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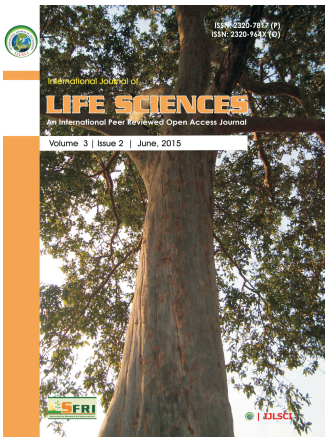

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Contact: +91-942 077 5527
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editorijlsci@gmail.com
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Cover image: ***Terminalia arjuna*** **(Roxb.) Wt & Arn.**

Description: The Arjuna is about 12–25 m tall; Branches glabrous; Smooth bark greenish white; Leaves sub-opposite, oblong or elliptic-oblong, rounded or sub cordate at base, Obtuse or sub-acute, shallowly crenate-serrate or subentire, glabrous, pale green above, brownish beneath; Petiole 6-10 mm long with 1-2 prominent glands at base of lamina; Flowers sessile, in short axillary and terminal panicles; Bractioles linear lanciolate, caducous. Calyx 4 mm long glabrous; teeth triangular,1.5 mm long. Disc clothed with yellowish or redish hairs. Drupes 3-5 cm long, ovoid-oblong, dark brown, with 5 hard wings(Naik & Associates,1998).

Locality: Near Sipna River bank, Semadoh Rest House

Distribution: Very Common through Maharashtra statealong banks of rivers, Mahabaleshwar, Khandala, Pachgani, Satara, Western Ghats, South India, Gujarat, Lingmala.

Photo By: Mr. Anand Oak, Research Scholar, Dept of Botany, Shri Shivaji Science College, Amravati

Contents

RESEARCH ARTICLES

SHORT COMMUNICATION

IJLSCI

ISSN:2320-7817(p) | 2320-964X(o)

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Editorial Board

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RESEARCH ARTICLE

In vitro fungitoxic effect of some plant growth regulators on spore germination and germ tube emergence of *Alternaria solani*

Bhajbhuje MN

Seed Pathology Lab., Department of Botany, Jawaharlal Nehru Mahavidyalaya, Wadi, Nagpur- 440 023 (M.S.) India. | e-mail : dr_mnbhajbhuje@rediffmail.com

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ABSTRACT

The inhibitory effect of five plant growth regulators including indol-3-acetic acid; indol-3-butyric acid; naphthalene acetic acid; 2,4-dichlorophenoxy acetic acid; and phenyl acetic acid was evaluated *in vitro* against the leaf blight pathogen, *Alternaria solani*. All test chemical inducers at 10^{-2} to 10^{-4}M conc. significantly reduced spore germination and germ tube growth of the test pathogen. Pathogenic fungus, *Alternaria solani* had sensitive response against 10^{-2} conc. of naphthalene acetic acid, reducing spore germination by 84% and germ tube growth by 14% over untreated control. Also, complete inhibition spore germination of test fungal pathogen was confined when all chemical inducers were evaluated at 10^{-1}M conc. Fungal spore germination and germ tube growth increased significantly as the conc. of chemical inducers was decreased. Among the test growth regulators, indol-3-acetic acid had least inhibitory effect against test pathogen of these parameters undertaken. The results of the present study revealed the possibility of usage plant growth regulators in inducing phytoalexin compound in susceptible plant cultivars.

Key words: Fungitoxicity, susceptible, abiotic elicitors, chemical plant resistance inducers.

INTRODUCTION

The Deuteromycetes ubiquitous fungal genus *Alternaria* comprises diverse saprophytic as well as Endophytic species and is known for its notoriously destructive plant pathogen members (Mamgain *et al*., 2013). Out of the total 299 known species representing genus *Alternaria*, majority of them lack sexuality, although few species have been found to have sexual stage in

their life cycle.The genus is characterized by the formation of polymorphous, multicellular and pigmented conidia either singly or in short or longer chains by asexual method. The conidia are broadest near the base; taper gradually to an elongated bead and provided with cross, longitudinal as well as oblique septa. It has been found to have a drastic effect on the members belonging in the plant families such as Cucurbitaceae, Brassicaceae, Solanaceae, Poaceae etc. which are having nutritional as well as economical food value. It is associated with a wide variety of substrates including seeds, plants, agricultural products, animals, soil and the atmosphere. It is also common allergen in humans, growing indoors and causing hay fever or hypersensitivity reactions that sometimes lead to asthma. Some species readily cause opportunistic infections in immuno-compromised people such as AIDS patients (Mamgain *et al*., 2013). Several taxa are also important postharvest pathogens, causative agents of phaeohyphomycosis in immuno-compromised patients or airborne allergens.

Some saprophytic species representing genus *Alternaria*, are agents of decay and decomposition and growing profusely on dead and decaying debries of plant and animal origin producing a variety of primary and secondary metabolites. Several pathogenic species mostly causing leaf blight infection considered the major problems in agricultural production throughout the world, reducing yield and quality of crops and produce more than 70 phytotoxins of host selective (host specific) and nonspecific types (Trivedi *et al*., 2013). Host-selective toxins (HSTs) are toxic only to host plants while nonspecific toxins can affect variety of non-host plants. The *Alternaria* HSTs involve a diverse group of low-molecular-weight substances such as alterotoxins, alternariol, tenuazonic acid; alternaric acid and were found in culture filtrates as families of closely related compound and were reported to play a crucial role in determining host specificity and contributing to disease development. The *Alternaria* HSTs cause necrosis on leaves of susceptible cultivars at concentrations as low as 10^{-8} to 10^{-9} M and no necrosis on leaves of resistant cultivars even at higher concentrations (Otani *et al.*, 1995) The toxin from secondary metabolites penetrate host tissues, directly act on living host cell protoplasm and damage the metabolically active cells to influence the course of disease development (Mamgain *et al*., 2013).

Majority species of *Alternaria* caused leaf spot and early blight resulting in defoliation, reduction in size and quality of fruits, ultimately adversely affects productivity (Mamgain *et al*., 2013).). The leaf spots may be control by foliar application of effective organic fungicides, but these are reported hazardous and their residual toxicity in plant parts, fruits poses carcinogenic disorders to consumers (Trivedi, *et al*., 2013) and also helps to increase level of air pollutants. To overcome these, the concept of screening for disease resistance has been developed (Eckadt, 2011). Phytoalexin accumulation at infection site in leaves, stem, cotyledons and hypocotyl (Ingham, 1982), in response to wounding (Rahe and Arnold, 1975), to interaction with micro-organism (Iriti & Franco, 2009) or to treatment with certain chemicals (Ismile *et al*., 1987; Bhajbhuje, 2013) makes a significant contribution to resistance, cultivars that are normally susceptible to a virulent race of pathogen thus providing protection in different plants. A little is known about induction of resistance by application of plant growth regulators in plants and control of leaf blight pathogen, it seemed to be worthwhile to report the fungitoxicity of plant growth regulators at variable concentrations against leaf blight pathogen, *Alternaria solani* (Ellis & Martin) Jones & Grout.

MATERIALS AND METHODS

The plant growth regulators of diverse chemical nature induce phytoalexin in plants, when applied in dilute concentration, were screened for fungitoxic effect against leaf blight causing pathogen *Alternaria solani*. The stock solution of

0.1M concentration for five plant growth regulators was prepared separately in volumetric flask and each was diluted to the concentration between 10^{-2} to 10^{-4}M. These solutions of different concentrations of test chemicals were screened for fungitoxic assay employing the slide germination technique (CMI, 2010). *Alternaria solani* was isolated from infested leaves and stored seeds of tomato as internal seed borne pathogen and maintained in laboratory on PDA nutrient medium at 25±1^{0}C. One drop of different conc. of test chemicals was placed on cover slip and added one drop of spore suspension of test pathogen in the drop. The spores were allowed to grow in drop of water serve as control. The coverslip with spores in drop was inversely placed on cavity glass slide in triplicate. Slides of different treatments were randomly distributed into large Petri dishes made into moist chamber and kept these for 24 hrs. in darkness. One drop of lecto-phenol was put on each spot to fix the germinated spores. Germination of spores was counted in terms of percentage on the basis of 300 spores and germ tube growth was measured on the basis of 90 germlings from each spot observed randomly. These concentrations were selected on the basis of their effectiveness in inducing resistance in plants (Bhajbhuje, 2014).

RESULTS AND DISCUSSIOINS

Altogether five plant growth regulators in aqueous dilute solution (10^{-2} to 10^{-4}M) are screened to study *in vitro* fungitoxic effect on spore germination & germ tube growth of pathogen following slide germination method (CMI, 2010). A drop of spore suspension in Czapek's Dox broth was placed 3 cm apart on each of three slides per treatment. The slides were randomly distributed into large Petri plates made into moist chamber and kept at room temperature in darkness. After 24 h of incubation, the percent spore germination was recorded from each spot on the basis of 50 spores and germ tube growth on the basis of 15 germlings.

The results presented in Table 1 revealed that an aqueous solution of all five plant growth regulators at different concentrations caused injury to spore of *Alternaria solani* inhibiting spore germination and germ tube growth. An absolute inhibitory effect was induced with naphthalene acetic acid (NAA) and phenyl acetic acid (PAA), when treated with 10^{-1}M aqueous solution of test chemical inducers. Naphthalene acetic acid (NAA) caused greater inhibitory effect at 10^{-2}M conc. reducing the spore germination by 84% over the untreated control, followed by phenyl acetic acid (PAA) causing reduction in this parameters by 71 %. Moderate inhibitory effect to the extent of 61% and 65% for spore germination was recorded with 2, 4-dichloro-phenoxy acetic acid (2, 4-D) and Indol-3-butyric acid (IBA) at 10^{-2}M conc. respectively. Indol-3-acetic acid (IAA) had least inhibitory effect at this conc. on spore germination (Table 1).

The inhibitory effect for all the plant growth regulators was declined with dilution of aqueous stock solution. The greatest declining of inhibitory effect was recorded with naphthalene acetic acid (NAA), reducing the spore germination by 35% and 6%, when treated with conc. 10^{-3} to 10^{-4}M. Least inhibition of spore germination was confined at 10^{-4}M with Indol-3-acetic acid (IAA) while remaining test chemical inducers indol-3-butyric acid (IBA); 2,4-dichlorophenoxy acetic acid (2,4- D); phenyl acetic acid (PAA) had considerable to moderate inhibitory effect at conc. 10^{-3} M while it was declined to 2-9% when treated with 10^{-3} M conc. over untreated control (Table 1).

The similar trend was confined for germ tube emergence with all concentration of the plant growth regulators tested. The spores of leaf blight pathogen; *Alternaria solani* remained dormant and did not produce germ tube, when treated with 10^{-1}M aqueous solution of all five plant growth regulators excepting indol-3-acetic acid (IAA). All the test chemical inducers excluding indol-3-acetic acid (IAA) at 10^{-2}M concentration had 10 to 18% inhibitory effect on

germ tube growth compared to untreated control. Indol-3-acetic acid (IAA) at 10^{-2}M induced 3% inhibitory effect. Phenyl acetic acid (PAA) and 2,4-dichlorophenoxy acetic acid (2,4-D) caused greater inhibition at 10^{-2}M conc., reducing the mean germ tube growth to the extent of 18 and 17% respectively while remaining test chemical inducers had 10-14% inhibitory effect on the same parameter over untreated control (Table 1). The inhibitory effect was declined with decrease in concentration of all the plant growth regulators tested. The greatest declining of inhibitory effect was recorded with Phenyl acetic acid (PAA) reducing the germ tube growth by 16% and 13% at conc. 10^{-3} to 10^{-4}M respectively. Indol-3-acetic acid (IAA) had little inhibitory effect; Naphthalene acetic acid (NAA) induced 9% inhibition while remaining test chemical inducers

Table 1: Effect of plant growth regulators at dilute concentration on spore germination and germ tube growth of *Alternata solani*

S. No.	Plant growth regulators	Percent spore germination			Mean germ tube growth		
		10^{-2} M	10^{-3} M	10^{-4} M	10^{-2} M	10^{-3} M	10^{-4} M
1.	Indol-3-acetic acid (IAA)	44 (-55.1)	71 (-27.6)	96 (-2.0)	92 (-3.1)	94 (-2.1)	95 (-1.0)
2.	Indol-3-butyric acid (IBA)	34 (-65.3)	64 (-34.7)	92 (-6.1)	86 (-10.4)	89 (-7.3)	91 (-5.2)
3.	Naphthalene acetic acid (NAA)	16 (-83.7)	48 (-51.0)	84 (-14.3)	83 (-13.5)	86 (-10..4)	87 (-9.4)
4.	2,4-dichlorophenoxy acetic acid (2,4-D)	38 (-61.2)	68 (-30.6)	94 (-4.1)	88 (-16.7)	92 (-4.2)	94 (-2.1)
5.	Phenyl acetic acid (PAA)	28 (-71.4)	56 (-42.9)	89 (-9.2)	79 (-17.7)	81 (-15.6)	84 (-12.5)
	Water (Control)	98	98	98	96	96	96

1. Results have been expressed as percentage in terms of control; 2.Average of 300 spores; 3.Average of 90 germlings; 4.Values in parentheses indicate percentage reduction or increase in terms of control

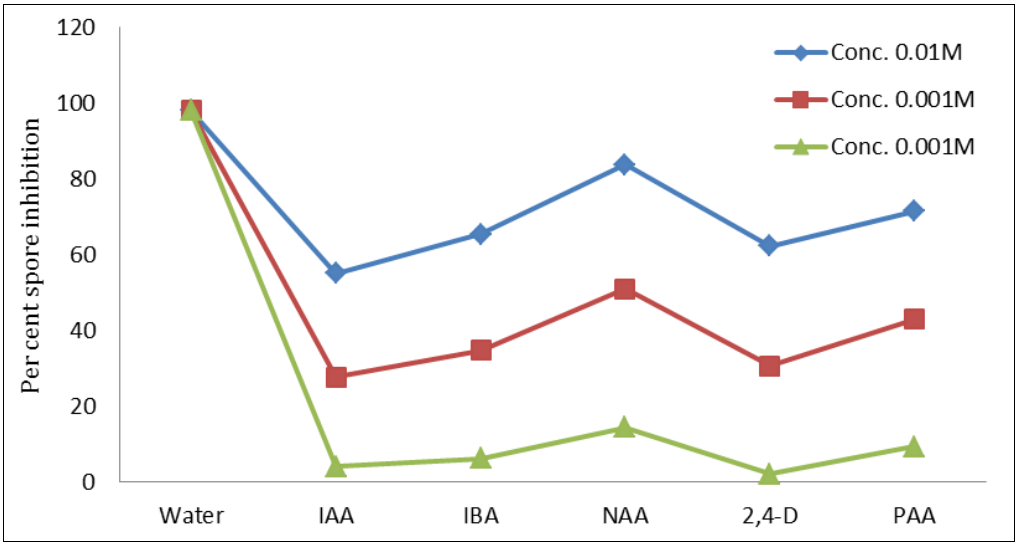

Fig. 1: Fungitoxicity of plant growth regulators on spore germination *Alternata solani*
(IAA= indol -3-acetic acid; IBA=Indol-3-butaric acid; NAA= Maphthalene acetic acid; 2,4-D+ 2,4-dichlorophenoxy acetic acid; PAA-phenyl acetic acid)

Indol-3-butyric acid (IBA); 2,4-dichlorophenoxy acetic acid (2, 4-D); phenyl acetic had mild inhibitory effect at conc. 10^{-4} M compared to untreated control (Table 1). Of the five plant growth regulators screened against *Alternaria solani*, naphthalene acetic acid (NAA) caused greater inhibition of spore germination, but had moderate inhibitory effect on germ tube emergence, while Phenyl acetic acid (PAA) induced considerable inhibitory effect on spore germination but had greater reduction in germ tube growth. Moderate to considerable inhibitory effect was recorded with Indol-3-butyric acid (IBA) and 2,4-dichlorophenoxy acetic acid (2,4-D) while Indole-3-acetic acid had least inhibitory effect on both parameters studied (Fig. 1). It is in agreement with earlier finding of Ashraf and Ali (2007) who reported inhibitory response of chemical inducers on microbial community.

Ezzouhri *et al*., (2009) reported the chemical tolerance level of some filamentous fungal organisms including *Alternaria alternata, Aspergillus niger, Geotrichum candidus, Penicillium sp*., and *Fusarium sp*. Swami and Alane (2013) screened crude extract of various parts of some botanicals containing variable concentration of chemicals against some dominant seed borne fungal pathogens of green gram and reported the inhibitory level of the test crude extracts against these pathogens including *Alternaria alternata, Phytophthora sp., Fusarium oxysporum, Aspergillus niger, Rhizoctonia solani, Curvularia lunata* and *Cladosporium* and reported the inhibitory level of the test crude extracts against these pathogens. The effectiveness of variable concentration of diverse group of chemicals was confirmed on spores of *Alternaria brassicicola* (Meena *et al*., 2011), *Alternaria porae* (Feofilova *et al*., 2012) and *Alternaria alternata* (Bhajbhuje, 2014).

Direct toxicity of heavy metal salts of varying origin to the fungal pathogen does not seem to explain the reduction of symptoms. Chlorides of copper and barium are non-toxic, provided stronger protection than mercuric and cadmium chloride, a highly toxic one. These test chemicals may exert inhibitory influence upon fungal spores germination and impose upon them exogenous dormancy. This is clearly shown by sensitivity of fungal spores to chemicals by several researchers. The inhibition of spore germination may be attributed to variable toxic effect of test chemicals. Similar findings were reported with conidia of *Alternaria tenuis* (Bhajbhuje, 1989); *A. tenuissima* (Singh *et al*., 2000); *A. alternata* (Meena *et al*., 2011; Bhajbhuje, 2014), *A. porae* (Feofilova *et al*., 2012), *A. solani* (Abdel-Kader *et al*., 2012). The hydrolytic products of the chemicals possibly at low conc. induced dormancy or may cause injuries to fungal spores by dissolving the protective thick wall layers and plasma membrane or ruptured them making porous. Aqueous solution of test chemicals diffused through ruptured cell wall and porous plasma membrane to cytoplasm, react with functional cytoplasmic components of spore and seems to disturb a series of physiological processes of spore germination leading to any of the change (i) an inhibitors of trehalose degrading enzymes is destroyed; (ii) the trehalose degrading enzyme is synthesized from its precursor, the conversion being analogous to the trypinogen-trypsin transformation; (iii) the enzyme is thought to be spatially separated from its substrate inside a dormant spores and activation may bring the two together and (iv) a series of interlocking enzyme reactions are shifted from one steady state level (Feofilova *et al*., 2012). In the present investigations, the variable inhibition of fungal spore germination and germ tube growth may be attributed to the differential toxic effect of the test chemicals.

CONCLUSION

The experimental findings reveal that an aqueous solution at 10^{-2}M concentration of plant growth regulators seemed to provide more vigorous defence response to virulent pathogen, *Alternaria solani*. These phytoalexin inducer test chemicals stimulated production of large amount of

fungitoxic substances in susceptible tissue on post-infection of virulent pathogen which make plant resistant to some extent and readily respond to infection. Of the test chemical inducers, naphthalene acetic acid at 10^{-2}M may serve as very promising compounds for use in plant disease control.

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RESEARCH ARTICLE

Effect of Domestic Sewage on Phytoplankton Community in River Rapti at Gorakhpur

Kushwaha VB and Agrahari M

Department of Zoology, D.D.U. Gorakhpur University, Gorakhpur-273008, U.P., India
E mail: vbkddugkp@gmail.com

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ABSTRACT

This paper aims to study the effect of domestic sewage on phytoplankton population in river Rapti at Gorakhpur, U.P. and India. An increase in free CO_2, bicarbonate alkalinity, nitrate, phosphate and BOD while a decrease in pH, DO and carbonate alkalinity was observed at sewage mixing point. However, these parameters gradually changed at the station away from sewage mixing point (downstream) and were within the limits of Indian standards. During the study period total 29 species of phytoplanktons were observed belonging to 4 families: Bacillariophyceae (11), Chlorophyceae (10), Cyanophyceae (6) and Euglenophyceae (2). In present investigation it was observed that population of phytoplankton was very low at station R_2 where sewage mixed into river.

Keywords: Sewage, River, Phytoplanktons, Bacillariophyceae, Chlorophyceae, Cyanophyceae, Euglenophyceae

INTRODUCTION

Water is indispensable for all living organisms on the earth any of the water on this planet is stored in ocean and ice caps, which is difficult to recover for our diverse needs. Any of our demands for water are fulfilled by rain water, which gets deposited in surface and ground water resources. Now days, both surface and ground water resources are contaminated by various sources like industrial effluents, agricultural discharge and municipal waste water associated with large amount of inorganic and organic toxic pollutants along with harmful pathogens (Okoh et al., 2007). Sewage,used water of community generally contains organic as well as inorganic wastes from residences, business houses and industries that might lead to the. It affects physical,

chemical and biological characteristics of the water. Hence, continuous monitoring of river's water quality is very essential to determine the state of pollution in our rivers. Communication of this information to general public and government can help to develop the policies for the conservation of our natural fresh water resources (Ali et al., 2000). Phytoplanktons are a predominant type of a plant found in aquatic bodies. The quality and quantity of phytoplankton is good indicator of water quality. Phytoplanktons (algae) are those microscopic plants contain chlorophyll-a that float or swim too feebly to maintain a constant position against a water current (Lee 2008). Many workers have studies the plankton diversity and effect of domestic sewage on it, (Palmer 1980; Arya et al., 1987; Acharjee et al., 1995; Jha et al., 1997; Mathivanan et al., 2007; Hassan et al., 2008). The objective of this work is to show the effect of domestic sewage on phytoplankton population in river Rapti a tributary of river Ghaghara at Gorakhpur, U.P., India.

MATERIALS AND METHODS

Sampling stations:

Three water sampling stations were selected over 5 kms stretch of river Rapti. Station I (R_1): upstream of sewage discharge, station II (R_2): sewage mixing point, here a continuous discharge of city sewage through a large cemented drain occurs on the bank of the river. About 3 kms away from the station I, station III (R_3): downstream of sewage discharge point, about 2 kms away from station II (Agrahari and Kushwaha ,2012).

Sample collection and their physico-chemical analysis:

The study of the river Rapti at Gorakhpur was done for 12 months (Dec 2009 to Nov 2010). Samples of river water were collected in winter (Dec 2009) and summer (Jun 2010) season from all three stations. Samples were collected in plastic bottles for physico-chemical analysis. For biochemical oxygen demand (BOD) and dissolved oxygen (DO) samples were collected in BOD bottles. Temperature, pH and dissolved oxygen were measured at the site (Agrahari and Kushwaha , 2012).

Methods for analysis of physico-chemical parameters:

The physico-chemical analysis was carried out using the methods given by APHA (1976). pH was measured using pH meter and temperature was measured using simple, mercury filled Celsius thermometer. Nitrate, phosphate and sulphate test kits were purchased from Hi-media Laboratories Pvt. Ltd., Mumbai, India (Agrahari and Kushwaha , 2012).

Collection and Preservation of phytoplanktons:

For the analysis of phytoplankton samples were collected by filtering 30 liters of water through standard plankton counting net. Phytoplanktons were counted with the help of Sedwicq Rafter slide. The phytoplankton samples were observed under Olumpus microscope. The phytopllanktons were identified by using books and journals viz. (Agrahari and Kushwaha,2014; APHA 1985; Jena et al., 2005; Pingale and Deshmukh, 2005; Hosmani, 2008; Perumal and Anand, 2008).

Calculation:

Abundance of phytoplankton was estimated as organisms/liter in the concentrated sample using the equation:

$$N \text{ (number of individuals)/Liter} = \frac{A \times 1000 \times C}{L}$$

Where,

A =Number of phytoplanktons/cc,

C=Volume of concentrated sample taken for counting

L=Volume of water in liter for collection of sample

1000 = Area of counting chambers

Data was subjected to Analysis of variance using software.

RESULTS AND DISCUSSIOINS

The average physico-chemical quality of river Rapti at three sampling stations in Gorakhpur during summer, winter and rainy seasons are presented in Table 1.

The maximum water temperature 33.8 ^{o}C was observed at station R_2 in summer season and minimum 19.9 ^{o}C at station R_3 in winter season. The pH value of the river water at different stations was recorded to be within highest 8.5 at station R_1 in summer season and lowest 7.0 at station R_2 in winter season. Highest value of electrical conductivity 404µmhos/cm was recorded at station R_2 in summer season and lowest value 198µmhos/cm was recorded at station R_1 in rainy season and highest value of TDS 286 ppm was recorded at station R_2 in rainy season and lowest value 102 ppm was recorded at station R_1 in winter season. Dissolved O_2 ranged between 10.2 ppm at station R_1 during winter to 2.4 ppm at station R_2 summer season. Low level of DO is again indicative of polluted nature of water body. Free CO_2 ranged between 20 ppm at station R_2 in summer season and 2 ppm at station R_1 and R_3 in winter season. Chloride concentration ranged between 12 ppm at station R_1 in rainy season to 285 ppm at station R_2 in summer season. The carbonate alkalinity varied from and 24.4 ppm at station R_1 in summer season and 3.5 ppm at station R_2 in rainy season. Bicarbonate alkalinity varied from 81 ppm at station R_3 in rainy season to 762 ppm at station R_2 in summer season. Total hardness, Ca hardness and Mg Hardness varied from a maximum of 492 ppm at station R_2 in summer season and 122 ppm at station R_1 in rainy season, 216 ppm at station R_2 in summer season and 44.6 ppm at station R_3 in rainy season and 62.28 ppm at station R_2 to 11.9 ppm at station R_1 in rainy season respectively. Nitrate was ranged between 0.019 ppm at station R_1 to 1.8 ppm at station R_2 in summer season. Nitrate is one of the most important indicators of pollution of water. Phosphate was found to be maximum of 1.6 ppm at station R_2 in summer season and 0.016 ppm at station R_3 in winter season. Sulphate concentration ranged between maximum of 18.5 ppm at station R_2 in summer season to a minimum of 10.4 ppm at station R_3 in rainy season. The BOD of river water varied from a maximum of 109.4ppm at station R_2 in summer season and 2.2 ppm at station R_3 in winter season. COD values ranged between 51 ppm at station R_2 in winter season to 19.2 ppm at station R_1 in rainy season.

Mean values of phytoplankton (units/ml) of river Rapti at three sampling stations (R_1, R_2 and R_3) in summer, winter and rainy seasons are presented in table 2. 29 species of phytoplanktons were observed belonging to 4 families: Bacillariophyceae (11), Chlorophyceae (10), Cyanophyceae (6) and Euglenophyceae (2).

Bacillariophyceae:

In present study the largest and diverse group is Bacillariophyceae. Diatoms were represented by 11 species *Amphora sp., Navicula sp., Fragilaria sp., Nitzschia sp., Gomphonema sp., Pinnularia sp., Syndra sp., Gyrosigma sp., Surirella sp., Diatoma sp.* and *Melosira sp.* . In the present study maximum number of species of Bacillariophyceae 1983 units/ml in summer season was recorded at station R_1. Minimum number of species 533.1 units/ml in rainy season was recorded at station R_2 where sewage mixed into the river.

Chlorophyceae:

Chlorophyceae was the second group after Bacillariophyceae in the number of identified species observed. In this group 10 species were recorded which are *Chlorella sp., Scenedesmus sp., Zygnema sp., Volvox sp., Ankistrodesmus sp., Ulothrix sp., Cosmarium sp., Mougeotia sp., Pediastrum sp.* and *Spirogyra sp.* In the present study maximum number of species of Chlorophyceae 1616.5 units/ml in summer season was recorded at station R_1. Minimum number of species 241.6 units/ml in rainy season was recorded at station R_2.

Table 1: Mean values of physical and chemical parameters at different sampling stations of the river Rapti at Gorakhpur during summer, winter and rainy seasons.

Tests	Stations									Between Stations	Between Seasons
	Summer			Winter			Rainy				
	R_{S1}	R_{S2}	R_{S3}	R_{S1}	R_{S2}	R_{S3}	R_{S1}	R_{S2}	R_{S3}		
Temp (^{0}C)	33.500	33.800	33.200	20.000	20.000	19.900	27.500	27.700	26.500	*(p<0.05)	**(p<0.01)
pH	8.500	7.400	8.200	8.000	7.000	8.200	8.200	7.200	7.500	*(p<0.01)	
Elec Cond, (µmhos/cm)	220.000	404.000	309.000	201.000	264.000	224.000	198.000	245.000	203.000	*(p<0.05)	*(p<0.05)
TDS (ppm)	122.000	260.000	182.000	102.000	207.000	164.000	140.000	286.000	185.000	*(p<0.01)	*(p<0.01)
DO_2 (ppm)	7.600	2.400	6.900	10.200	3.600	8.200	8.800	3.600	8.000	**(p<0.01)	*(p<0.01)
Free CO_2 (ppm)	8.000	20.000	6.000	2.000	6.000	2.000	4.000	18.000	5.400	*(p<0.01)	*(p<0.05)
Cl_2 (ppm)	35.000	285.000	46.000	20.000	270.000	48.000	12.000	109.000	20.200	*(p<0.01)	
CO_3 (ppm)	24.400	8.000	14.000	14.400	4.000	8.400	6.800	3.500	4.000	*(p<0.01)	*(p<0.01)
HCO_3 (ppm)	372.000	762.000	608.000	208.000	660.000	301.000	92.000	115.000	81.000	*(p<0.05)	*(p<0.01)
Tot Hard (ppm)	200.000	492.000	268.000	188.000	424.000	220.000	122.000	450.000	198.000	**(p<0.01)	*(p<0.01)
Ca Hard (ppm)	65.400	216.800	68.600	49.700	95.600	69.600	54.500	65.800	44.600		
Mg Hard (ppm)	24.160	54.380	39.640	21.880	62.280	24.000	11.900	51.100	25.760	*(p<0.01)	
NO_3 (ppm)	0.038	1.800	0.190	0.019	0.072	0.038	0.049	0.480	0.071	*(p<0.01)	
PO_4 (ppm)	0.060	1.600	0.400	0.020	0.380	0.016	0.040	1.540	0.600	*(p<0.01)	
SO_4 (ppm)	11.600	18.400	12.600	14.800	18.500	14.500	11.900	12.900	10.400	*(p<0.01)	*(p<0.01)
BOD (ppm)	6.800	109.400	5.900	3.200	69.400	2.200	4.000	70.400	5.000	*(p<0.01)	
COD (ppm)	23.200	46.500	32.500	22.000	51.000	39.800	19.200	49.600	30.800	*(p<0.01)	

**(p<0.01) indicates highly significant differences

*(p<0.01) and *(p<0.05) indicates significant differences

Table 2: Mean values of phytoplanktons (units/ml) at different sampling stations in the river Rapti at Gorakhpur during summer, winter and rainy seasons.

Tests		Stations									Between Stations	Between Seasons
		Summer			Winter			Rainy				
		R_{S1}	R_{S2}	R_{S3}	R_{S1}	R_{S2}	R_{S3}	R_{S1}	R_{S2}	R_{S3}		
Bacillariophyceae	*Amphora sp.*	183.30	125.00	75.00	241.60	166.60	100.00	183.30	58.30	150.00	*(p<0.05)	
	Navicula sp.	266.60	166.60	166.60	233.30	200.00	66.60	225.00	100.00	83.30	*(p<0.01)	
	Fragilaria sp.	316.60	283.30	283.30	250.00	100.00	175.00	66.60	83.30	83.30		*(p<0.01)
	Nitzschia sp.	183.30	58.30	66.60	100.00	-	-	58.30	66.60	50.00	*(p<0.05)	
	Gomphonema sp.	208.30	175.00	141.60	200.00	166.60	100.00	100.00	66.60	-	*(p<0.01)	**(p<0.01)
	Pinnularia sp.	175.00	66.60	91.60	266.60	83.30	100.00	100.00	58.30	-	*(p<0.01)	*(p<0.05)
	Synedra sp.	225.00	200.00	166.60	-	-	-	100.00	-	83.30		*(p<0.01)
	Gyrosigma sp.	125.00	75.00	83.30	-	-	-	141.60	100.00	100.00	*(p<0.05)	*(p<0.01)
	Surirella sp.	-	-	-	-	-	-	100.00	-	66.60		*(p<0.01)
	Diatoma sp.	133.30	66.60	75.00	125.00	-	100.00	-	-	-		*(p<0.05)
	Melosira sp.	166.60	-	100.00	-	-	-	-	-	-		*(p<0.05)
	Total	**1983.00**	**1216.40**	**1249.60**	**1416.50**	**716.50**	**641.60**	**1074.80**	**533.10**	**616.50**		
Chlorophyceae	*Chlorella sp.*	175.00	-	66.60	-	-	-	-	-	-		
	Scenedesmus sp.	133.30	50.00	175.00	150.00	-	200.00	150.00	66.60	91.60	*(p<0.01)	
	Zygnema sp.	275.00	83.30	141.60	116.60	-	58.30	-	-	-		*(p<0.01)
	Volvox sp.	133.30	66.60	91.60	-	-	-	150.00	50.00	83.30	*(p<0.05)	*(p<0.01)
	Ankistrodesmus sp.	300.00	166.60	225.00	250.00	75.00	66.60	-	-	-		*(p<0.01)
	Ulothrix sp.	100.00	58.30	75.00	200.00	75.00	91.60	150.00	50.00	100.00	*(p<0.01)	
	Cosmarium sp.	133.30	50.00	166.60	66.60	75.00	100.00	-	-	-		*(p<0.01)
	Mougeotia sp.	200.00	66.60	100.00	-	-	-	141.60	75.00	83.30		*(p<0.01)
	Pediastrum sp.	-	-	-	166.60	50.00	100.00	-	-	-		*(p<0.01)
	Spirogyra sp.	166.60	58.30	200.00	200.00	-	75.00	-	-	-		*(p<0.05)
	Total	**1616.50**	**599.70**	**1241.40**	**1149.80**	**275.00**	**691.50**	**591.60**	**241.60**	**358.20**		

Table 2: Continued...

Tests		Stations									Between Stations	Between Seasons
		Summer			Winter			Rainy				
		R_{S1}	R_{S2}	R_{S3}	R_{S1}	R_{S2}	R_{S3}	R_{S1}	R_{S2}	R_{S3}		
Cyanophyceae	*Microcystis sp.*	291.60	300.00	100.00	133.30	166.60	150.00	-	-	-		*(p<0.01)
	Oscillatoria sp.	200.00	266.60	100.00	166.60	166.60	75.00	100.00	125.00	100.00	*(p<0.05)	
	Anabaena sp.	200.00	200.00	200.00	166.60	175.00	50.00	100.00	133.30	91.60		*(p<0.05)
	Merismopedia sp.	100.00	200.00	100.00	-	-	-	-	-	-		*(p<0.01)
	Spirulina sp.	100.00	100.00	66.60	-	-	-	-	-	-		*(p<0.01)
	Nostoc sp.	166.60	266.60	183.30	75.00	158.30	66.60	-	-	-	*(p<0.05)	*(p<0.01)
	Total	**1058.20**	**1333.20**	**749.90**	**541.50**	**666.50**	**341.60**	**200.00**	**258.30**	**191.60**		
Euglenophyceae												
	Euglena sp.	200.00	75.00	66.60	100.00	-	66.60	-	-	-		*(p<0.05)
	Phacus sp.	100.00	100.00	175.00	150.00	-	100.00	-	-	-		*(p<0.05)
	Total	**300.00**	**175.00**	**241.60**	**250.00**	**-**	**166.60**	**-**	-	-		

**(p<0.01) indicates highly significant differences

*(p<0.01) and *(p<0.05) indicates significant differences

Cyanophyceae:

Cyanophyceae was represented by 6 species of which *Microcystis sp., Oscillatoria sp., Anabaena sp., Merismopedia sp., Spirulina sp.*and *Nostoc sp.* In the present study maximum number of species of Cyanophyceae 1333.2 units/ml in summer season was recorded at station R_2. Minimum number of species 191.6 units/ml in rainy season was recorded at station R_3.

Euglenophyceae:

Only two species of Euglenophyceae were recorded that is *Euglena sp*. and *Phacus sp.* In the present study maximum number of species of Euglenophyceae 300 units/ml in summer season was recorded at station R_1. Minimum number of species 166.6 units/ml in summer season was recorded at station R_3. Group Euglenophyceae was absent in rainy season.

DISCUSSION

In present investigation an increase in electrical conductivity, TDS, free CO_2, bicarbonate alkalinity, total, Ca and Mg hardness, chloride, nitrate, phosphate, sulphate, BOD and COD while a decrease in pH, DO_2 and carbonate alkalinity was observed at sewage mixing point. However, these parameters gradually changed at the station away from sewage mixing point (downstream). A highest value of electrical conductivity was recorded at station R_2 in summer season. This might be due to the addition of sewage into it. An increase in electrical conductivity is regarded as pollution indicator in water bodies (Das et al., 2006; Agarhari and Kushwaha, 2012). An increase in TDS at station where sewage meets river water indicates an increase in pollution. Water with high dissolved solid is of inferior quality and may induce adverse response in the body of the consumer (Agarhari and Kushwaha, 2012; Mahor, 2011). Low level of DO is again indicative of polluted nature of water body. Such low level of oxygen was also noted by Iqbal et al. (2006). At station R_2 saturation level of dissolved O_2 was very low in winter and rainy season. It may be due to high rate of oxygen consumption by oxidizable matter coming in along with sewage. Free CO_2 present in large amount at station R_2 can be attributed to high BOD load that comes with consumption of oxygen and release of CO_2 by the respiratory activity of the living organisms. Maximum values of free CO_2 recorded at station R_2 during summer might be due to acceleration in the rate of decomposition of organic matter by microbes, decrease of photosynthetic activity and high rate of respiration by benthic biota and microorganisms as observed by Hedge and Bharti (1985) and Sinha (1988). Maximum values of bicarbonates alkalinity recorded at station R_2 and R_3 were probably due to the input of domestic sewage. Shah (1988) noticed higher concentration of bicarbonate alkalinity in the domestic sewage during the study of river Jhelum. High fluctuation of Ca, Mg and total hardness were recorded at station R_2. In the present study higher values of Ca, Mg and total hardness observed at all the three sampling stations (Table 1 and 2) may be due to input of domestic sewage which contains organic matters. Cl_2 was found to be highly marked, higher values of Cl_2 recorded at station R_2 was due to the continuous influx of contaminated domestic sewage. Similar results are reported by Sinha (1988) in the case of river Yamuna and river Damodar. The maximum values of phosphate observed at station R_2, in comparison to other stations throughout the study period may be due to the discharge of contaminated domestic sewage containing decayed organic matter (Shah, 1988; Rana and Palria, 1988). Higher values were recorded at station R_2, owing to high amount of organic matter in domestic sewage (Paramshivam and Sreenivasan (1981) and Somashekar (1985) also reported that an increase in BOD and bacterial level as indicative of increasing pollution, which is supported by Sinha (1988). Station wise, maximum values of COD were recorded at station R_2 indicating presence of organic wastes in sewage.

In present investigation it was observed that population of phytoplankton was low at station R_2 where sewage mixed into river. This lowering of phytoplankton population at station R_2 is due to the presence of organic and inorganic matters of sewage that affects the physico-chemical quality of water as evidenced earlier by many workers (Kang et al., 2004; Shirodkar et al., 2010; Bhardwaj et al., 2010). And physico-chemical properties show effect on the phytoplankton diversity. Many previous studies showed the effect of physico-chemical parameters on plankton community. Hassan *et al*., (2008) studied the effect of chemical and physical properties of River water in Shatt-AI-Hilla on phytoplankton communities, Sukumaran and Das, (2002) reported that the basic process of phytoplankton production was dependent upon temperature, turbidity and nutrients. The role of temperature in the development of algae has been emphasized by many workers from time to time (Palharya et al., 1993). Limnological studies of water quality are based on the principle that every type of aquatic ecosystem is associated with one specific community of organisms, the living communities that develop in aquatic ecosystems depend on specific physico-chemical characteristics of water and are noticeably modified when those conditions change (Kushwaha and Agrahari, 2014). Factors such as dissolved oxygen, transparency, depth, salinity, pH, temperature and nutrients influence the occurrence, abundance and distribution of Planktonic organisms (MBO, 2007) and effect their composition and distribution from place to place and year to year due to the dynamic nature of the aquatic system (FAO, 2006). In present observation temperature shows a moderate value in summer season which is favorable for growth of phytoplankton. Nutrients (nitrates and phosphates) showed low values in both seasons where as it increases at station-R_2 due to presence of domestic sewage. Dissolved Oxygen decreases at station R_2 that causes lowering of phytoplankton population. Presence of Dissolved oxygen is essential to maintain the biological life in the water Palhrya, et al., 1993). In this observation it was found that group Cyanophyceae showed higher population at station R_2 in comparison to other station because member of Cyanophyceae group are known to be highly adaptive and can colonize even the polluted area (Palhrya, et al., 1993). Present observation also showed seasonal variations in phytoplankton communities, phytoplankton productivity was high during summer and low during winter season as evidenced earlier by Sadguru et al., (2002), Sharma et al., (2011) and Agrahari and Kushwaha (2012). The lowering of population of phytoplankton in winter can be attributed to low temperature (Sadguru et al., 2002; Hassan et al., 2008; Gross and Pfiester, 1988).

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RESEARCH ARTICLE

Impact of Heavy metal, Arsenic trioxide on Biochemical profile of teleost, *Clarias batrachus* (Linn.)

Pundir Garima[1]* and Pundir Himanshu[2]

[1]Department of Zoology , R. G. (P.G) College, Meerut UP, India

[2]Department of Computer Science and Technology, Radha Govind Engineering College Meerut UP, India,

Address for correspondence Email: gaarimaa112@yahoo.co.in

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ABSTRACT

The present investigation aims at evaluating the toxic effect of heavy metal, Arsenic trioxide on biochemical profile of *Clarias batrachus* after 30, 45 and 60 days of post treatment with experimental chemical , arsenic trioxide. Lc 50 value calculated for sublethal study of arsenic trioxide was 8.7 mg/l. Following biochemical parameters were analysed: Serum Protein, Serum Cholesterol. Serum Glucose, Acid phosphatase and Alkaline phosphatase**.** Decrease in serum protein observed in present study was due to liver cirrhosis or nephrosis. Increase in cholesterol indicated environmental stress in *Clarias batrachus* .The significant reduction in plasma glucose levels during acute treatment indicates hypoxic condition. Increased stimulation of alkaline phosphatase corresponds to pathological processes as liver impairment, kidney dysfunction and bone disease.

Keywords: Arsenic trioxide, *Clarias batrachus*, Lethal concentration , Acid phosphatase, Alkaline phosphatase.

INTRODUCTION

The effect of heavy metals on aquatic organism is currently attracting wide spread attention particularly in studies related to industrial pollution. High toxicity of industrial pollutions have been known since long time, but their hazardous nature as pollution of aquatic environment has been matter of concern only after a large number of deaths of fishes occurring in different areas due to different metals. In aquatic environment, fishes are usually regarded as organisms of choice for assessing the effects of environmental pollution on aquatic ecosystems

Gernhofer *et al.* (2001). Despite progress made in environmental waste management, heavy metals still pose immense health hazards to humans and biota unlike other classes of pollutants, which can be biodegraded and destroyed completely. The name "Arsenic" is derived from the Greek word "arsenikon", which means yellow orpiment. Arsenic compound have been mined and used since ancient times. The extraction of the element from arsenic compound was first reported by Albertus Magnus in 1250 A.D. Emsley (2001**)** Arsenic, a heavy metal ranks 20th in earth's crust, 14th in sea water and 12th in human body. Arsenic exhibit metallic as well as non-metallic properties. Arsenic is aknown chemical element that has the symbol 'As' and atomic number 33. Its atomic mass is 74.92 and is prevalent in the environment, occurring both naturally and as a result of environment pollution.

Sources of arsenic include treatment of wood using chromate copper arsenate, burning of coal in thermal power plants, operation of gold minning, as treatment of land with arsenical pesticides. Arsenic occurs naturally and its use is possibly aggravated by the use of over powering aquifers and by phosphorous from fertililizers, production of dyes from tanneries, application of some herbicides and insecticides. It is present in effluents from Laundring Tamaki and Frankenbeger (1992) Arsenic, an important environmental contaminant, is present in the aquatic environment as a result of geogenic and anthropogenic processes, Gonzalez *et al.* (2006); Singh and Banerjee (2008). Biochemical characteristics of blood are among the important indices of the status of internal environment of the fish organism (Luskova, 1997). The present study focuses on the impact of arsenic on biochemical profile of *Clarias batrachus* .Arsenic generally exists in the inorganic form in water samples. Under different redox conditions arsenic is stable in the +5, +3, -3, and 0 oxidation states. The pentavalent (+5) arsenic or arsenate species include AsO43-, and H2AsO4-. The trivalent (+3) arsenic or arsenite species include As (OH)4-, AsO2(OH)2-, and AsO-3. The pentavalent arsenic species are predominant and stable in the oxygen-rich aerobic environment, whereas the trivalent arsenic species are predominant in the moderately reducing anaerobic environment such as groundwater.

MATERIALS AND METHODS

Test fish:

Healthy living specimen of teleost, *Clarias batrachus* were collected from local fish market of Meerut. Fish measuring 15 ± 2cm in length and 60 ± 8 gm in weight were selected for the present study. Selected fishes were acclimatised to the laboratory conditions for period of 15 days.

Preparation of stock solution and determination of 96 hr LC 50 value of Arsenic trioxide:

1gm of arsenic trioxide stock solution was prepared by dissolving arsenic trioxide in 1N HCl under constant heating. The pH was adjusted to 7.4 by adding 1N NaOH dropwise and the solution was filtered by passing through filter paper. For the determination of median tolerance limits or LC 50, different concentrations of arsenic trioxide (20, 30, 40, 50, 60, 70, 80 and 90 mg/l) were prepared from the stock and added in separate glass aquaria containing 50 L of water.

Chemical exposure and Experimental design:

Fishes were divided into 4 equal groups each comprising of 30 fishes. Each group was kept in separate glass aquaria of 250 litre capacity. First group was treated as control group. Fishes of other 3 groups were treated with sub-lethal concentration 8.7mg/l arsenic trioxide for period of 30, 45 and 60 days. Water in the aquariums were renewed after 24 hours and fresh solution of the toxicants were added to bring the concentration to the desired level.

Biochemical studies:

All biochemical studies were performed with the serum of control as well as treated groups of fishes.

Preparation and preservation of serum:
Fish blood was centrifuged at the speed of 3000 rpm. The serum was separated and preserved in the refrigerator at -20^{0}C in the deep freezer. These vials were properly labelled according to the experimental design. Whenever the serum was required, it was first of all brought to the room temperature and then further estimations were done.

1. Determination of Serum Protein
Total serum protein was determined by Kjeldahl's digestion

2. Determination of Serum Cholesterol
Serum cholesterol was estimated with the help of one step method (Wybenga and Pilleggi).

3.Determination of Glucose
Glucose level was estimated by Kit method (End point o-toludine).

4. Determination of Acid Phosphatase – According to Kind and King's kit method.

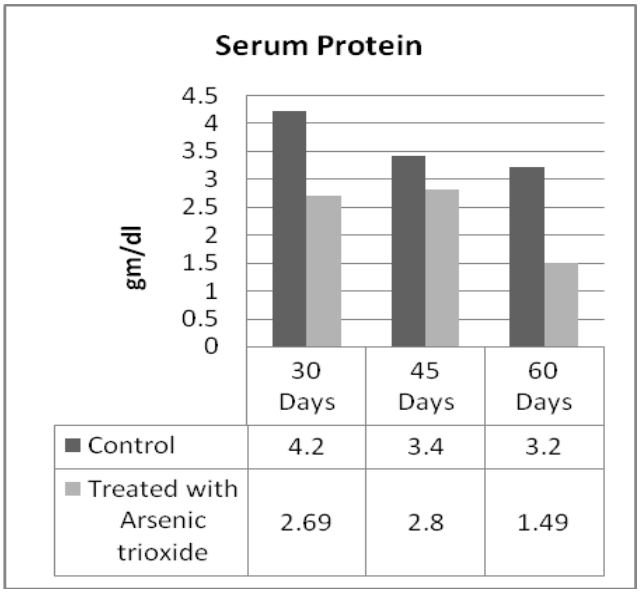
5. Determination of Alkaline Phosphatase:
For the estimation of serum alkaline phosphatase Kind and king's

RESULTS AND DISCUSSIOINS

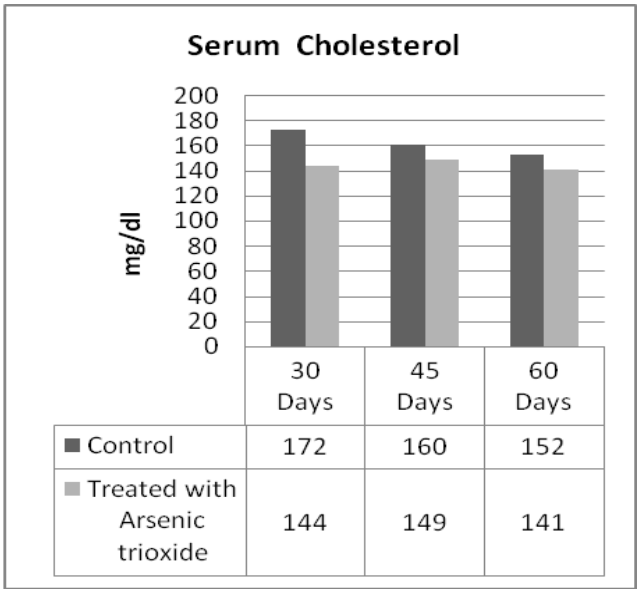
The Serum protein was observed to be 2.69 gm/dl after 30 days of PT with arsenic trioxide. The observed value showed difference in parameter when ($p<0.05$), compared with control values after 30 days . The Serum protein showed increase of 2.80 gm/dl after 45 days of PT with arsenic trioxide. While this parameter showed decline of 1.49 gm/dl after 60 days of PT with arsenic trioxide.

In the present investigation the decrease in Serum protein during acute and sublethal treatment is supported by the reports of Nandi *et al.*, 2005. Palaniappan and Vijayasundaram (2009) suggesting that the decrease in plasma protein may be due to liver cirrhosis or nephrosis or might be due to alteration in enzymatic activity involved in protein biosynthesis. Pazhanisamy (2002) reported change in total protein content of various tissues in different fishes exposed to different heavy metals. Jana and Bandyopathyay (1981); Jatyajit (1996); Baskaran and Palanichamy (1995) have reported such a reduction in protein content when the fish *Channa punctatus* has been exposed to heavy metals such as mercury, arsenic and lead and *Channa striatus* exposed to mercury cadmium and lead. Gagnon *et al.* (2006) reported that due to metal complex formation, normal functioning of cell is disturbed and that in turn may result in variation on physiological and biochemical mechanisms of animals. Serum Cholesterol was observed to be 144mg/dl after 30 days of PT with arsenic trioxide. The observed value was found to decline when ($p<0.05$), compared with control values after 30 days. This parameter showed increase 149 mg/dl after 45 days of PT with arsenic trioxide. 60 days of PT with arsenic trioxide showed decline of 141 mg/dl. The observed value showed decrease when ($p<0.05$), compared with control values after 60 days. Serum Cholesterol showed initial increase after 45 days and decline was noted after 60 days. Heavy metals are known to have hazardous effects on cell structure, especially on the membranes. Therefore, it becomes evident that increase in cholesterol may be the indications of environmental stress. The present findings are in agreement with studies of Murray (1991); Gill and Epple (1993); Sastry and Shukla (1994) who pointed that hyper cholestrolemia observed in *Clarias* may be due to impairment of liver and inhibition of enzymes, which converts cholesterol into bile acid.

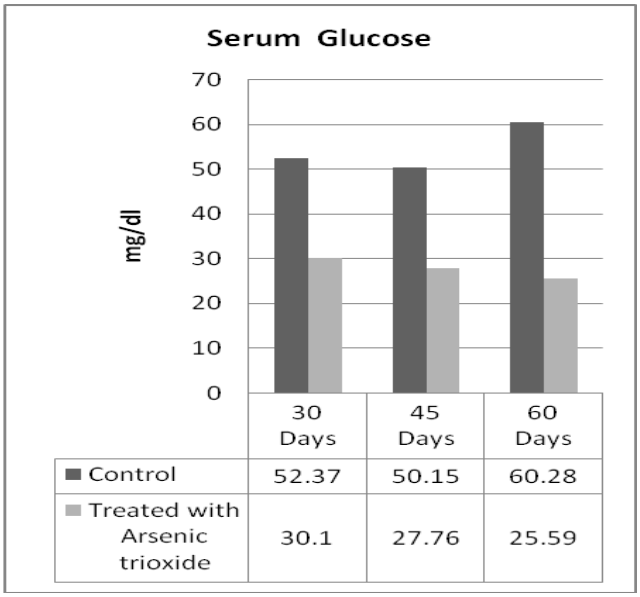
Serum Glucose was observed to be 30.10 mg/dl after 30 days of PT with arsenic trioxide. The observed value was found to decline when ($p<0.05$), compared with control values after 30 days. This parameter showed decline of 27.76 mg/dl after 45 days of PT with arsenic trioxide. While after 60 days of PT with arsenic trioxide



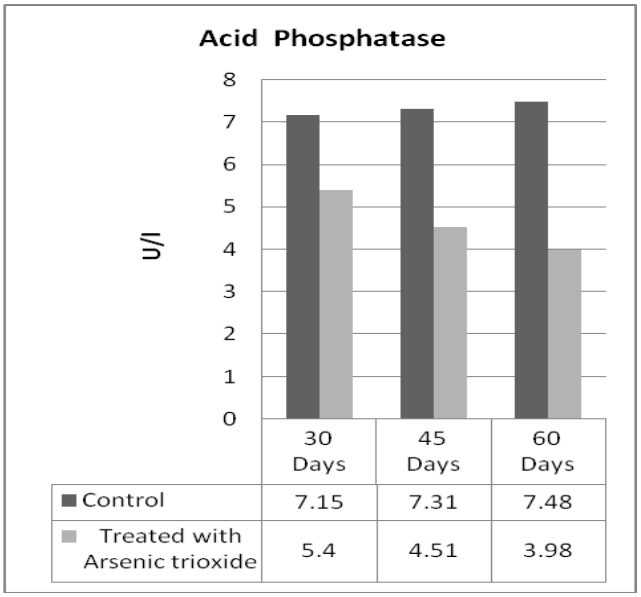
	30 Days	45 Days	60 Days
Control	4.2	3.4	3.2
Treated with Arsenic trioxide	2.69	2.8	1.49



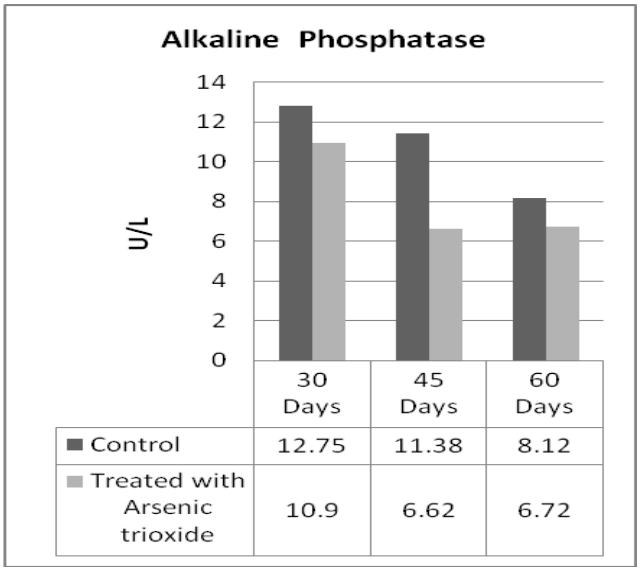
	30 Days	45 Days	60 Days
Control	172	160	152
Treated with Arsenic trioxide	144	149	141



	30 Days	45 Days	60 Days
Control	52.37	50.15	60.28
Treated with Arsenic trioxide	30.1	27.76	25.59



	30 Days	45 Days	60 Days
Control	7.15	7.31	7.48
Treated with Arsenic trioxide	5.4	4.51	3.98



	30 Days	45 Days	60 Days
Control	12.75	11.38	8.12
Treated with Arsenic trioxide	10.9	6.62	6.72

this parameter showed further decline of 25.59mg/dl. The serum glucose showed decline after 45 and 60 days of exposure period Very little attention is paid on effect of arsenic on Serum glucose level in *Clarias batrachus* . Tseng., 2004 reported that chronic exposure of arsenic or its methylated metabolites induced diabetes mellitus in rats and this condition may be responsible for hyperglycemia. Thus an elevation of blood glucose level in the present study during sublethal treatment might be due to gluconeogenesis to provide energy for the increased metabolic demands imposed by arsenic stress. The significant reduction in plasma glucose levels during acute treatment might be

due to hypoxic conditions caused by arsenic leading to an excess utilization of stored carbohydrates.

Acid Phosphatase was observed to be 5.40U/L after 30 days of PT with Arsenic trioxide. The observed value was found to decline when ($p<0.05$), when compared with control values after 30 days of exposure period. After 45 days of exposure period the observed value showed decline of 4.51 U/L, further decline in parameter was observed to be 3.98U/L after 60 days of PT with arsenic trioxide. Sastry and Gupta (1979) reported elevation in activity of acid phosphatase in *Channa punctatus* under lead exposure. The rise in the activities of acid phosphatase due to lead toxicity leads to hepatocellular damage in the organism Sharma (1999). This increase is associated with liver damage as this enzyme is known to be associated with lyososomal activity. It has been suggested that the acid phosphatase elevation causes proliferation of lysosomes in an attempt to sequester the toxic xenobiotic (Gill and Epple, 1992).

Alkaline phosphatase was noted to be 10.90U/L after 30 days of PT with arsenic trioxide. This value was found to be declined when ($p<0.05$), compared with control values after 30 days. This parameter showed decline of 6.62 U/L after 45 days of PT with arsenic trioxide. The observed value showed decline in parameter when ($p<0.05$), compared with control values after 45 days. Alkaline phosphatase showed increase of 6.72U/L after 60 days of PT with arsenic trioxide. Since very less work has been reported directly on this metal but the findings of present work coincides with findings of workers on other heavy metals. The result is in agreement with findings of Agarwal and Sastry (1979) who have observed significant increase in activity of ALP in *Channa punctatus* after 96 hr of post treatment with mercuric chloride. Gill *et al.*, 1991 and Ranjeeta (2008) recorded an increase in alkaline phosphatase activity in *Puntius conchonius* and *Clarias batrachus* under mercuric chloride intoxication and endosulfan exposure. Ilyas et al., (2007) also noticed the same result in *Labeo rohita*. Such result might be due to increase in osteoblastic activity or intra and extra hepatic obstructions of biliary passage Jyothi and Narayan (1999).

CONCLUSION

In the present study variations in all biochemical parameters were recorded with duration of exposure to experimental chemicalArsenic trioxide. Thus it is indicated that heavy metal arsenic trioxide is causing harmful alterations in biochemical profile of economically important food fish, *Clarias batrachus.*

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RESEARCH ARTICLE

Food and feeding of an economically important estuarine fish, *Sillago sihama* (forsskal)

Yeragi SS* and Yeragi SG

Department of Zoology, K.J. Somaiya College of Science, Vidhyavihar, Mumbai-400 077, MS, India

*Corresponding author E-mail : dryeragi@gmail.com.

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ABSTRACT

The present investigation revealed that the stomach content of both juvenile (<130 mm SL) and adult (>130mm SL) *Sillago sihama* (Forsskal) in Mithbav (L.16^0 20' N.L.17^0 25')estuary of Sindhudurg District, Maharashtra from April 2011 to March 2012. The gut analysis was carried out using frequency of occurrence and point methods. Diatoms were found to be the most preferable food of plant origin in both the life stages like juvenile and adult in all the season, Diatoms, blue-green algae and dinoflagellactes constituted main food of plant origni. Diatoms were found to be the most preferable food during starvation period of monsoon due to unexpected water currents. During monsoon, the habit of food and feeding was distured for short time. They do not remain in main stream of water but move towards the bank, bay, as well as in lagoons of the estuary. During monsoon it occurred in more than 11.3% of food item by point method. The feeding intensity of juvenile was notice that on increasing along with increase in size group. The quality and quantity of food items were fluctuated seasonwise to season and juvenile to adult stages. Crustacean including shrimps, crabs, their larvae, copepods eggs, and larval forms comprised the maximum part of the food of animal origin. It was concluded that *sillage sihama* in the coastal waters of Mithbav is planktonivorous and feeding on a wide range of food of planktonic and benthic organisms.

Key words: *Sillago sihama*, feeding, Mithbav.

INTRODUCTION

Creeks, estuaries are an aquatic environment provide numerous commercially important resources that solve the problem of

bread and butter of poor coastal natives. It is but natural, that these resources support the human life in different ways. During monsoon, the sea fishery is totally stopped for short time hence food remains the basic need of manking for survival. The organisms like fish, shrimps, oystere, clams mussles crabs are harvested on large scales, of all trophic levels are available in estuarine ecosystem. Those of second, third and highest levels are known to be rich in protein in terms of quantity and quality particularly in eight amino acids necessary for human being. These amino acids are easily consumed through all through edible resource organisms.

In an aquatic ecosystem, every resource organism can serch for favourable habitat to fulfil the demand of their food and safety. There they live, breed to form the nursery ground. Feeding ecology is the significant aspect of the life history strategy of every living speeies to understand the functional role of the fish within their ecosystem (Patole, 2009). It is an important aspect in an aquacultural practices. *Sillago sihama* (Indian, whiting fish) is the commercially important high market prised protein rich fish for coastal people. However without knowledge of the food requirements, feeding behavior pattern and predator-Prey relationships is not possible to understand the predieted changes that might result from any natural or anthropogenic intervention (Yeragi, 1997). *Sillago sihama* (family-Sillaginidae), is commercially and recreationally important estuarine and near shore speeies of Mithbav coastal zone. It is eco-sensitive fish and in future could be used for exploitation of blue revolution to solve the problem of basic food. It is better to understand their hydrological status, selectivity of food and life history in coastal waters. In pre-monsoon time, their schools are migrating in Mithbav estuary for breeding, spawning as well as for nursing purposes. They always prefer sandy-muddy ground for quick burrow to avaid predation. It is golden yellow in colour with sharp mouth which enhance for burrow in soft sandy regions. It has two dorsal fin, first with 10-15 slender spines, second with one leading spine and 16-27 soft rays. Anal fin is long with two leading spines, with 14-20 soft rays. Family-sillaginidae includes 33 species but only one genus. This is the exceptional family amongst the finfish. Larvae and juveniles are pelagic and feeding on plankton. The body encloses two bladder with two anterior and two posterior extension. Sexual maturity attended about 13-19 cm. SL. Maximum length (SL) observed in this estuary was above 30-35 cm. but commercially markatable size was 20 cm and age is around seven years. The spawning was recorded in the month of july-August. In low salinity the growth is faster and within two month period development reached to fingerling stage. The first sexual maturity was observed at L130-140mm. at the age of one year. The ovulation is once in a year.

The objective of the present study were.

1) to explain the stomach contents of juvenile (130 mm<) and audit (130 > mm) of *S.sihama*
2) to justify dietary difference amongst variable size classes of juveniles
3) to determine seasonal changes in the diets of adults of said species
4) to compare feedng habits between the juvenile and adult.

MATERIALS AND METHODS

The study site, Mithbav estuary opening broadly to the west coast of India. Individuals of *Sillage sihama* of 130 mm. in standard length (SL) or more were difined as adults, following histological examination of the gonads. To examine seasonal dietary differences, adults were collected monthly from cast net, gill net and filter (Yendi) net. The fishery was conducted within the estuary from April 2011 to March 2012. The juveniles were collected 3-4 cm. length samples with help of Yendi along with *Penaeus indicus* (white shrimp) on mud-flat region of mangrove swamp. The adults were collected through the bottom fishery of gill-net (Tiyana), cast net and others throughout the year.

In the laboratory (Local), immediately of collection, SL and body weight were measured for each juvenile and adult specimens to the nearest 1mm and 0.1 gm, respectively. Juveniles were sorted into 5 size classees (≤ 10mm SL 11-40 mm SL, 41-70 mm SL, 71-100 mm SL and 101-129mm SL). Food items from the stomach contents of each specimen were identified to the lowest possible tax on and the percentage volume of each in the diet visually estimated under a binocular microscope.

RESULTS AND DISCUSSIOINS

In the present investigation, many live specimens of *Sillago sihama* were used for examination. The species were dissected out to collect the guts carefully under hygenic condition and after word they retured to the coastal people. The length range for adult was 14.21.-21.80 cm (>130mm SL) and for Juveniles (<130mm SL). Bacillario-phyceae (diatoms), Cyanophyceae (blue-green algae) and Dinophyceae (dinoflagellates) constitute the main food of plant origin in monsoon and post-monsoon seasons for both the size groups. The juveniles voraciously feed more than adult on cyanophyceae and Dinophyceae. The detritus was observed highest 11.15% in juvenile during monsoon period and then gradually decline upto pre-monsoon time. In monsoon adults also consumed more detritus to avoid starvation due to speedy water current and flood condition. The percentage composition of detritus was 19.15% in monsoon and lowest 1.17% in pre-monsoon.

Table 1 : Seasonal occurrence of food items of adult *Sillago sihama* in the coastal waters of Mithbav estuary.

Food Categories	Monsoon %	Post monsoon %	Pre-monsoon %
Diatoms	11.13	29.17	07.18
Blue-green algae	05.11⁰	04.28	01.17
Dinoflagellates	04.21	05.19	03.15
Decapods	23.15	24.17	21.15
Mollusca	0.5	01.51	04.17
Foraminifera	01.21	0.15	01.18
Copepods	12.15	26.51	28.91
Polychaetes	01.25	02.12	03.17
Detritus	19.15	02.15	01.17
Animal derivatives	11.19	13.85	15.36
Eggs	08.71	03.15	05.18
Fish	02.24	07.78	08.21

Table 2 : Showing seasonal variation in food items of Juvenile *S. Sihama.*

Food Categories	Monsoon %	Post monsoon %	Pre-monsoon %
Diatoms	17.13	18.24	06.17
Blue-green algae	07.15	09.19	04.45
Dinoflagellates	08.32	09.26	02.18
Decapods	20.18	18.27	17.62
Mollusca	01.71	02.51	08.91
Foraminifera	0.8	02.15	03.31
Copepods	13.18	21.63	24.19
Polychaetes	0.19	01.92	02.61
Detritus	11.15	3.25	03.21
Animal derivatives	07.93	8.26	13.65
Eggs	08.84	03.12	09.18
Fish	03.42	02.22	04.52

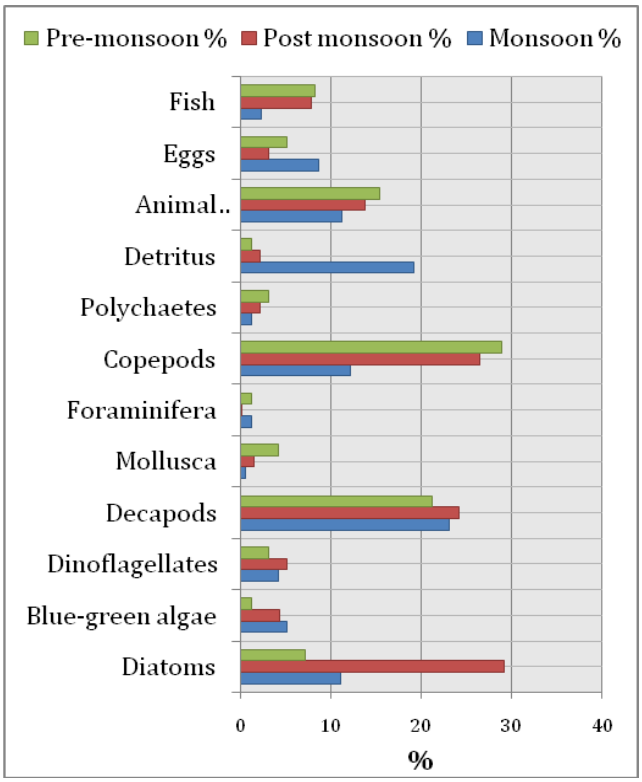

Fig.1: Histogram Showing seasonal variation in food items of Adult *S.Sihama*

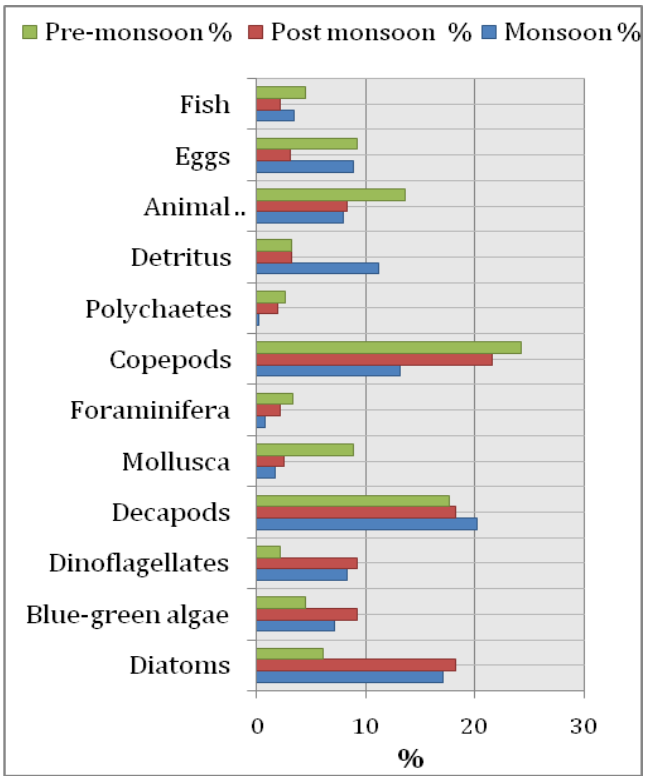

Fig. 2: Histogram showing seasonal Variation in food items of Juvenile *S.sihama.*

During flood time these fish move towards the bank of estuary to avoid unfavorable conditions. The coastal living people catch the fish easily in lagoon, bays and near by the bank with help of cast net. The major food items found in the stomach of *S. sihama* are shown in Table. 1 & 2 In point method, diatoms were found to be the most selective food of plant origin. It was occurred in more than 17.13% in monsoon, 18.24 in post monsoon while decline in pre-monsoon to 6.17%. It was noticed that during pre-monsoon the juveniles voraciously feed on copepods whose percentage composition was 24.19% highest in all the seasons. It is also seen that the Diatoms and copepods were inversely proportion as food items. In aduit the parentage of Diatom as food item was hightest in monsoon (11.13%) and lowest (7.18%) in pre-monsoon due to availability of copepods. Decapods, like juvenile shrimps, crabs, hermit crab were found to be highest in adult during post monsoon (24.17%) while in juverules maximum in monsoon (20.18%). The percentage compositions in both the groups were found to be slightly changed from season to season. It also observed that the highest peak was in monsoon followed by post monsoon. Copepods and animal derivatives as a groups contributed about 11.19% monsoon and highest 15.30% in pre-monsoon in adult while in juvenile lowest 7.91% in monsoon and maximum 9.18% in pre-monsoon. The percentage compositions of food items like mollusks, foraminifera, were least in all the seasons. The molluscan food items was highest in both during pre-monsoon because of clams, oysters, mussels, solri, *Perna*, larvae found to be plenty in pre-monsoon season. The juveniles shrimps were detected heights in monsoon and pre-monsoon period. Animal derivatives (eggs, scales, crustaceans appendages, etc) were peak during post monsoon and pre-monsoon. The choises of food items were found to be maximum in post and pre-monsoon their monsoon than. The intensity, of feeding is directly corelated to season

as well as size groups. The juveniles spend more time in feeding than adults. The adults were restricted to the feeding ground and prefer deep water areas, while jureniles spend more time towards the bank of estuary.

From the above mentioned observation, it is clearly understood that this speeies could be esily cultivated in fish ponds like Shrimp. The *S.Sihama* are tasty fish having high prised therefore the coasted people should used this fish for fish farming to get highest yield production to solve the problem of food. (Yeragi 2004).

The dietory compositions of juveniles of *S.sihama* changed progressively with increasing body size. The change included a shift from the ingestion of small zooplankton, such as calanoid copepods by small juveniles to the consumption of larger benthic prey, such as polychaetes, shrimps, similar to those of audlt of the species. The overall feeding habits of juvenile S. sihama $\leq$10 and 11-45mm. SL size classes preferred major food items of calanoid copepods. In large size classes, however, this prey item was replaced by polychaetes and mollusks. The results also indicated that the percentage of copepods differed significantly between season wise and size wize.

CONCLUSION

S. sihama is ecologically and economically important estuarine fish. Cultivation of this fish is likely to be profitable because of the consumer demand both in local and export markets. It has high rate of tolerance and planktivores. The growth is very fast. The rate of fecundity is also high. It is advisable to local native that they can easily make the fish farm in the adjacent vacant mud-flat region to get high yield production to solve the problem of required amino acids to mankind. considering high rate of tolerance, fast growth, high population dynamic, it is better used for fish farming as model example.

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RESEARCH ARTICLE

Antioxidant and antimicrobial properties of *Adhatoda vasica* L. Nees

Wankhede TB

Department of Botany, Shri Shivaji Science College, Amravati – 444604, MS, India

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ABSTRACT

Plant *Adhatoda vasica* L. Nees (Acanthaceae) commonly known as Malabar nut is an evergreen two – three m tall shrub, sometime used as hedge, branches opposite and stem yellowish. Leaves simple, 10-20 cm long and 3 to 7.5 cm or sometime much more broad, elliptical, ovate-lanceolate, and tapering towards apex. Inflorescence terminal or sub terminal spikes, flowers white bilabiate and fruits two-valve capsule, which dehisces when mature, or dry. The plant leaves, bark and root known for traditional medicinal use in Ayurveda. The plant parts generally bitter and useful in cough, bronchitis, asthma, skin disease, eczema and scabies. The leaves extensively employed in preparations indicated in respiratory ointments and particularly in cough syrups. An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfer electrons from a substance to oxidizing agent oxidation reaction can produce free radicals, which start chain reaction that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates and other oxidation reaction by being oxidize themselves. In present investigation preliminary antioxidants evaluated from the experimental plant and antimicrobial sensitivity test carried out against some human pathogenic microbial strains to support out the potential compounds of pharmacognostic interest.

Keywords: Antioxidants, antimicrobial sensitivity, pharmacology

INTRODUCTION

Antioxidants remarkably occur in plants having potential to protect the plant from severe damage. More danger of free

radicals, plants produces more antioxidant. Antioxidant are nutraceuticals whose deficiency states are associated with variety of dreaded conditions, viz. cardiovascular diseases, diabetic, cataracts, rheumatoid arthritis, Alzheimer's disease and others. The medicinal properties of plants have been investigated in the recent scientific development, throughout the world, due to their antioxidant activities, no side effect and economic viability (Anndy *et al.,* 2003). Flavonoids and phenolic compounds widely distributed in plants which have been reported to excess multiple biological effect, including antioxidant, free radicals scavenging abilities, anti-inflammatory, anti carcinogenic etc. (Miller,1996). They are also suggested to be iron chelator i.e. the novel natural's antioxidant and radicals scavenging properties (Boyer *et al*., 1988: Havsteen 1983) Basic clinical and epidemiological research has suggested a potential protective effect of antioxidant nutrients such as (Vitamin C) Ascorbic acid, Anthocyanin, β carotene, Lycopene, chlorophyll etc. on the risk of cancer cardiovascular diseases and aging (Aviram, 2000). Ascorbic acid is an important antioxidant essential for the normal regulation of the colloidal condition of connective tissue, osteaid tissue, dentine and the intercellular cement substance of the capillaries. It is concerned in the hydroxylation of proline and hydroxyproline as an important constituent of collages. Severe ascorbic acid deficiency produces scurvy. Lycopene is a powerful antioxidant that categorically retards damage caused to DNA and protein. Lycopene offers distinctly and appreciable much better skin protection against the UV-light than beta-carotene. It especially accumulated in the various segments of human body viz. skin, adrenal gland, prostrate glands, testes etc. It also renders adequate protein against cancer. Lycopene noticeably arrest the insulin like growth factor-1 stimulation of cancerous growth (Xianquan *et al*., 2005). In recent year, numerous studies have shown that anthocyanin displays a wide range of biological activities including antioxidant, anti-inflammatory, anti-microbial and anticarcino-genic activities, improvement of vision, induction of apoptosis and neuroprotective effects. In addition, anthocyanin displays a variety of effects on blood vessels and platelets that may reduced the risk of coronary heart diseases (Mazza*,* 2007). Chlorophyll is nowadays a solution of many problems like vitamin deficiency, pollution after effect, countering heart and diabetic diseases. It prescribed in the form of leaf juices, liquid chlorophyll, chlorophyll tablets and leafy pastes. This all due to biochemical nature of chlorophyll which on dissociation and substitution reaction gives rise to various essential organic compound like hemoglobin and vitamin B12 (Ursula *et. al.,* 2005). Phytol tail of chlorophyll dissociates into two beta- carotene molecule which is a precursor of vitamin A. Hence, plant pigments especially of medicinal plants acts as sources of antioxidant (Hsu *et al*., 2013). *Adhatoda vasica* reported to prevent oxidative damage of carbon tetrachloride induced hepatotoxic effect in rats (Pandit *et al*., 2004). Maurya and Singh, (2010) accounted highest amount of phenolic compounds which scavenging the free radicals and exhibits greatest antioxidant activity. The positive effect of gamma irradiation on the natural antioxidants of *Justica Adhatoda* showed release of phenolic compounds (Rajurkar *et al.*, 2012). The ethanolic extract of *A. vasica* showed high antioxidant activity with cytoprotective potential in cell culture (Mamta *et al.*,2013). Interestingly, the methanolic and aqueous extract of *A. vasica* has potential phytochemical composition of flavonoids, phenols with antioxidant and cytotoxic effect (Rao *et al.*, 2013). Recently, (Kumar *et al.*, 2014) evaluated pharmacological screening of leaf extract of *A. vasica* against dysentery and diarrhea due to presence of chemical compounds tannins, alkaloids, saponins and flavonoids.

MATERIALS AND METHODS

Fresh leaves, stem and root are metabolically active parts of the plant and site of synthesis for many chemical compounds hence chosen for analysis. Plant materials collected from Melghat

forest areas brought laboratory cleaned and preserved. Estimation of antioxidants like (Vit-C) Ascorbic acid, Anthocyanin, Lycopene, Chlorophyll from plant materials carried out as per the protocols of Thimmaiah (1999).

The plant parts cut in small pieces, cleaned carefully and washed under tap water to remove impurities followed by shade drying. Dried plant parts crushed in blender, powdered and preserved in airtight bottles. Soxhlet extraction process followed in petroleum ether ethanol, methanol, and acetone and different solvent fractions obtained. Dried extracts were stored in labeled sterile wide mouthed screw capped bottles at 4^0C and used for further study (Parekh and Chanda, 2008). The standard pathogenic bacterial and fungal strain obtained from Microbial Type Culture Collection and Gene Bank (IMTECH), Chandigarh, India. The bacteria rejuvenated in Nutrient broth (Hi-media laboratories, Mumbai, India) at 37^0C for 18 hrs and then stored at 4^0C on Nutrient agar. The fungal organisms were sub cultured on Sabaroud's dextrose agar. Four bacterial strains like gram-negative *Proteus vulgaris* (MTCC-744), *Shigella flexneri* (MTCC-1457), *Salmonella typhimurium* (MTCC-98), gram-positive *Staphylococcus aureus* (MTCC-96) and one fungal pathogen *Aspergillus niger* (MTCC-28) were selected. Disc diffusion method was used for the antibacterial sensitivity test by following the standard methods (NCCLS, 1990). The results were compared with the standard bacterial antibiotics like (10 µg/ml) Tetracycline and Nystatin for fungi.

RESULTS AND DISCUSSIOIN

Determination of antioxidants

Antioxidants mostly known to protect our body from the formation of free radicals. Ascorbic acid is not synthesized in human being and dietary or oral consumption only provide this vitamin. The high quality of ascorbic acid was found in fresh leaves of *Adhatoda vasica* showed 1200 µgm of ascorbic acid content (Table- 1). The normal human body when fully saturated contains about 5000 mg of vitamin C, at which 30mg found in adrenal glands, 200mg in extra cellular fluids & really distributed in varying concentrations throughout the cells at the body. (Danne, 1990). Lycopene is one of the over 600 or more carotenoids pigments. Some studies reported that lycopene could inhibit the growth of cancer and endometric cancers (Rao and Agarwal 2000). The moderate lycopene value of 0.84 µgm found in *Adhatoda vasica* (Table -1). The moderate quantity of anthocyanin was found in fresh leaves of *Adathoda vasica* i.e. 62.25 µgm. The antioxidant activity (Scavenging free radicals metal chelation; protein binding) of anthocyanin including the protection of LDL against oxidation has been demonstrated in a number of *In vitro* systems. (Aviram, 2000) The total chlorophyll content in *Adhatoda vasica* found 0.60 µmg from the fresh leaves of the plant (Table-2). Chlorophyll has anti inflammatory, antioxidant and wound healing properties. It is efficient delivery of magnesium helps the blood to carry oxygen to cell and tissues. Chlorophyll also removes carbon dioxide and carbon monoxide, and has been found to

Table 1: Observations for *Adhatoda vasica*

Sr. No	Name of the compound	Plant part taken for analysis	Weight of plant part	Vol. of extract	Vol. of extract taken for analysis	Absorbance (nm)	Value found in µgm
1.	Ascorbic acid	Leaves	1 g	10 ml	1ml	0.193	1250 µgm
2.	Lycopene	Leaves	1 g	10 ml	1ml	0.028	0.87 µgm
3	Anthocyanin	Leaves	2g	10 ml	1ml	0.249	62.25 µgm
4.	Chlorophyll	Leaves	1g	Total chlorophyll		0.545	0.555 µmg

Table 2: Preliminary antimicrobial sensitivity test of *Adhatoda vasica*

Sr. No.	Solvent Extract	Zone of Inhibition [mm]				
		***Proteus vulgaris* [MTCC-744]**	***Shigella flexneri* [MTCC-1457]**	***Staphylococcus aureus* [MTCC -96]**	***Salmonella typhimurium* [MTCC -98]**	***Aspergillus niger* [MTCC-281]**
1.	Petroleum Ether	08	06	09	09	15
2.	Ethanol	12	11	10	15	17
3.	Methanol	09	08	10	11	13
4.	Acetone	09	13	07	11	12
5.	Tetracyclin [control]	27	29	34	30	-
6.	Nystatin [control]	-	-	-	-	31

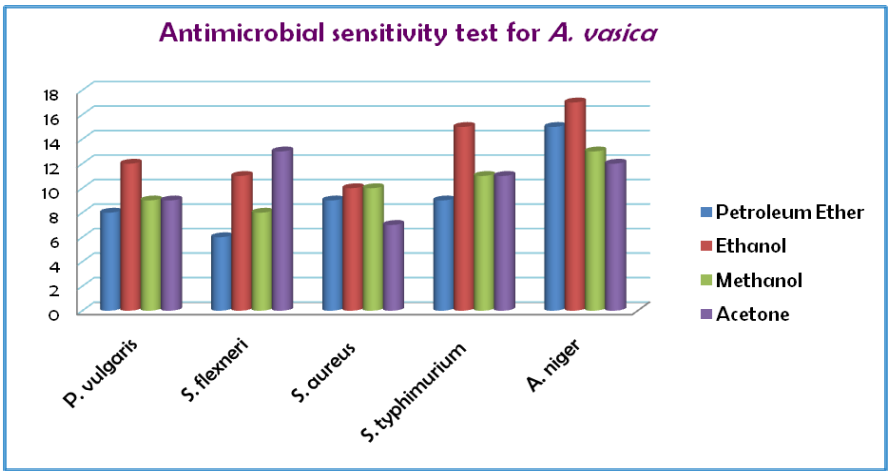

Fig. 1: Analysis of antimicrobial sensitivity test

reduce fecal, urinary, and body odor. Chlorophyll may reduce the binding of carcinogens to DNA in the liver and other organs (Hsu *et al.*, 2013).

Antimicrobial sensitivity of *Adhatoda vasica*

The various types of extract showed consistently positive result against maximum microbial pathogens (Table 2, Fig. 1). The petroleum ether extract of the plant exhibited significant interaction with fungi *Aspergillus niger* with 15mm zone and less with *S. flexneri* pathogens. Subsequently, the ethanol extract found much sensitive with positive results against microorganisms like *P. vulgaris, S. aureus, S. typhimurium and A. niger* with maximum zone of 17 mm. The greenish black coloured extract of methanol was found sensitive to various pathogens with positive interaction like *P. vulgaris, S. aureus, S. typhimurium* and *A. niger* with 13mm zone but less reactive to *S. flexneri* with 8mm zone (Rao *et al.*, 2013) The acetonic extract of the plant showed least response against microorganisms *P. vulgaris* and *S. aureus,* and moderate active against *S. flexneri S. typhimurium* while greatest against fungi *A. niger* with zone 12mm (Table-2, Fig -1). The ethanol and methanol extract of the plant found more sensitive to all the pathogens as compared to the, petroleum ether and acetone extract. As compare to bacterial strains the fungal strain *A. niger* showed highest and remarkable antifungal sensitivity (Mamta *et al.*, 2013).

CONCLUSION

From the analysis and results it revealed that *Adhatoda vasica* is important medicinal plant with rich antioxidant potential and antimicrobial

sensitivity against pathogenic microorganisms. Its noteworthy that the conventional drugs more sensitive to gram positive bacteria (*S. aureus*) but in present investigation the extracts were more sensitive to gram negative bacteria (*P. vulgaris, S. typhimurium,* and *S. flexneri*). Hence, besides conventional drug practice more advance exploration needed for pharmacognostic uses.

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RESEARCH ARTICLE

Seasonal Variation of Physicochemical and Microbial Parameters of water of Nal-Damayanti Sagar Dam, Morshi, Dist. Amravati, MS, India

Ghaware AU and Jadhao RG*

Department of Zoology, Shri Shivaji Science College, Amravati, 444603 Maharashtra. India.

*Correspondent author- Dr. R.G. Jadhao, Associate Professor, Department of Zoology, Shri. Shivaji Science College, Amravati

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ABSTRACT

This study was aimed to estimate current status of Physico-chemical characteristics and level of pollution indicator bacteria and their variation at whole stretch of dam. Some environmental parameters such as Temperature, pH, Turbidity, Dissolved oxygen, Sulphates and Nitrates were monitored. In addition, the microbial analyses involved total viable bacterial and fungal counts. The results of physicochemical parameters showed varied value; from conclusions revealed that large number of sewage drains in Morshi and agricultural discharge is mainly responsible for pollution in Nal-Damyanti Sagar Dam.

Keywords: Environment, DO, Sulphate, Agricultural Discharge.

INTRODUCTION

Water is one of the abundantly available substances in nature, which man has exploited more than any other resources for the sustenance of life. Water of good quality is required for living organisms. Dams are the most important water resource. Unfortunately, the dams are being polluted by indiscriminate disposal of sewage, industrial wastes and human activities. The dams are always the victims of the negative impacts of urbanization. Most water bodies become contaminated due to incorporation of untreated solid and liquid waste. Now a days due to increased human population and man-made conditions, the water quality is deteriorating everywhere Jayabhaye (2008).

Water quality provides current information about the concentration of various solutes at a given place and time. Water quality parameters provide the basis for judging the suitability of water for its designated uses and to improve existing conditions. For optimum development and management for the beneficial uses, current information is needed which is provided by water quality programmers. Prevention of river pollution requires effective monitoring of physicochemical and microbiological parameters Chandra et al. (2006). In most countries, the principal risks to human health associated with the consumption of polluted water are microbiological in nature WHO (1997). The bacteriological examination of water has a special significance in pollution studies, as it is a direct measurement of deleterious effect of pollution on human health APHA (1981). Coliforms are the major microbial indicator of monitoring water quality (Brenner et al., 1993; Grant, 1997). The detection of *Escherichia coli* provides definite evidence of fecal pollution. This work aimed to assessment of the water quality of Nal-damyanti Sagar dam and relates the physicochemical characteristics and microbial quality of water with standard guidelines for safe consumption or usage.

MATERIALS AND METHODS

Water samples for physic-chemical analysis were collected from Nal-damyanti sagar Dam, geographical coordination Longitude 21° 16' 35" N and Latitude 78° 3' 26" E. Morshi, (M.S) India, during Feb 2010-Jan 2011 in the early morning between 8 am to 11 am in the first week of every month from Feb10-Jan11. The samples were collected in acid washed plastic container from a depth of 5-10 cm. below the surface of water. Samples were aseptically collected in sterile brown bottles (500 ml capacity), transported to laboratory and stored at 4^0C until bacteriological analysis completed within 6 h of sampling.

Physicochemical Analyses: The physico-chemical characteristics of the dam water like water temperature, turbidity, pH, Sulphates and nitrate were determined in summer, monsoon and winter according to standard methods APHA (2005); Trivedy and Goel (1984).

Bacteriological analysis: Spread plate method was used for enumeration of Total Viable Bacterial Count at 37^0C and Fungal count at 25^0C.

RESULTS AND DISCUSSIOINS

Physicochemical Analyses

Temperature: The temperature of water affects some important physical properties and characteristics of water such as density, viscosity, conductance, salinity, solubility of dissolved gases etc. and also, chemical and biological reaction rates increase with temperature.

In present investigation, the maximum value was recorded 25.45 ± 1.06 (°C) recorded during summer; minimum value was recorded 19.72 ± 0.58 (°C) recorded during winter. Low temperature recorded in winter may due to lesser solar radiation, low atmospheric temperature and high temperature in summer because of low water level, high solar radiation and clear atmosphere. Similarly, results have been reported by (Anita *et al.,* 2005; Jawale and Patil, 2009).

pH: In present investigation, the maximum value was recorded 7.50 ± 0.35 recorded during summer; minimum value was recorded 6.45 ± 0.02 recorded during monsoon. pH range shows that the water of all sampling sites of dam was slightly alkaline and acidic in nature. High value of pH during summer might be due to low water levels and concentration of nutrient in water. pH shows high significant positive relationship with water temperature. Similar trend was also reported (Narayana *et al.,* 2008; Reddy Vasumathi *et al.,* 2009; Kadam *et al.,* 2007; Anita, 2002).

Table 1 : Seasonal variation in physic-chemical and microbial parameters of Nal-Damyanti Sagar Dam, Morshi (During Jan10-Dec 11)

Parameter	Summer	Monsoon	Winter
Temperature	22.45 ± 1.06	22.77 ± 0.28	19.72 ± 0.58
pH	7.50 ± 0.35	6.85 ± 0.21	6.45 ± 0.02
Dissolve oxygen	6.4 ± 0.06	6.8 ± 0.18	7.3 ± 0.075
Turbidity	42.26 ± 0.47	47.62 ± 1.16	43.56 ± 0.86
Sulphate	17.69 ± 0.18	19.48 ± 0.39	16.03 ± 0.79
Nitrate	0.65 ± 0.024	0.73 ± 0.06	0.67 ± 0.028
Total Bacterial Count	25 ± 4.4	26.5 ± 2.87	15.5 ± 1.65
Total Fungal Count	2 ± 0.70	6.25 ± 1.6	10 ± 1.58

Dissolved oxygen: It is a very important water quality parameter and is also an index of physical and biological processes going on in water. In present investigation, the maximum DO value was recorded 7.3 ± 0.075 mg/l during winter and minimum value 6.4 ± 0.06 mg/l during summer. Kataria et al. (2006) reported that depletion of dissolve oxygen in water is due to high temperature and increased microbial activity, on their study on water quality of Dahod dam, India. The level of DO was found minimum in summer. This is because of the low solubility of gases at high temperature (Hynes, 1978).

Turbidity: Suspension of particle in water interfering with the passage of light is called Turbidity. Turbidity has been long known to hinder disinfection by shielding microbes, some of them perhaps pathogens. In the present investigation, the maximum turbidity value was recorded 47.62 ± 1.16 during monsoon and minimum turbidity value 42.26 ± 0.47 was during summer. These observations were also supported by (Prasanna and Ranjan, 2010, Shraddha *et al.,* 2008; Trivedi *et al.,* 2009). High values of turbidity in monsoon may be due to influx of rain water from catchments area, cloudiness, less penetration of light, washes silts, sand, high organic matter and low transparency due to suspended inert particulate matter. However, low values of turbidity in summer may be due to clear atmosphere, evaporation of water and high light penetration.

Sulphate: Sulphate is present in fertilizers they contribute to water pollution and increase sulphate concentration in water body. In the present investigation, the maximum sulphate values obtained 19.48 ± 0.39 mg/l during monsoon and minimum value 16.03 ± 0.79 mg/l during winter. Maximum sulphate concentration during monsoon may be due to the dilution and utilization of sulphate by aquatic plants. However, the low sulphate concentration was noted during winter may be due to biodegradation and low water level. Similarly, results have been reported (Reddy *et al.,* 2009; Telkhade *et al.,* 2008; Shanthi *et al.,* 2006).

Nitrate: Nitrate is the most highly oxidized form of nitrogen compounds commonly present in natural waters, because it is a product of aerobic decomposition of organic nitrogenous matter. In the present investigation, maximum values of nitrate obtained 0.73 ± 0.06 mg/l during monsoon and minimum value obtained 0.65 ± 0.024 mg/l during winter season. Nitrate levels in surface water often show a marked seasonal fluctuation with higher concentration being found during monsoon month compared to winter months. Similarly results have been reported (Gohram, 1961, Rajashekhar *et al.,* 2007).

Microbial analysis:

Total bacterial count: High bacterial density in water indicates sewage contamination. As long as *E. coli* is present in water, there is every possibility of the presence of some pathological bacteria in water and this will affect or alter the diversity of organisms and sometime obstruct the aquatic organisms especially fishes and crabs. Bahadoor *et al.,* (2004); Obiri *et al.,* (2003) reported the interaction between coliform bacteria and its aquatic environment. In the present investigation, maximum bacterial count in CFU/ml obtained 26.5×10^4 during monsoon and minimum value obtained 15.5×10^4 during winter season. Continuously increase in population of Morshi around the dam area is mainly responsible for increased level of pollution. Most of sewage water often added from residential area. Higher bacterial population during monsoon is due to increased land run off and higher faecal inputs in to dam from connecting rivers and various sources. An increase in the bacterial level after rainfall was reported by (Shehane *et al.,* 2005). THB load in the present study is significantly correlated with dissolve oxygen.

Total fungal count: In the present research shows that maximum fungal count in CFU/ml obtained 10×10^3 during winter and minimum count value obtained 2×10^3 during summer season. Total fungal count found to be higher in winter season than the respective level found in summer and monsoon. Shridhar and kaveriappa (1989) also observed that the total number of water fungi was lowest during summer season. Occurrence of maximum number of fungal species during winter and spring season in the present study might be due to moderate temperature and slightly higher percentage of organic and inorganic matter.

CONCLUSION

The obtained results of the present study concluded that the water quality along the studied area in Nal-Damyanti Sagar Dam was remarkably influenced by wastewater discharge from drains located on its sides regarding both physicochemical and microbial characteristics. Agricultural and sewage wastes are the key factors in this environmental problem. The water of Nal-Damyanti Sagar Dam is subjected to fecal pollution and continuous monitoring of microbial quality of water is recommended to control the spreading of pathogens transmitted by contaminated water.

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RESEARCH ARTICLE

Effect of moisture content on the production of protease by *Fusarium oxysporum* using agroindustrial waste

Vidhale NN and Deshmukh Rupali R

Department of Microbiology, Shri. Shivaji Science College, Amravati 444604, M.S., India
Email: vidhalenana@gmail.com

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ABSTRACT

The effect of moisture content on the production of protease from *Fusarium oxysporum* was studied using agro industrial waste as substrates such as dal mill waste, oil mill waste, molasses, fruit waste and vegetable garbage under solid state fermentation. Dal mill waste, oil mill waste and vegetable garbage produced maximum protease activity in presence of all the types of moistures (25%, 35%, 45%, 55%, 65%, & 75%) after 96 hrs of incubation. However molasses and fruit waste gave highest protease production in presence of 55 % and 65% moisture content after 7th day of incubation. Among all the substrate dal mill waste and oil mill waste were promising in being utilized faster for the production of protease enzyme.

Keywords: Agoindustrial waste, *Fusarium oxysporum,* protease

INTRODUCTION

Protease is one of the most commercial enzyme used in food processing, detergent industry, dairy industry, silver recovery, medical purpose, leather making, meat processing, and chemical industry as well as in waste water treatment (Negi and Benerjee, 2006). This enzyme occurs widely in plants and animals, but commercially proteases are produced exclusively from microorganisms. Molds of the genera *Aspergillus, Penicillium* and *Rhizopus* are especially used for producing proteases (Sandhya *et al*., 2005). Solid state fermentation has gained tremendous attention for the low cost production of industrially important enzymes by utilization of various types of waste as agro industrial waste. In search for cheaper fermentation processes with a high enzyme yield, SSF was found to be more attractive (Kota *et al*., 1999). Different mticroorganisms were utilized for

the production of protease fungi such as *Aspergillus ,Penicillium , Rhizopus, Chrysosporium, Mucor, Scedosporium* and bacteria like *Bacillus licheniformis, Bacillus firmus, Bacillus alcalophilus, Bacillus subtilis, Bacillus thuringiensis, Streptomyces spp*. etc.Hence study was carried out to study protease production potentiality of Fungus *Fusarium oxysporum.*

MATERIALS AND METHODS

Substrates preparation

Agro industrial waste such as dal mill waste, oil mill waste, Molasses, fruit waste and vegetable garbage were collected and powdered to size about 2 mm in homogenizer and then sieved through 20-40 mesh screens to obtain a particle having diameter between 0.42 to 0.85 mm. Each of such substrate was supplemented with 0.83 gm K2HPO4 and 0.16gm $MgSO_4$, 1.5% agar-agar and 10 ml distilled water and autoclaved for 15 min. at 15 lb/inch ^{2}. Initial utilization of these substrates for production of protease by *Fusarium oxysporum* strain was studied under Solid state fermentation, incubated for 7 days at room temperature (25 to 30^{0}C). Initially 20 g of 50% moistened substrate was sterilized and thoroughly mixed with 1ml spore suspension of 7 day old culture of *Fusarium oxysporum*. This substrate along with spore suspension was poured in sterilized Petri dish and allowed to incubate at room temperature for 7 days.

Enzyme estimation

After every 24 hrs of interval 1gm fermented substrate was harvested from petri plate and transferred to test tube containing 5ml phosphate buffer. The contents were homogenized and centrifuged at 2000 rpm for 30 min to remove all particulate matter. Protease activity was assayed as suggested by Keay and Wrildi (1970). To 1 ml of culture filtrate, 1ml of 2% casein solution was added and the mixture was incubated at 37^{0}C for 10 min. The reaction was terminated by adding 2ml 0.4 M TCA (Trichloro acetic acid), again incubated at 37^{0}C for 20 min. and filtered through Whatman filter paper no1. One ml of the filterate was added to 5 ml of sodium carbonate (0.4M) and 1ml Folin- Ciocalteus's reagent and incubated at 37^{0}C for 30 min. The Absorbance was measured at 660 nm. in Spectrophotometer .

Effect of Moisture content

All the five substrates were provided with different moisture percents such as 25%, 35%, 45%, 55%, 65% and 75%. Protease production in SSF in all the fives substrate under above moisture content was studied. Protease production was estimated from second day of incubation up to 7 days.

RESULTS AND DISCUSSIOINS

All the agro industrial waste substrates were provided with moisture 25%, 35%, 45%, 55%, 65% & 75% and incubated for 7 days at room temperature. Dal mill waste, oil mill waste and vegetable garbage produced maximum protease activity in presence of all the types of moistures after 96 hrs of incubation. However molasses and fruit waste gave highest protease production in presence of 55 % and 65% moisture content after 7th day of incubation. Among all the substrate dal mill waste and oil mill waste were promising in being utilized faster for the production of protease enzyme. Results of enzyme estimation are summarized in Table-1 and fig- 1, 2, 3, 4, and 5. The highest enzyme production (121.50 ug^{-1}) was obtained at 60% initial moisture content by *Streptomyces sp*. (N902) (Lazim *et al*. 2009). A similar observation has been reported in case of *Streptomyces sp*. 594 protease production by De Azerodo *et al*. (2005). Study of Germano *et al*. (2003) indicated the requirement of 55 and 63% initial moisture content for maximum proteases production by *Penicillum* LPB- 9 and *A. falvus* (Malathi and Chakroborty (1991) respectively, in SSF. In the study of Chutmanop *et al*. (2008), the optimum initial moisture level was about 50%

Table 1: Effect of moisture percent on production of protease by *Fusarium oxysporum* in a solid state fermentation using agro industrial waste as substrate.

Type of waste	% of moisture	Production of protease (µg/ml)at different Incubation period						Mean ± SD Value
		24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.	144 hrs.	
Dal mill waste	25	7.2	7.8	7.9	10.9	10.5	10.3	9.1 ±1.49
	35	6.3	7.4	8	10.9	10.7	10.3	8.93±1.78
	45	6.4	7.6	8.1	10.9	10.8	10.6	9.07±1.78
	55	6.5	7.7	8.4	10.8	10.7	10.4	9.08±1.65
	65	6.4	7.4	8.2	10.8	10.3	9.2	8.72±1.55
	75	6.4	7.3	8.3	10.9	10.4	9.1	8.73±1.60
Oil mill waste	25	6.6	11.1	9	10.9	9.6	9.7	9.48±1.48
	35	5.8	11.1	10.1	10.8	8.9	9.8	9.42±1.77
	45	6.2	10.9	10.3	10.7	8.1	10.3	9.42±1.71
	55	6.7	8.7	10.4	10.8	7.8	10.3	9.12±1.51
	65	6.4	8.7	10.3	11	8	10.1	9.08±1.57
	75	7	8.4	10.3	10.9	6.6	5	8.03±2.07
Molasses	25	2.3	2.8	2.4	4.2	7.4	7.4	4.42 ±2.20
	35	1.9	1.6	2.1	5.1	6.2	6.3	3.87 ±2.04
	45	1.4	1.3	2	4.5	7.4	7.5	4.02 ±2.65
	55	1.3	1.2	2	5.2	7.8	7.8	4.22 ±2.86
	65	1	1.1	1.6	4.6	7.7	7.8	3.97 ±2.93
	75	2.5	1.3	2.2	4	7.6	7.5	4.18 ±2.51
Fruit waste	25	2.2	3.3	3.6	4.9	7.6	7.7	4.88 ±2.11
	35	2.2	3.2	4.2	5.6	7.9	8	5.18 ±2.21
	45	2.4	2.7	3.5	5	7.1	7.4	4.68 ±1.99
	55	2.2	3.4	4.3	5.4	8.5	8.6	5.4 ±2.43
	65	2.1	3	3.7	4.9	8.6	8.6	5.15 ±2.58
	75	2	3.5	3.8	6.1	8.1	8.2	5.28 ±2.35
Vegetable garbage	25	3.7	3.2	6.1	8.1	8.3	9.5	6.48 ±2.37
	35	2.8	4.2	6.9	9.8	9.3	8.9	6.98 ±2.65
	45	3.3	4.1	6.1	9.1	8.6	6.6	6.3 ±2.13
	55	3.5	3.6	7.2	6.9	7.3	7.5	6 ±1.74
	65	3	3.9	5.9	9.7	9.7	8.2	6.73 ±2.66
	75	3.8	3.5	7.1	8.7	8.7	7.6	6.57 ±2.14

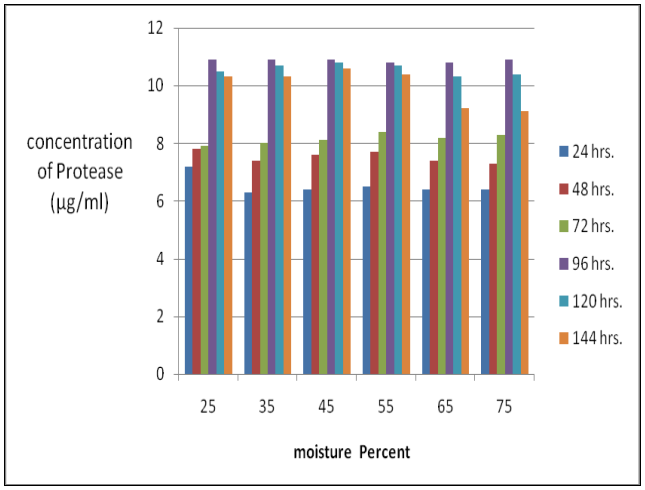

Fig 1: Effect of moisture percent on production of protease by *Fusarium oxysporum* in a SSF using dal mill waste as substrate.

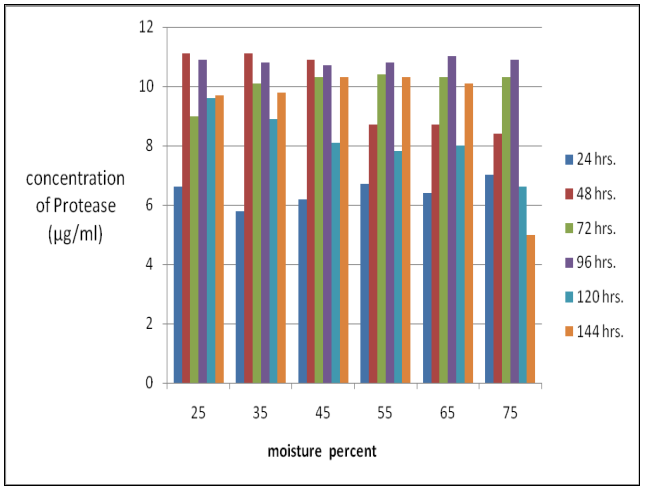

Fig 2: Effect of moisture percent on production of protease by *Fusarium oxysporum* in a SSF using oil mill waste as substrate.

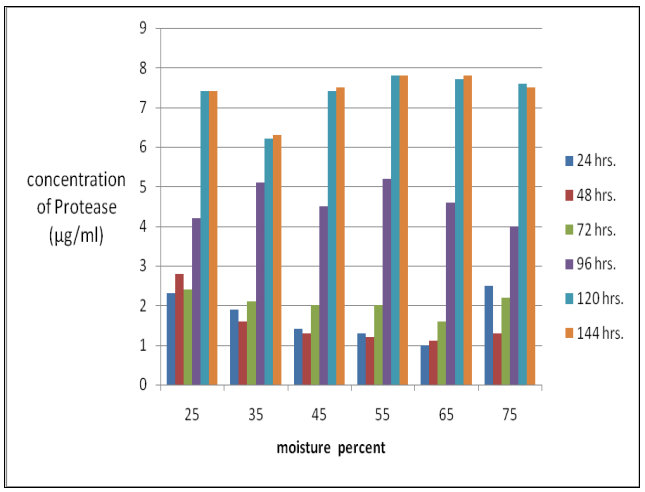

Fig 3: Effect of moisture percent on production of protease by *Fusarium oxysporum* in a SSF using molasses as a substrate.

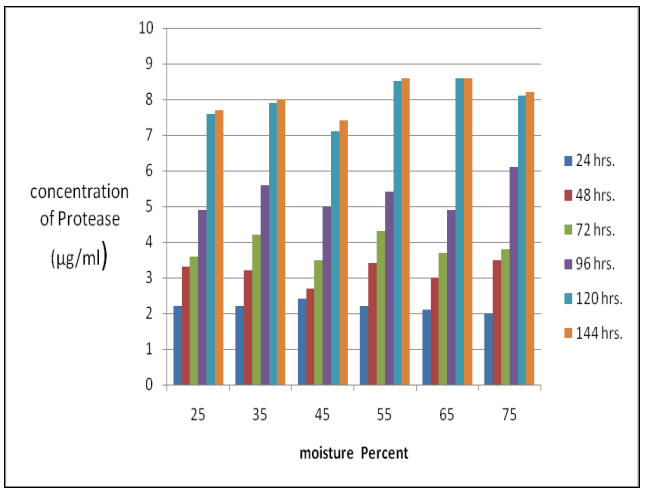

Fig 4: Effect of moisture percent on production of protease by *Fusarium oxysporum* in a SSF using fruit waste as a substrate.

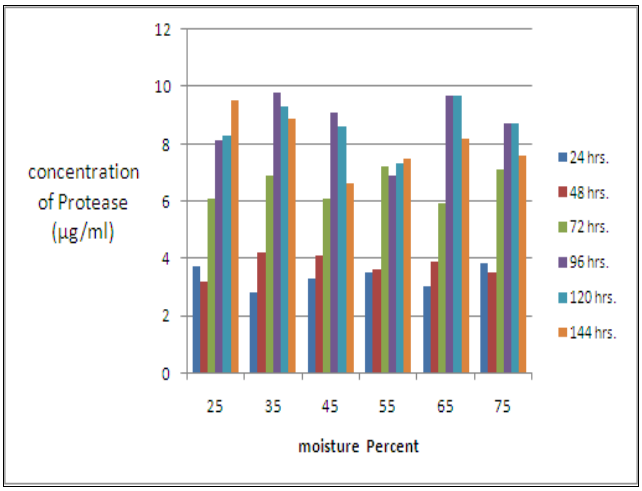

Fig5: Effect of moisture percent on production of protease by *Fusarium oxysporum* in a SSF using vegetable garbage as substrate.

which afforded a high protease activity value.. In the study carried out by Sajeed and Vidhale (2013) the effect of initial moisture on protease production was maximum at initial moisture level of 50% (w/w).

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RESEARCH ARTICLE

Rotifers diversity in Kudla Dam near Umri Nanded, MS, India

Bhoyar VV

Department of Zoology, L. B. D.G. College, Umri, Dist Nanded (MS) India.

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ABSTRACT

The water quality and nutrients influence population of zooplanktons. The population observed in the distribution of zooplankton is due to abiotic factors (e.g. temperature, salinity DO and other dissolved ions), to biotic factors (e.g. nutrients). Many species of zooplanktons are limited by temperature, dissolved oxygen, salinity and other physico-chemical factors. Zooplanktons constitute an important link in food chain as (primary or secondary consumers) and serve as food for fishes directly or indirectly. Therefore any harm to these will be harmful to the fish populations. The occurrence of zooplankton depends upon its productivity, which is influenced by physico-chemical parameters and the level of nutrients in water. Hence the present study was carried out on kudla dam for a period of one year from Jun 2013 to May 2014. In the present study 19 species of Rotifers were found.

Key words: zooplanktons, dam, water quality

INTRODUCTION

Most important energy source to all living organisms is the sun. The solar energy is converted into organic compounds by the process of photosynthesis. This process of converting carbon dioxide and water into carbohydrates is performed by primary producers. These primary producers in water are called as phytoplanktons. These are consumed by zooplanktons, which are again consumed by fishes and so on. These all organisms depend upon water for their growth and reproduction. Therefore any change in the quality of water affects the nature and productivity of these organisms. Change in physico-chemical parameters and nutrient content of water body plays an important role in the production of plankton which act as the natural food of many species of fishes, mainly zooplanktons form important food

source of many fishes and support the necessary amount of diet for the growth of Larval forms (Rahman and Hussain, 2008)). Phytoplankton being the primary producers forms the lowest trophic level in the food chain of freshwater ecosystem , moreover, number and species of phytoplankton's serves to determine the quality of water body (Bahura, 2001). Because of interference of man with nature, the water clarity becomes less and with the addition of nutrients and organic matter, the primary production in water increased. Zooplanktons form the important link between phytoplankton and fishes. The productivity of zooplanktons is influenced by physic-chemical para-me ters and organic contents in water. Zooplanktons feed upon the phytoplankton and make them available to fishes in the food chain (Michael, 1973).

MATERIALS AND METHODS

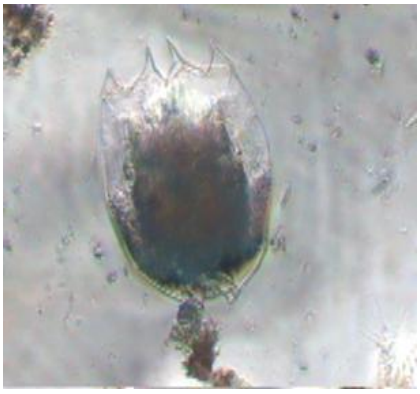
The samples were collected from four sampling stations of the dams. The present study was conducted in Kudla dam for period of one year i.e.- from Jun 2013 to May 2014. Plank tonic samples were collected on monthly basis from four stations A, B, C and D. Planktons were collected using plankton net made up of bolting silk cloth (Trivedi and Goel, 1986). Filtered samples were fixed and preserved by adding 4% formalin. For counting planktons a Sedgwick Raftor Plankton Counting Cell was used. Identification of planktons was done with the help of methods described by Sehgal (1983); Battish (1992); Dhanapathi (2000) (APHA, 1998).

RESULTS AND DISCUSSIOINS

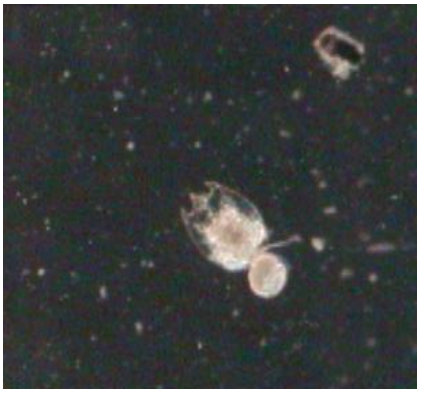
During the study period the high incidence of rotifers in summer season indicating the influence of temperature on positive co-relation between temperature and rotifers population. Similar observations were made by Sinha and Sinha (1983); Singh (2000); Kaushik and Sharma (1994), while working with other reservoir. The maximum density of rotifers was observed during spring peak in April. This was followed by a marked decrease in abundance during the clear water phase in May, 2014.

Table 1 : Zooplanton species abundance

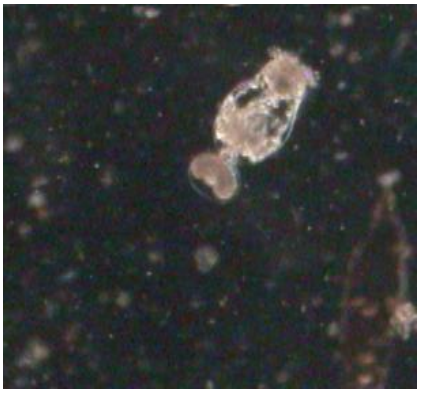
Zooplankton	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
Rotifera												
Rotararia	-	-	-	+	+	-	+	-	+	+	-	+
B.calyciflorus	+	+	+	+	+	-	+	+	+	+	+	+
Euchlanis brahmae	+	-	+	+	+	+	-	+	-	+	-	+
Asplanchana	-	-	+	+	-	+	-	+	+	+	+	+
Keratella quadrata	+	+	+	+	+	+	+	+	+	+	+	+
Keralella cochleris	+	+	+	-	-	+	+	+	+	+	+	+
Nothalca squmala	-	-	+	-	+	+	+	+	-	+	-	+
Plationus Platulus	-	+	+	+	+	+	+	+	+	+	-	-
Lecane (monostyla) bulla	-	-	-	+	+	+	+	+	+	+	+	+
B. bidentata	+	+	-	-	+	+	+	+	+	+	+	+
L.Papuana	+	+	-	-	+	+	+	+	+	+	+	+
L.doryssa	-	-	-	-	-	+	+	+	+	+	+	+
Pseudoeuchlanis longipedis	-	+	+	+	+	-	+	-	-	+	-	+
B.caudatus	+	+	-	+	+	-	+	+	+	+	+	-
B. c.v. hymani	-	-	+	+	+	+	-	-	-	+	+	+
B.plicatilis	+	-	+	-	-	+	+	+	+	+	+	-
B. quadridentatus	+	+	+	+	-	+	+	+	+	+	+	-
B. durgae	+	+	+	+	+	-	+	+	+	+	+	+
Aaplancha brightwelli	-	-	-	-	+	+	-	+	-	+	-	+



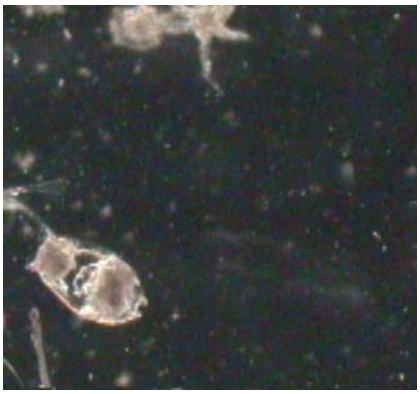
B. Plicatilis



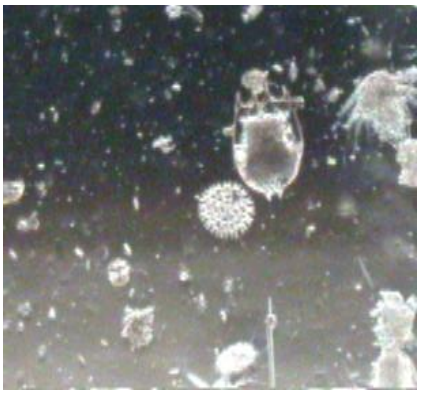
B. calyciflorus with egg



female rotifer sp.



B. Ureoceolaris sp.



B. Calyciflorus borgerti sp.

Fig.1: The selected species of Zooplanktons

Rotifers play an important role as grazers, suspension feeders and predators within the zooplankton community. The difference in population density of different rotifers can be analyzed by the biotic interactions. The monthly variations of rotifers were ranged between 04 to 07/mL at Station-A, 05 to 15/mL at Station B, 06 to 14/mL at Station C and 06 to 17/mL at Station D, in the year 2013-14. The minimum population rotifers were recorded in the month of August and the maximum population of rotifer was recorded in the month of April, 2014.

High rotifer population indicates population from organic matter due to direct entry of untreated domestic sewage from catchments area (Arora, 1967). Rao (1982) has reported less effect of abiotic factors on the abundance and fertility of pelagic rotifers. Chanadrasekhar (1962) observed that in summer and monsoon, the factors like water temperature, turbidity, transparency and dissolved oxygen (play an important role in controlling the diversity and density of rotifers).

Abdus and Altaff (1995) studied on qualitative and quantitative analysis of zooplankton population of a tropical pond during summer and rainy season and observed that the zooplankton during the month of July and November shows difference in the density of planktonic species. Dominance of rotifers over other groups has also been reported in other water bodies of the world (Michael 1968; Singh and Sahai, 19768).

The sequence of dominance of various groups was Rotifera > Cladocera > Copepoda. According to George (1966) the abundance of Rotifers is followed by cladocera is an indication of the

eutrophic nature of water bodies. The abundance of Rotifers may be attributed to their dependence on phytoplankton detritus matter and bacteria as food. Higher plankton number was recorded during summer due to an increase in Rotifer number. Present findings also support this.

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RESEARCH ARTICLE

Diversity of Zooplankton in some Reserviours in and around Karwar- Uttara Kannada District Karnataka

Vasanthkumar B[1] and Kapsikar Gangadhar B[2,]

Dept of Zoology, Govt. Arts and Science College, Karwar- Karnataka

Address for correspondence e mail: ugc.bvk@gmail.com

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ABSTRACT

The present work was carried out from October 2012 to December 2013. The main aim of the study was to analyse the diversity, evenness and richness of zooplankton species in some Reserviours of Karwar. The samples were collected during morning hours and were analyzed monthly for different parameter. Results showed that the larval forms were dominant throughout the study period followed by copepods and protozoa group.

Key words: Zooplankton, Hydrobiology, Karwar, Diversity, Correlation

INTRODUCTION

Zooplankton are the heterotropic component of the plankton community, which range from microscopic to few feet in size. Even though there are many permanent members, much of its composition is made by the juveniles of some animal groups. They form an important link in the aquatic food chain as 'secondary producers' accumulating the carbon for transferring to the other level of food chain, the consumers. Kali River exhibits different type of biotopes such as estuary, backwater, fresh water and mangrove etc. Five reserviours were selected for study. The environmental parameters of aquatic biotope fluctuate periodically dependable on the three conspicuous seasons, pre-monsoon (February-May), southwest monsoon (June-September) and post-monsoon (October-January). The pre monsoon season is identified by high temperature and salinity, the south- west monsoon season is characterized by heavy

rainfall and the post-monsoon season is known for stable environmental conditions and a high biological productivity rate.

MATERIALS AND METHODS

Water samples were collected on monthly basis. Water samples were collected using a clean plastic container for the study of various physic-chemical and biological parameters.

The plankton samples were collected on monthly basis. Plankton samples were collected by filtering 100 litres of water through plankton net made up of bolting silk. The zooplankton samples were preserved in 5 percent formalin. The preserved samples were brought to the laboratory for qualitative and quantitative analysis and the identification was done with the help of methods described by Pennak (1953), Arora (1963), Sehgal (1983), Battish (1992) and Murugan *et al*. (1998).

Community structure analysis: Three indices were used to obtain the estimation of species diversity, species richness and species evenness.

1. Shannon and Weaver (1949) and Simpson (1949) diversity index value was obtained using the following equation:

$D = \sum Pi^2 (\log Pi)$ (Shannon's index)

$i = I$

$D = \sum Pi^2$ (Simpson index)

$i = I$

Where

Pi = is the proportion of the first species. The proportions are given Pi=ni/N

2. Species richness (D or R1 and R2) was obtained using the equation.

$R1 = (S - 1) / \log N$ (Margalef, 1951)

$R2 = S \sqrt{n}$ (Menhinick, 1964)

Where:

R = is the index of species richness

S = total number of species

N = total number of individuals

3. Species equitability or evenness was determined by using the expression of Pielou (1966) and Sheldon (1969).

$$E1 = \frac{N1}{N0}$$ (Pileou's Evenness)

$$E1 = \frac{N1}{N0}$$ (Sheldon evenness)

Where:

N0 = number of species in the sample

N1 = number of abundant species in the sample.

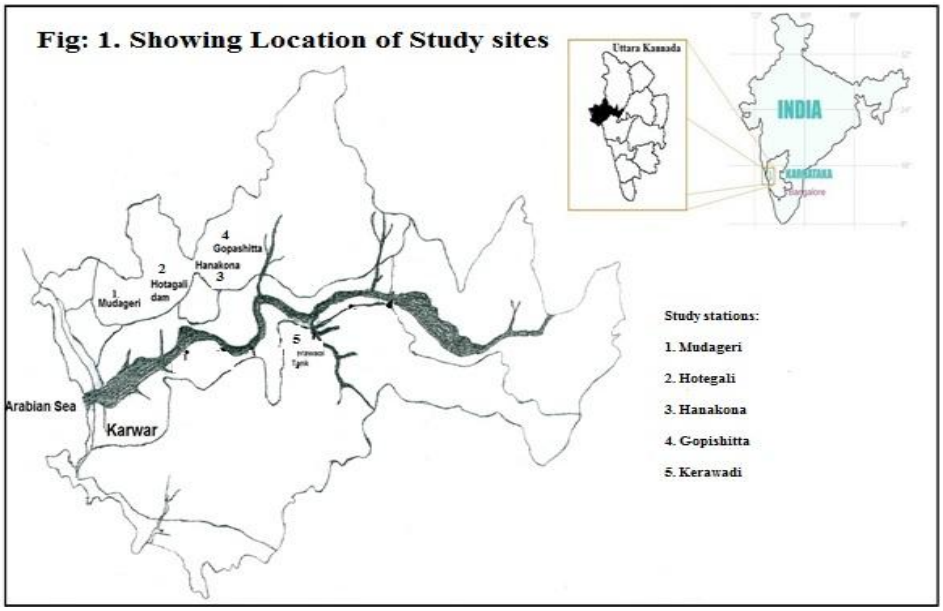

Table: I Study stations with Lat/Long.

Sl.No	Name of the Reservoir	Distance from Karwar	Geographical position
1	Mudageri	13.3km	14⁰-53'-52.27" N latitude/ 74⁰-07'-55.06" E longitude
2	Hotegali	15km	14⁰-54'-13.45" N latitude/ 74⁰-10'-12.08" E longitude
3	Hanakon	17km	14⁰-54'-05.29" N latitude/ 74⁰-11'-28.25" E longitude
4	Gopashitta	19.3km	14⁰-54'-39.24" N latitude/ 74⁰-13'-08.24" E longitude
5	Kerawadi	33km	14⁰-52'-46.32" N latitude/ 74⁰-15'-46.63" E longitude

RESULTS

Table 2: Monthly Variation in Hydrographical Parameters at Station I

Parameters	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D
Water temp (°c)	28	28	27	26	28.	29	30	32	28	26	28	28	29	27	27
pH	6.9	7.5	7.8	7.6	7.2	7.5	7.5	7.2	7.3	6.6	7.1	6.6	7.4	7.5	7.8
D.O (ml/l)	5.3	6.0	5.5	5.3	5.2	5.1	5.3	5.2	6.1	6.5	5.5	5.8	5.9	5.1	5.4

Table 3: Monthly Variation in Hydrographical Parameters at Station II

Parameters	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D
Water temp (°c)	29	28	29	29	31	28	30	29	26	28	28	28	28	28	29
pH	6.9	7.1	6.5	6.6	7.6	7.8	7.5	7.7	6.8	6.3	6.7	6.7	7.0	6.8	6.7
D.O (ml/l)	5.7	6.1	5.8	5.5	5.4	5.1	4.9	5.1	6.5	6.4	6.6	5.8	5.5	5.5	5.1

Table 4: Monthly Variation in Hydrographical Parameters at Station III

Parameters	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D
Water temp (°c)	29	28	28	28	29	30	29	30	29	28	27	29	29	28	29
pH	6.9	6.9	7.0	6.7	7.3	7.5	7.9	8.2	7.2	6.3	6.4	6.9	7.3	7.5	7.2
D.O (ml/l)	5.6	5.9	5.4	5.4	5.5	5.2	5.5	21.2	6	6.36	6.2	5.7	5.8	5.3	5.3

Table 5: Monthly Variation in Hydrographical Parameters at Station IV

Parameters	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D
Water temp (°c)	27	27	26	26	27	30	30	30	27	26	28	28	29	28	28
pH	7.0	7.3	7.8	7.5	7.3	7.3	7.6	7.7	7.6	6.6	6.9	7.2	7.3	7.5	7.1
D.O (ml/l)	5.3	6.0	5.3	5.6	5.4	5.1	5.0	5.5	6.2	6.3	5.9	5.6	6.2	5.4	5.7

Table 6: Monthly Variation in Hydrographical Parameters at Station V

Parameters	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D
Water temp (°c)	28	27	28	27	29	30	30	30	27	27	26	28	28	28	28
pH	7.6	7.9	7.9	6.3	7.4	7.6	7.9	8	6	6.4	6.5	7.1	6.8	6.7	6.8
D.O (ml/l)	5.7	5.9	5.5	5.2	5.6	5.4	4.9	4.8	6.1	6.4	6.7	6.3	6.0	5.1	5.2

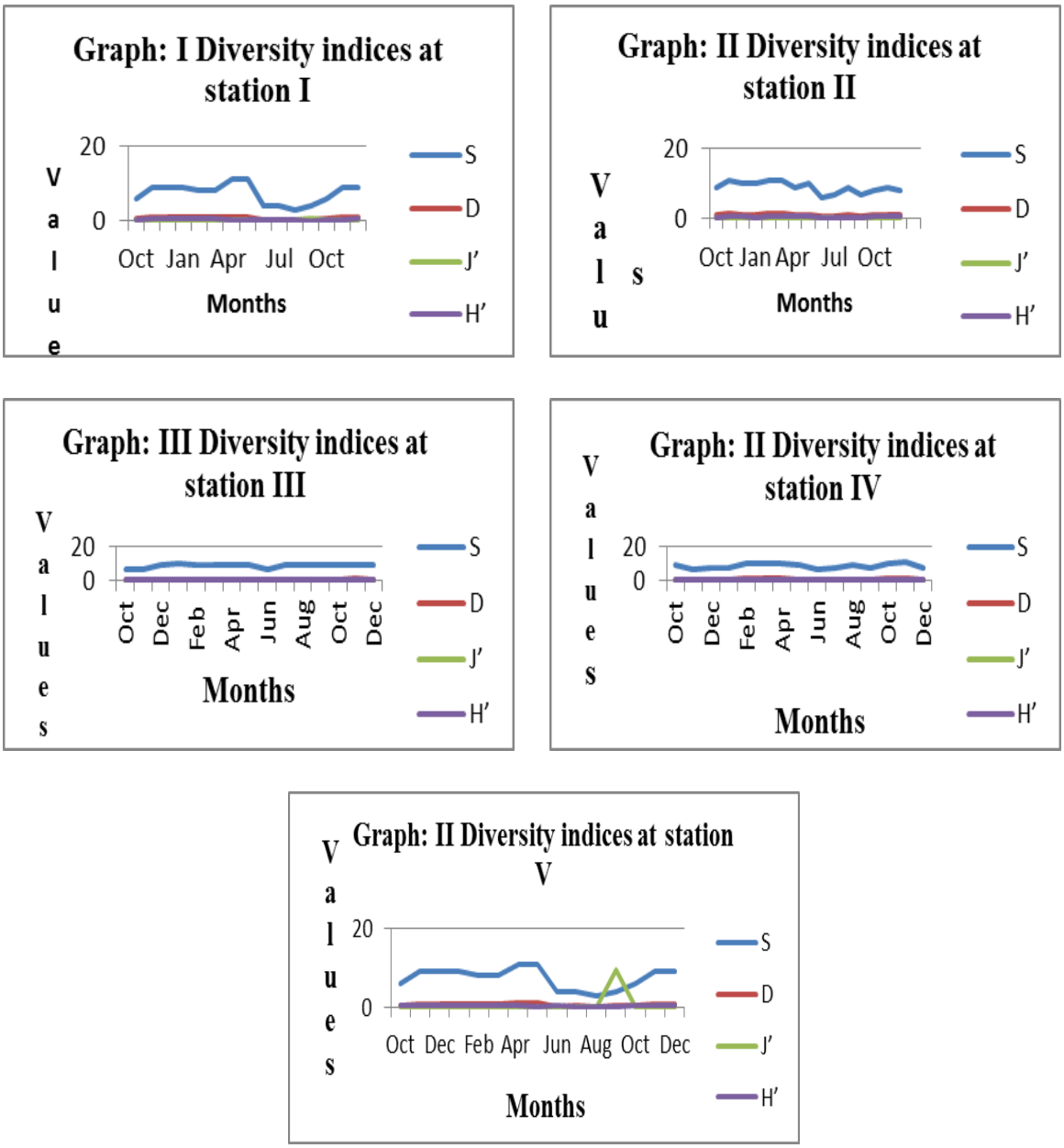

Fig. 1 : Results of Diversity Indices, Species Richness and evenness of zooplankton species

DISCUSSION

Totally 12 groups of zooplankton were identified during the present study. The larval forms ranked 1st (1264-3067/m3) followed by copepods (97-1420/m3) and *protozoa* (41.54/m3). The larval forms constituted about 83-85% of the total species present in all the stations. Copepods constituted 11-13% while *protozoa* constituted only 2-3%.Other groups constituted about 15-17% of the zooplankton diversity. From the study it is clear that the zooplankton population of the study region was found to be dominated by larval forms followed by copepods and protozoans.

Although zooplankton exists under a wide range of environmental conditions, yet many species are limited by DO, temperature and other physico-chemical factors. According to (Magurran 1988) the diversity indices based on 1. Stable

communities have diversity value and unstable ones have low diversity and 2. Stability in diversity is an index of environmental integrity and wellbeing. Species diversity indices of the Zooplankton groups showed Margalef index (d) ranged between 0.4 and 1.68 and Shannon-Weiner (H) ranged between 0.40 and 0.78 and Evenness (J') ranged between 0.20 and 0.32. In all the stations highest numbers of zooplankton species were observed in the month of May and lowest in the month of October. According to Mcdonald (2003) the value of diversity indices ranging between 1.5 and 3.4 represents low diversity and species richness value above 3 indicates high diversity. In the present study, the value of Shannon-Weiner (H) ranged 0.40 and 0.78.

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RESEARCH ARTICLE

NF1 gene Analysis: New paradigm by computational approach

Jadhav VA[1,2] and Laeequr Raheman[3]

[1]Department of Biophysics, D.B. College, Bhokar, Nanded, MS, India -431801

[2]School of Life Science, SRTM University, Nanded, 431606, MS, India.

[3]MGM'S college of CS & IT, Nanded, 431601, MS, India

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Abbreviation:
NF1 : Neurofibromatosis
GRD: GAP related domain

ABSTRACT

Now a day, we are having good stimulation and regulation due to small piece of evolutionarily developed nucleotides working dynamically called gene (eg.NF1). The malfunctioning of NF1is autosomal dominant condition, contributes a set distinct genetic disorder that that cause tumors to grow along various types of nerve. In addition, it can affect the development of non-nervous tissue such as bone and skin. The NF1 gene, encodes for protein called neurofibromine, belongs to family of protein that serve as negative regulators ras oncogene. The GRD region encoded by exons 20-27a, is the function ascribed region. We are aiming to identify and analyze with structure prediction.

Keywords: structure prediction, autosomal dominant, NF1, GRD.

INTRODUCTION

Neurofibromatosis type 1(NF1) is one of the most common genetic disorders in human and is characterized by neurofibromas (Riccardi, 1992) It encompasses a set of distinct genetic disorder within neurons, brains, bones, skins etc that cause tumors to grow various nerves and non-nervous tissue. Neurofibromatosis cause to tumor to grow anywhere on or in the body. The NF1codes for protein neurofibromine, it posses a region that shares a high homology with the family of GTPase–activating proteins, which are negative regulators of RAS function and thereby control cell growth and differentiation (Serra *et al*., 1997). NF1 patients show 'two hit' hypothesis with one allele inactivated and another somatically mutated. While considering importance of impaired regulation (Sebastian, 2011)

We are analyzing NF1 locus in benign neurofibromas in NF1 gene. The further research will helpful in active site prediction and possible outcomes for pharmacokinetics.

MATERIALS AND METHODS

In this analysis, we have retrieved nucleotide as well as protein sequence of NF1 gene from NCBI Gene database (http://www.ncbi.nlm.nih.gov/gene) and Protein database (http://www.ncbi.nlm.nih.gov/protein). After retrival of protein sequence of NF1 gene we analyzed primary structure protein using ProtParam tool, which computes various physico-chemical properties of given protein sequence. It is available online in proteomics category of ExPASy sever http://web.expasy.org/protparam. The secondary structure analysis was carried out by ANTHEPROT integrated protein sequence software. It provides analysis by different method, out of which GOR and DPM method were used in secondary structure analysis. In consequence we predicted motif, domain, coiled region of NF1 protein sequence using Pfam (http://pfam.xfam.org/search/sequence) and Inter Pro Scan (http://www.ebi.ac.uk/Tools/pfa/iprscan5). The PDB File format was used to analyzed active region i.e. Motif and domain for the basis of protein ligand interaction.

RESULTS AND DISCUSSIOINS

Neurofibromin is cytosolic protein with molecular weight of 280kDa. Atomic composition of neurofibromin protein shows 2818 total amino acid. The physico-chemical parameter are specific volume 0.74cm^2 cm/g, Extinction Coefficient 282685/m cm, Estimated half-life >10 hours (E.coli, in vivo), Instability index computed to be 43.35 and GRAVY value is estimated to be -0.129 (Table 1).

Table 1: Physico-chemical parameter of Neurofibromin

Number of amino acids: 2818 Molecular weight: 317032.5 Theoretical pI: 6.90 Total number of positively charged residues (Arg + Lys): 290 Atomic composition: Carbon C 14141 Hydrogen H 22457 Nitrogen N 3813 Oxygen O 4158 Sulfur S 144 Formula: C14141H22457N3813O4158S144 Total number of atoms: 44713	Total number of negatively charged residues (Asp + Glu): 299 Extinction coefficients: Extinction coefficients are in units of M-1 cm-1, at 280 nm measured in water. Ext. coefficient 282685 Abs 0.1% (=1 g/l) 0.892, assuming all pairs of Cys residues form cystines Ext. coefficient 278810 Abs 0.1% (=1 g/l) 0.879, assuming all Cys residues are reduced Estimated half-life: The N-terminal of the sequence considered is M (Met). The estimated half-life is: 30 hours (mammalian reticulocytes, in vitro). >20 hours (yeast, in vivo). >10 hours (Escherichia coli, in vivo). Instability index: The instability index (II) is computed to be 43.35 This classifies the protein as unstable. Aliphatic index: 94.36 Grand average of hydropathicity (GRAVY): -0.129

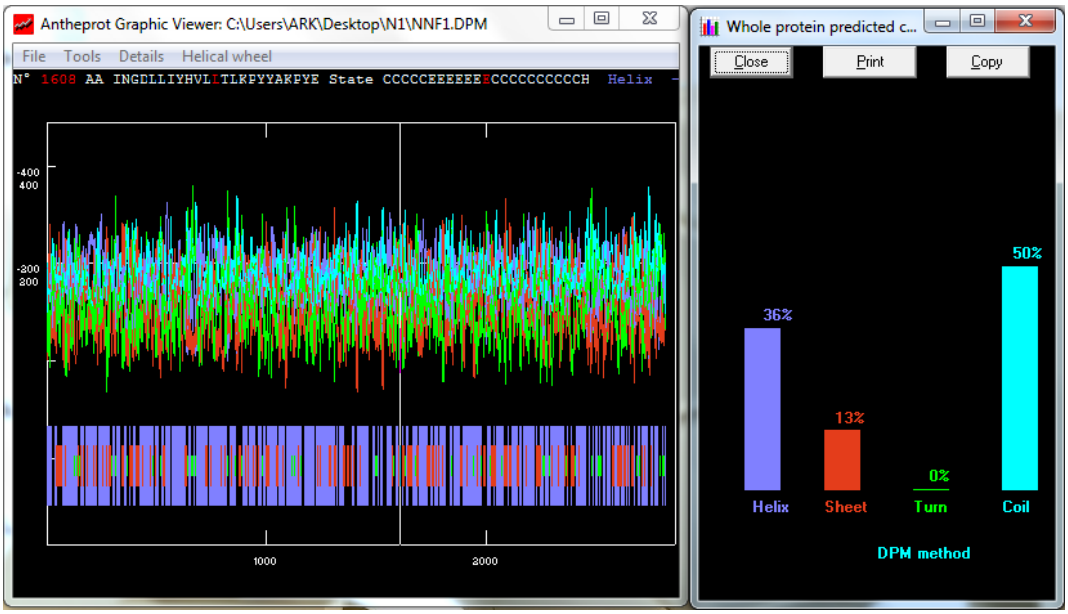

Fig.1: Secondary Structure prediction using Antheprot (a) By DPM method

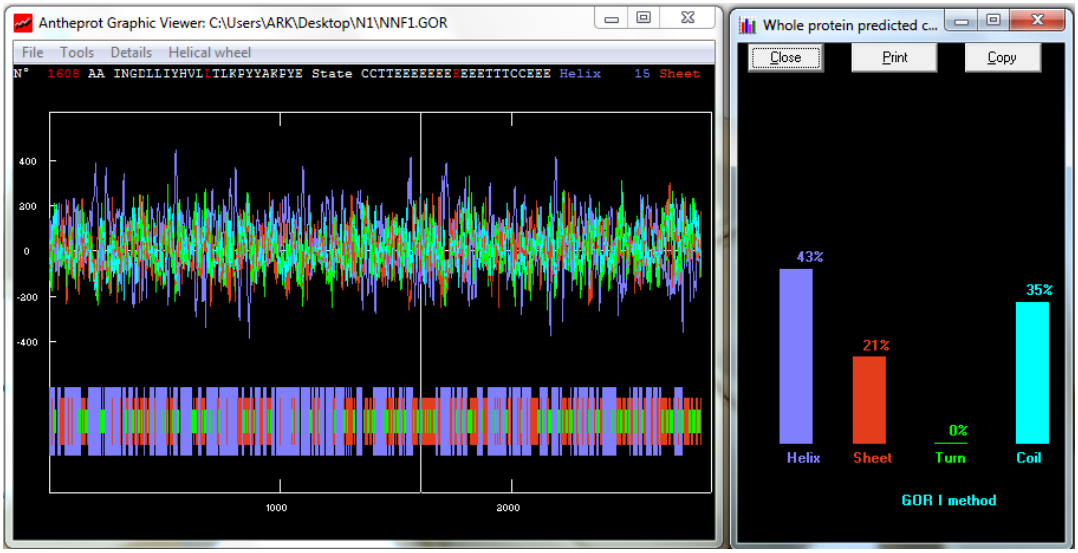

Fig .1: (b) By GOR methods

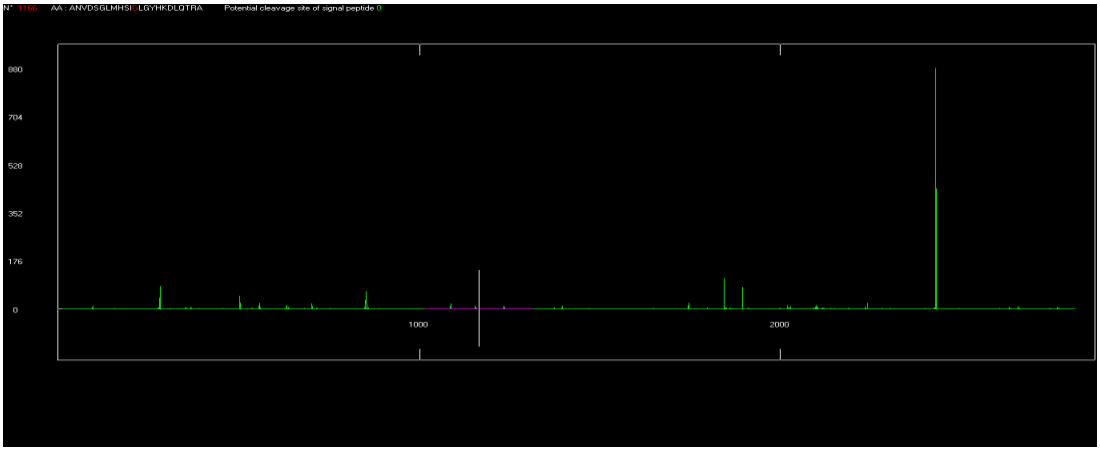

Fig.2: Potential Cleavage Site Using Antheprot (Eukaryotes)

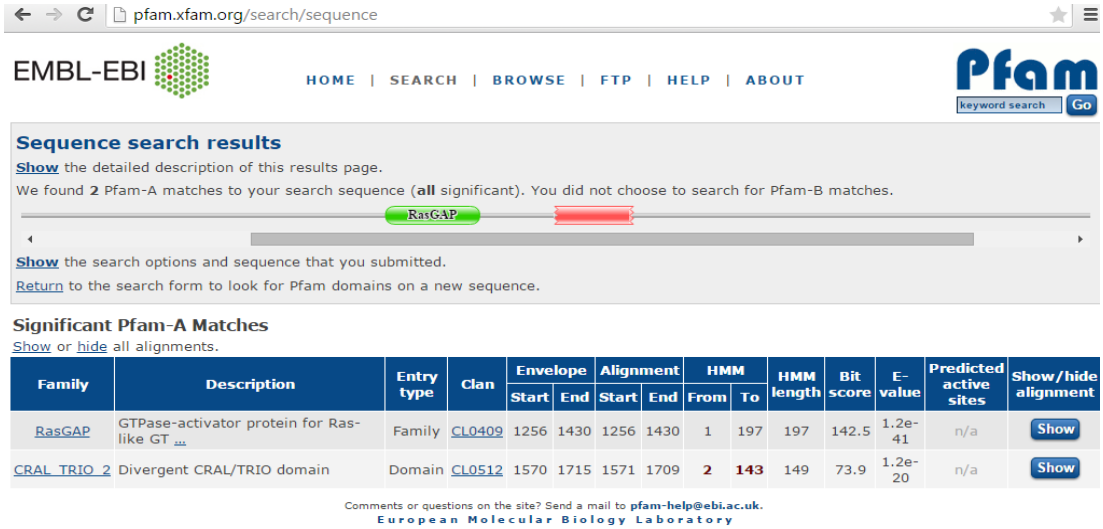

Fig 3: Pfam result showing RasGap related Protein

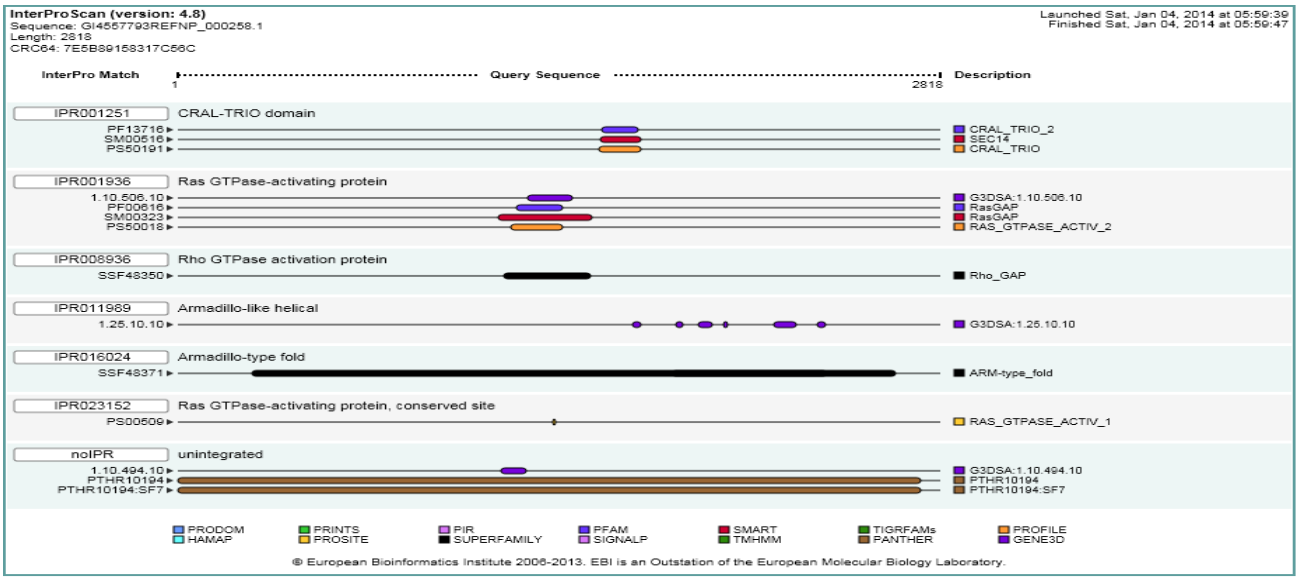

Fig.4: InterPro Scan showing different domain in prote

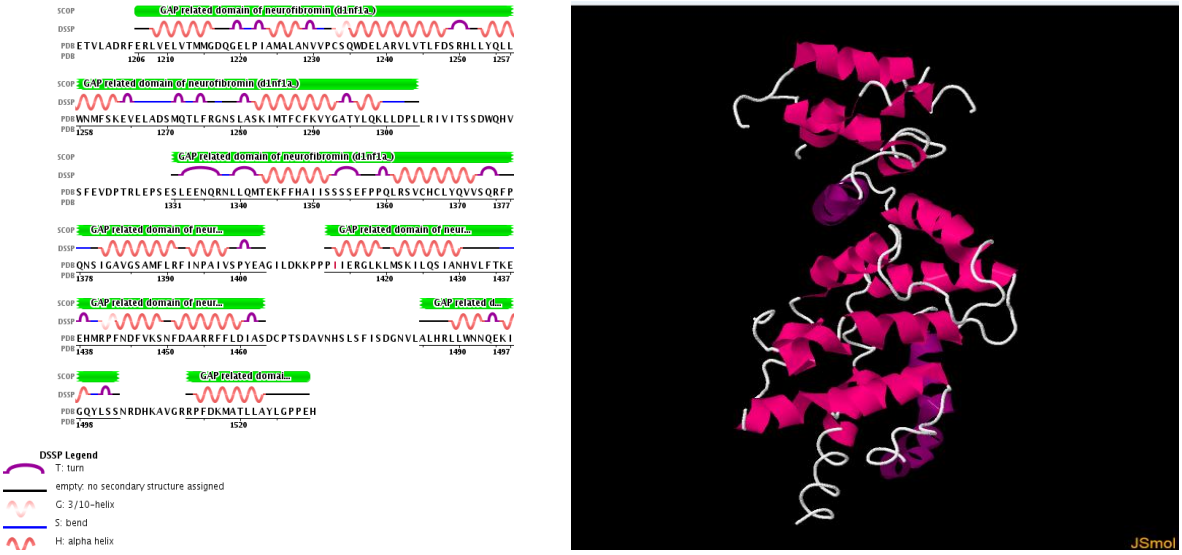

Fig.5: GAP related domain of neurofibromin and 3D view of GAP related domain of neurofibromine using JSmol

The secondary structure comprises alpha helix, β-sheets, turns and coiled region. The analysis shows 36% Helix, 13%Sheet, 0% Turns, 50% coiled region whereas 43% Helix, 21%Sheet, 0% Turns, 35% coiled region according to DPM and GOR method respectively (fig. 1.a & b). The potential cleavage site of signal peptide (Eukaryote) shown in (fig 2). We have found 11 Pfam-A matches to our search sequence (2 significant and 1 insignificant). The graphics below shows (fig.3) the arrangement of matches on our sequence. InterproScan showing different domain in protein (fig.4) GAP related domain of neurofibromin consist of 260 residues (fig.5). 3D Structure of GAP related domain of neurofibromin showing in fig.5.

CONCLUSION

The various protein parameters give significant information about atomic composition, bonding, interactions etc. so it can use to regulate the functioning of neurofibromin protein. This approach is important in new paradigm of computational drug design.

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SHORT COMMUNICATION

Unusual sighting of Yellow- wattled Lapwing (*Vanellus malabaricus*) in Lucknow District, Uttar Pradesh, India

Kumar Adesh * and Kanaujia Amita

Biodiversity & Wildlife Conservation Lab, Department of Zoology, University of Lucknow, Lucknow, UP, India

*Corresponding author Email:adesh.science@gmail.com

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ABSTRACT

The present study concerns the survey of Yellow-wattled Lapwing in Lucknow district. It is a wader and its habitats preferences includes any sort of open ground, dry fields and the largest concentrations are found in and near wetlands. It is medium-sized pale brown waders with a black crown which is separated from the brown on the neck by a narrow white band and large yellow facial wattles. It is obligate visual forager, meaning catch its prey at the substrate boundary layers, by picking small invertebrates from the surface or from low vegetation cover. Yellow-wattled Lapwing plays a prominent role in ecosystems. The Yellow-wattled Lapwing contributes to maintaining ecosystem food chain because they regulate and maintain the populations of many invertebrates (Pests) which are harmful for agricultural crops. Yellow-wattled Lapwing is a good example of territorial and social behaviour. The present survey was carried out at winter and summer season during February 2014- March 2015.

Keywords: Ecosystem, Yellow-wattled Lapwing, Social behavior.

INTRODUCTION

Yellow- wattled Lapwing is prominent and unique wader with a black crown, white supercilium, large yellow facial wattles and tail is white, tipped black belong to family Charadriidae and order Charadriiformes. The long legs are yellow in colour. The breeding season is March-May to the lead of the monsoon. Its habitats preferences include any sort of open ground, dry fields and the largest concentrations are found in and near wetlands fringes (Kumar, 2015). They make short distance movement in

response to rain. It is obligate visual forager, meaning catch its prey at the substrate boundary layers, by picking small invertebrates from the surface or from low vegetation cover. Yellow-wattled Lapwing plays a prominent role in ecosystems. The Yellow-wattled Lapwing contributes to maintaining ecosystem food chain because they regulate and maintain the populations of many invertebrates (Pests) which are harmful for agricultural crops. Yellow-wattled Lapwing is a good example of territorial and social behaviour. There appears to be slightly decreases in its status over the past few decades and is considered as least concerned bird. Only few works have done by several workers on Yellow-wattled lapwing (Jayakar and Spurway, 1965; 1968; Dhindsa, 1983; Gupta and Kaushik, 2010; Santharam, 1980; Sethi et al., 2010). Though, no one has studied Yellow-wattled Lapwing so far in Lucknow in context of status, ecology and threats, hence the present study was primarily effort.

MATERIALS AND METHODS

The Geographic coordinates of Lucknow is 26.8470^0 N and 80.9470^0 E. The study area involves Lucknow and its associated areas (up to 100 km). The capital of Uttar Pradesh is situated 123 meter above sea level. In summer temperature ranges from 25-45°C while in winter from 2-20 °C, the average annual rainfall is about 896.2 mm (35.28 inch).Lucknow covers an area of 2528 sq.km.

Ecological survey of Yellow-wattled Lapwing was carried out at a fixed time- interval from February 2014- March 2015. Species was observed and monitored twice in a day in the morning and evening hours. Observations and monitoring were done with the aid of an Olympus 10x50 binocular and photography was done with 60 D SLR Cannon camera.

RESULTS AND DISCUSSIOINS

The yellow-wattled lapwing (*Vanellus malabaricus*) is a lapwing and a group of largish waders in the family Charadriidae that is endemic to the Indian Subcontinent. Dr. Amita Kanaujia along with research scholar Mr. Adesh Kumar had gone to wetlands survey and bird watching on 2nd February 2014 at Magaiyapurva Jheel, Gosaiganj (N 26˚38.707′ and E 081˚03.237′) in district Lucknow of Uttar Pradesh. They saw a flock of medium size birds looking rather common Myna or others waders but with long yellow legs and brown colour (Fig. 1 & 2). For few moments they show like little ringed plover but size was larger than plover. On comparison

Fig 1: Yellow- wattled Lapwing in resting and in flight

Fig 2: A flock of Yellow- wattled Lapwing

with colour plates in the Pictorial Guide of Ali & Ripley (1995), Grimmett *et al*., (2011), Ali (2002) identified the waders as Yellow- wattled Lapwing (*Vanellus malabaricus*). Total 31 lapwings were sighted here. However Yellow- wattled Lapwing was not sighted before at this site. Thereafter a follow up was kept every year at the Magaiyapurva Jheel, Gosaiganj in winter and sighted 46 lapwings. Kumar (2015) observed nesting biology of Yellow- wattled Lapwing in agricultural environment of Punjab.

On 17th January 2015, the authors visited a wetland near Sanjay Gandhi Post Graduate Institute of Medical Science (SGPGI, N 26˚44'24.6" and E 080˚57'16.2") and Behda pond in Nagar Chaungwa, Mahona (N 27˚05.700′ and E 080˚53.980') in Lucknow district of Uttar Pradesh. Again authors visited Sanjay Gandhi Post Graduate Institute of Medical Sciences (SGPGI) and Behda pond on 2nd February 2015 and 17th March 2105. Then we visited the backside of the Deen Dayal Upadhayaya Park (SGPGI) and we were delighted to see a flock of around 25-35 Yellow- wattled Lapwing and 22-40 from Behda pond. Although Yellow- wattled Lapwing seen in past but very little in number but these study sites number were quite much. A study on threats to nests of Yellow- wattled Lapwing in Kurukshetra has performed by Gupta and Kaushik (2012). A.S.F.L Lok and Subaraj (2009) studied the status of Lapwings in Singapore.

CONCLUSION

The gathering of large number of Yellow-wattled Lapwings at above study sites in Lucknow for feeding, resting and roosting is due to the abundance of food such as macrophytes, macrobenthic organisms and insects. Accessibility to food resources and availability of exposed mudflats and shorelines of wetlands provides an ideal location feeding and roosting. Still, no one has studied Yellow-wattled Lapwing so far in Lucknow in context of status, ecology and threats; hence the present study was primarily effort. Preliminary study performed in the discussed area would reveal a baseline data which is important for further research and conservation.

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