8 (11.81 t/ha), T_7 (11.04 t/ha), T_3 (10.49 t/ha), T_6 (9.90 t/ha) and T_5 (9.05 t/ha), respectively. The treatment T_2, T_6, T_3 as well as T_8 and T_7 were statistically at par. Significantly minimum fruit yield per hectare was obtained in T_7 (9.05 t/ha). The results are directly correlated with fruit yield per plant and similar results are in close confirmity with the finding of Borthakur *et al.* (1988) in guava.

Days to 50% flowering

It is vivid from the table that significantly maximum days were taken to 50% flowering under the treatment T_5 (39.75), followed by T_6 (37.75), T_3 (36.21), T_7 (35.47), T_8 (34.45), T_1 (33.13), T_2 (32.93) and T_4 (32.57). Significantly minimum days were taken to 50% flowering under the treatment T_4 (32.57). The temperature plays a key role in flower growth, development and fruit set in guava. Drip irrigation along with the mulch affect the temperature of micro climate around the plants. The greater influence of temperature and increased photosynthesis might have influenced to the initiation of first flowering, 50% flowering, number of flowers per plant, fruit set percentage due to different levels of drip irrigation and mulching. Similar results were corroborated with the findings of Patil and Patil (2001) and Patra *et al.* (2003) in guava crop.

Days to fruit maturity

It is evident from the data that significantly maximum days to fruit maturity were noted under the treatment T_5 (78.77) followed by T_6 (77.65), T_3 (74.76), T_7 (71.76), T_8 (68.95), T_1 (68.15), T_2 (67.94) and T_4 (65.70), while, minimum days to fruit maturity was counted under the treatment T_4 (65.70). An earlier ripening in T_5 may be due to higher soil temperature and plastic mulch enhancing the rates of physiological functioning. The finding are in line with the result of Obminskaya (1991) in strawberry crop.

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MEDICINAL VALUES OF SKUNK VINE *(Paederia foetida* L.*)* - AN OVERVIEW

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ABSTRACT

Paederia foetida L. is a climber found in various parts of the world and is well known for curing various diseases like asthma, diarrhoea, constipation and expulsion of the placenta etc. Its pharmacological properties like anti-inflammatory, anti-arthritic activity, anti-helminthic activity, hepatoprotective activity, antispasmodic activity and anticancer activity have been well established. IUCN enlisted *Paderia foetida* in near threatened category. This present paper described the various pharmacological and therapeutic as well as morphological features of this plant.

Key words: Phytoconstituents, *Paederia foetida,* pharmaceutical properties

INTRODUCTION

Paederia foetida L. commonly known as skunk vine, Chinese fever vine, Lesser Malayan stinkwort, Stink vine belongs to the family Rubiaceae of the order Gentianales and sub-kingdom Angiosperm (Blatter *etal.,* 1981; Nandkoni, 2002; Steinmetz, 1961; Khare, 2007). The medicinal properties of the species for curing asthma, diarrhoea, constipation and expulsion of the placenta etc. made the species very important from the pharmaceutical point of view (Hynniewta and Kumar, 2008). According to the ISSG database, the global distribution of *Paederia foetida* is divided into two ranges like alien range and native range. The alien range comprises of Brazil and United States whereas the native range extends from Bangladesh, Bhutan, Cambodia, China, India, Nepal, Indonesia, Japan, Korea (Christian, 1991) ,Lao People's Democratic Republic, Macao (Macau) Malaysia, Myanmar (Burma), Singapore, Taiwan, Thailand, Vietnam to Philippines. Moreover, Skunk vine occurs as a non native species in Hawaii and the south-eastern United States (Weber, 2003; Pemberton and Pratt., 2002). In Florida, skunk vine population occurs from the northern countries of Suwannee and Gadsden south to Broward Country (Langeland *et al*., 2006) particularly abundant in Florida's Hernando and Pasco countries (Garland, 2004). In India, Skunk vine is found in the Himalayan ranges up to an altitude of 1800 m from sea level. It is also found in Bihar, Odisha, Bengal and Assam. Wide availability of the species is towards the north-eastern parts and the Himalayas are the main habitats having natural forests, wetlands and hillsides along the streams (Pratt *et al*., 2001). The plant species is commonly found in various parts of Odisha viz Ganjam, Gajapati, Kandhamal, Baripada, Keonjhar, Dhenkanal, Nayagarh, Baudha, Malkanagiri, Koraput, Rayagada, Phulbani and Anugul. It was found that this plant is near threatened category. (Pattanaik *et al*., 2009 and Franco and Narasimhan, 2009).

MORPHOLOGICAL CHARACTERISTICS

The plant is a perennial, climbing or trailing vine can grow up to 9 m long. Stems grow up to 7 m or more, climbing, or prostrate and rooting at the nodes. Leaves opposite, oblong-lanceolate to ovate, 4.5-14 cm long, 2-5 cm wide, glabrous except lower surface with tufts of hairs in axils of primary veins, margins entire, ciliate when young, becoming glabrate, apex acuminate or acute, base cuneate to rounded or subcordate, petioles 2-5 cm long, stipules ca. 1.5-1.7 mm long, short-acuminate, short-fimbriate-ciliate to ciliate. Flowers in cymes 2-12 cm long, sometimes several grouped together " with subtending leaves and forming panicles up to 30 cm long; hypanthium ca. 1.5 mm long; calyx lobes deltate, ca. 0.4-0.5 mm long; corolla white to pale yellow with a dark rose purple center.

The tube ca. 7-11 mm long, densely floccose externally, purplish within with long, clavate, purple hairs, the lobes scarcely spreading, margins crisped-undulate; stamens inserted at different levels, Fruits are shiny brown, and nearly round capsule, and are typically 0.7cm wide. Seeds are black, round and often dotted with white needle-shaped crystals

Fig. 1 : Flowering twig of *Paederia foetida* L.

Fig. 2 : Entire plant of *Paederia foetida*

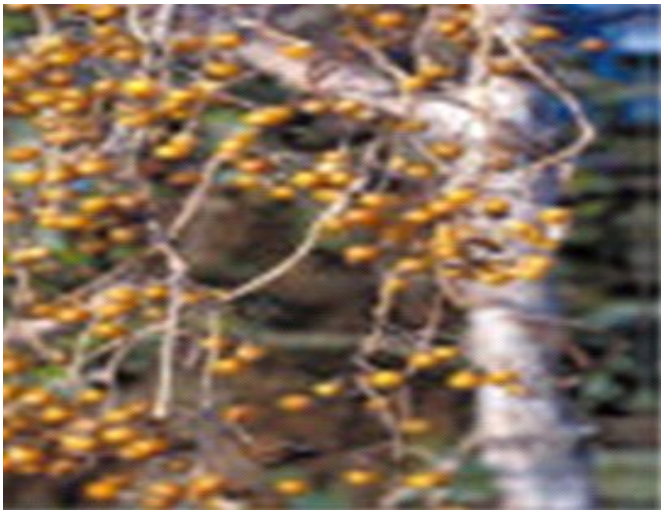

Fig. 3 : Fruit of *Paederia foetida* L.

Macroscopically, the fresh leaf of *Paederia foetida* L. is 10 to 15 cm long, 5 to 6 cm width and petiole 1.2 to 6 cm, surface is glabrous and mostly ovate, green having a characteristic odour indistinct bitter taste. The leaf is distinctly dorsi-ventral with prominent midrib and lamina. Epidermal trichomes are sparsely seen both on the upper and lower epidermis. Mesophyll composed of single layered palisade cells and 3-4 layered spongy tissue; in margin of the leaf mesophyll replaced by thick walled cells. Trichomes are present on both surfaces elongate to produce uniseriate trichomes. Mid ribs composed of single layered epidermis covered with cuticle; ground tissue consisting of 2-5 layered of collenchyma towards upper and lower side and rest parenchyma; a large median crescent-shaped vascular bundle consisting usual elements with xylem towards upper side and phloem towards lower side.

DISCUSSIONS

Pharmacognostic properties

The species has tremendous medicinal properties as mentioned above. Whole plant, leaves, roots are used in the preparation of medicine and various herbal formulations (Evans, 1989). The plant contains various phytoconstituents in various plants of the plant. The plant is rich in perennial Triterpenoids, Saponins, Ursolic acid, Epifriedelinol and Friedelin (Shukla, Y.N.; Lloyd, H.A.; Morton, J.F.; Kapadia, G. J. ,1976 , Wong, K.C.; Tan, G.L.,1994.). The leaf and stem contain oxygen-containing monoterpenes, linalol, sulphur-containing compounds like dimethyl disulphide, hentriacontane, hentriacontanol, ceryl alcohol, 2,3-dihydro-benzofuran, benzofuran. iridiod glucosides like Asperuloside, paederoside, scandoside, sitosterol, stigmasterol and campesterol (Kirtikar.K.K and Basu B.D,1993). Whereas the aerial parts of the plant contain Quinones like embelin and Alkaloids like a-Paederine and â-paederine (Prieto P, Pineda M, and Aguilar, M (1999), Wong, K C ; Tan G L,1994). The leaf of the species is rich in Mixture of fatty acids including non-ionic, capric, lauric, myristic, arachidic and palmitic acids,carotenes and vitamin – C (Ghani A, 1998, *The wealth of India* (1962)). It has other physic-chemical properties given in the Table-1.

Table-1: Physico chemical properties of *Paederia foetida Linn.*

Sl. No.	Physical parameters	Contents (%)
1.	Moisture	11.50
2.	Ash	10.00
3.	Acid insoluble	1.50

Traditional uses

Many tribes and rural people as well as traditional Vaidyas use it in various healing purposes**.** Roots are considered to be emetic, emollient and carminative, useful in colic, spasm, rheumatism and gout. Leaves and roots are considered to be a tonic. It stimulates the central nervous system. Leaf alleviates spasms and shows anticancer activity. Plant shows immunosuppressive and anti-inflammatory activity. The bark, leaf, root and whole plant have been used to treat maggots in wounds, abscesses, urethral calculi, repeat oestrus in cows and buffaloes, asthma, diarrhoea, constipation and expulsion of the placenta. Tribal peoples in Odisha use the whole plant in rituals. (Panda, 1987).

Pharmacological action

Root is used in curing Hemorrhoids, in piles, inflammation of spleen, liver and chest pain. Root and leaf is used in the preparation of tonic. Whole plant is used in treatment of rheumatism. Leaves are used as poultice to relieve distension of abdomen due to flatulence and also used in Herpes. Leaf juice is given to children for the treatment of diarrhea and the decoction of leaf is used for vesical calculi. The plant showed anti-tussive activity. The main use of the plant is for arthritis and rheumatic disorders. The fruits are used to prevent toothache. The whole plant showed tonic, astringent and anti-phlogistic actions and has been used in tenesmus. The plant also exhibited anti-inflammatory, anti-arthritic activity, anti-helmintic activity, hepato-protective activity, antispasmodic activity and anticancer activity (Blatter *et al.,* 1981).

Various Study have shown that the plant *Paederia foetida* reduces the purging index in a dose-dependent manner in magnesium sulfate-induced diarrhoea by reducing gastro-intestinal motility and enhanced morphine-induced reduction of motility. It has been also established that *Paederia foetida* has anti diarrheal activity by inhibiting intestinal motility supporting its use in traditional medicine The ethonomedicinial anti-inflammatory use of *Paederia foetida*,butanol fraction of the methanol extract of the defatted leaves of this plant shown significant inhibition of granulation in cotton pellet implanted rats. This plant also has shown decreased activity of liver trans-aminase and anti-rheumatic activity. Antispamodial activity had reported from the ethanolic extract of *Paederia foetida* in isolated guinea- pig ileum. Leaf juice of *Paederia foetida* have shown significant effect against bovine helminths– Strongyloides spp, Trichostrongylus and Haemonchus. Ethhanolic extract of *Paderia foetida* has a cough suppressant effect, the effect was less than codeine but similar to non-narcotic dropropizine. Nosaovaa *et al.,* 2007 related the effect to its demonstrated anti-inflammatory activity. *Paderia foetida* has high antioxidant activity, with fresh samples having higher phenolic contents and better antioxidant activity than the dried samples. Osman *et al.,* 2009 suggests *Paederia foetida* could be a significant source of natural antioxidant compounds. De *et al.,* 1993 investigated that the methanol extract of *Paederia foetida* have moderate hepatoprotective activity . (Afroz *et al.*, 2006, De *et al.*, 1994;Singh *et al* ,1994;Srivastav *et al* ,1973).

CONCLUSION

Paderia Foetida is a climbing vine with varied medicinal importance including the food value ,Its nutritional and therapeutic properties cover a various disorders and ailments i.e rheumatism , exhibited Anti-inflammatory, Anti-arthritic activity, Anti-helmintic activity, Hepato-protective activity, Antispasmodic activity and anticancer activity due to the ignorance and overexploitation of this plant it is now under the threat therefore it's the need of the time for the conservation biologists to ensure and formulate an effective strategy to conserve this plant. Though few attempts have been made to conserve it and studies are being made to standardize the in vitro protocols for its conservation simultaneously at its natural habitats by educating the community for its long term medicinal and economical importance.

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Manuscript should be typed in double-spacing on one side of Bond Paper (A-4). Tables must not exceed 12 vertical columns. Leave liberal margins on both the sides. Arrange the manuscript in the order of title, author(s), address of each author, abstract (approx.200 words), key words introduction, materials and methods, results and discussion, acknowledgement (if any) and references.

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2.5. **Key words.** Maximum of 5-6 key words should be provided for subject indexing.

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2.7. **Materials and Methods.** A full technical description of the methods followed for the experiment(s) should be given, providing enough information. Detailed methodology should be given when the methods are new while for standard methods, only references may be cited.

2.8. **Results and Discussion.** In this section, only significant results of the experiment(s) should be reported. Along with the tables and figures, the discussion should deal with interpretation of results and relate the author's findings with the past work on the same subject. The conclusions drawn should be explicitly listed at the end of this section.

3. **References.** Refer this copy as sample for references. For ex.*:* Thankppan A, Das BK, Barman HK and Samal SK (2008) Genetic fingerprinting of *Aeromonas hydrophila* isolated from diseased fresh water fishes of eastern India; e-planet, 6(2): 01-06.Distinction for the same author and same year be done as e.g. 1969a, 1969b. Unpublished data, and personal communication are not acceptable as references but may be referred to parenthetically in the text.

4. **Tables.** Number the tables consecutively in Arabic numerals. Tables should have comprehensible legends. Conditions specific to a particular experiment should be stated. Zero results must be represented by 0 and not determined by n.d. The dash sign is ambiguous. For values <1, insert a zero before the decimal point.

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