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Original Article

Alterations in Protein Content in three Different Vital Organs of a Freshwater Snail, *Pila globosa* Exposed to Cadmium Chloride

Bibhas Guha*, Onkar Nath Ghosh**

Abstract

Alterations in protein content was studied in freshwater bivalve,*Pila globosa* exposed to three different concentrations (*viz*., 0.002%, 0.005% and 0.01%) of cadmium chloride ($CdCl_2$) in mantle, foot, and liver at three different fixation intervals (*viz*., 7d, 14d and 21d). There was a significant decrease in protein content in all the three vital organs of *P. globosa* under experimentation as the concentration of $CdCl_2$ increases. The depletion of protein content was due to the toxicity of $CdCl_2$ and this toxicological stress might have increased the proteolysis activities in the cells.

Keywards: Protein Content; *Pila globosa*; Toxicity; Cadmium Chloride.

Introduction

Increasing level of pollution and its impact on living organisms has become a subject of great concern. Heavy metals pose a serious threat to the aquatic environment because of their toxicity, persistence, tendency to accumulate in organism and undergo food chain amplification (Weis and Weis 1977 a, b).They cause severe damage to the aquatic fauna, including molluscs, fishes etc, thereby telling up on their health and population. Cadmium (Cd) is one of the most toxic and widespread heavy metal, and is a recognized carcinogen in mammals (Pruski and Dixon, 2002). Cd reaches the water bodies from combustion of fuels, and plastics, phosphate fertilizers, pesticides, domestic wastes, oil refineries and electroplating industries. Amongst the heavy metals, the chloride, sulphate and nitrates of cadmium are soluble compounds whereas carbonate and hydroxides are not. Nica et al. (2012) advocated that Cd pose serious threats to environmental health because they tend to bioaccumulate in terrestrial ecosystems. Cd has also attracted a lot of attention as a soil pollutant because of its persistence, toxicity and bioaccumulative potential along terrestrial trophic chains (Veltman et al., 2008; ATSDR, 2012). It affect the activity of biologically active molecule such as glycogen, protein and lipid of target organisms (Ghosh and Chatterjee 1985; Devaraj and Devaraj 1987).

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Among soil-dwelling invertebrates, land snails closely adhere to preconditions for serving as a pertinent bioaccumulator for soil Cd (Berger and Dallinger, 1989; Gomot, 1997; de Vaufleury et al., 2006). These molluscs are able to sequestrate and detoxify Cd via complexation to specific Cd metallothioneins (Dallinger et al., 2001), and hence can accumulate Cd far above environmental concentrations without showing any metabolic disorders (Dallinger and Rainbow, 1993). Rao Ramana and Ramamurthi (1980) have observed the effects of sumithion on biochemical constituents in *Pila globosa*, but reports on such effects of $CdCl_2$ is limited.

Even though some reports are available on the effects of Cd toxicity in different groups of animals, but a comparative study of the effect of different concentrations of $CdCl_2$on the protein content of some vital organs of *Pila globosa,* an important food chain invertebrate of fresh water body are scanty, thus the present experiment was undertaken.

Materials and Methods

Live specimens of *Pila globosa* (Phylum: Mollusca; Class: Gastropoda; Family: Ampullariidae) weighing between 20 and 25 g were collected from a local fresh water pond and acclimated in concrete vat containing fresh tap water for ten days before using them for experimentation. After that the specimens were removed into 15 L glass aquaria each containing 10 L tap water (pH 7.25±0.14, total hardness 95±2.08mgL^{-1} as $CaCO_3$, dissolved oxygen 6.56±0.75 mgL^{-1}, temperature 28 - 30^{0}C) and five number of *Pila*. Prior to experiment they were cleaned to remove the fouling algal biomass and mud. Healthy and mature snails of approximately equal size were selected. Five live specimens each kept in the aquaria with three different optimally low doses that produced fairly quantifiable changes (obtained through range-finding trials) of Cadmium chloride ($CdCl_2$) (Sigma Aldrich, Cat. No. 10108-64-2): (1) 0.002 %; (2) 0.005%; (3) 0.01%. *Pila* kept in fresh tap water was served as control. The specimens were fed with *ad libitum* with pieces of an aquatic plant *Hydrilla* till sacrificed. After 7d, 14 d and 21 days of treatment the experimental *Pila* were sacrificed and three different vital organs, namely, mantle, foot and liver were removed for protein estimation. As the foot and mantle of *Pila* directly or indirectly exposed to the waterbodies and liver is the main site of metabolism, thus taken for the experiment. For protein estimation the collected tissues were homogenized in 0.9% NaCl. After centrifugation at 3000g for 15 minutes, the supernatants were collected. For estimating the quantity of protein, the techniques of Lowrey et al (1951) using folin-ciocalteu reagent was followed. A standard curve was constructed from the different known concentration of Bovine Serum Albumin (BSA) against OD values. The amount of unknown protein (mg/gm tissue) was calculated in the routine manner (at 550 nm) against the standard curve in the U-V spectrophotometer (Simatzu, Japan).

Statistical Analysis

For calculation of the differences between control and treated series, Student's t-test was conducted and the level of significance determined by using the Fisher and Yates statistical tables (Fisher and Yates, 1963).

Results and Discussion

Heavy metals, particularly Cd has been identified as the major source of aquatic pollution and detected in alarming quantities in many water bodies, particularly at or near industrial localities where effluents are routinely discharged. Tumorigenic, carcinogenic, mutagenic, teratogenic, and other cytotoxic effect of $CdCl_2$ have been extensively studied in both microorganisms and higher animals, particularly in mammals (Kalinina and Ploukhina. 1977; Felten, 1978; Bruce and Heddle, 1979; Deknudt and Gerber, 1979 Leonard, 1979), but such data are extremely limited for molluscs (Shuhaimi-Othman et al., 2012; Nica et al., 2012; Venkata Chandrudu and Radhakrishnaiah, 2008, 2013).

In the present experiment alterations of total protein content of *P. globosa* was found in all the three tissues examined (*i.e.*, foot, mantle and liver) at three different concentrations (*i.e.*, 0.002%, 0.005% and 0.01%) of $CdCl_2$. A critical analysis of the data reveals that the protein content was maximum in the liver tissues as compared to foot and mantle (Table-1, 2 and 3). It was found that in foot the amount of protein was decreased as the concentrations of the $CdCl_2$ increases in all the consecutive fixation intervals (*viz.*, 7d, 14d and 21 d), and the results was found to be significant ($p < 0.05$; 0.01; 0.001) for all the doses (see table-1). More or less similar trend of result was found for mantle and liver tissues (see Table-2 and 3). Previously, the significant decrease in total protein content in foot, hepatopancreas and gills of the fresh water mussel, *Lamellidens corrianus* on exposure to organochlorine insecticide, hildan have been advocated by Kulkarni, et al (2005). In fact, Hightower (1991)

Table 1: Showing the amount of protein (mg/gm) in the foot of *P. globosa* in three different doses of $CdCl_2$ in respect of control at three different fixation intervals

Days intervals	% of doses of $CdCl_2$ in respect of control			
	Control	0.002%	0.005%	0.01%
7 days	23.87±0.79	22.78±0.62	21.62±0.19^a	17.87±0.61^c
14 days	25.90±0.40	23.20±0.49^b	24.29±0.11^b	20.09±0.47^c
21 days	22.69±0.72	22.08±0.39	20.95±0.41	18.34±0.52^b

Number of individuals examined in each series/fixation intervals =5; ^{a} $p < 0.05$; ^{b} $p < 0.01$; ^{c} $p < 0.001$.

Table 2: Showing the amount of protein (mg/gm) in the mantle of *P. globosa* in three different doses of $CdCl_2$ in respect of control at three different fixation intervals.

Days intervals	Control	% of doses of $CdCl_2$ in respect of control 0.002%	0.005%	0.01%
7 days	14.66±0.36	12.73±0.57[a]	12.43±0.59[a]	11.79±0.60[b]
14 days	15.75±0.48	13.86±0.30[a]	11.82±0.28[c]	11.63±0.45[c]
21 days	15.03±0.25	13.22±0.41[b]	12.50±0.57[b]	10.10±0.41[c]

Number of individuals examined in each series/fixation intervals =5; [a]$p < 0.05$; [b]$p < 0.01$; [c]$p < 0.001$.

Table 3: Showing the amount of protein (mg/gm) in the liver of *P. globosa* in three different doses of $CdCl_2$ in respect of control at three different fixation intervals

Days intervals	Control	% of doses of $CdCl_2$ in respect of control 0.002%	0.005%	0.01%
7 days	35.23±0.08	34.71±0.22	33.62±0.52[a]	30.96±0.31[c]
14 days	36.67±0.04	35.98±0.45	35.53±0.80	33.91±0.38[c]
21 days	34.12±0.39	32.33±0.19[b]	32.10±0.39[b]	31.71±0.57[b]

Number of individuals examined in each series/fixation intervals =5; [a]$p < 0.05$; [b]$p < 0.01$; [c]$p < 0.001$.

introduced the term "proteotoxicity" as a central aspect of toxicity, which takes place at the level of protein. A marked fall in the protein level in all the tissues indicates a rapid initiation of breakdown of protein due to the proteotoxic effect of $CdCl_2$. On the other hand, the decrease in average total protein content of tissue after treatment suggests enhancement of proteolysis to meet the high energy demands under Cd or other stress (Patil, 2011). The depletion of tissue protein content was might be due to diversification of energy to meet the impending energy demand under toxic stress (Vincent et al., 1995). The result of the present experiment clearly demonstrated that the depletion of protein content in the vital organs of *Pila* was due to the toxicity of $CdCl_2$.

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Original Article

Effect on Growth and Yield of Summer Green Gram (*Vigna Radiata* L. Wilczek) under Different Hydrothermal Regimes

Ravi Kiran

Abstract

Effects of changed hydrothermal regimes was studied on summer green gram (*Vigna radiata* L. Wilczek) cv SML – 134 at Punjab Agricultural University Ludhiana in summer season of 1999 and 2000. The treatments included 3 dates of sowing viz. 12th April (D_1), 19th April (D_2) and 26th April (D_{-3}) (in main plots); 3 irrigation levels viz. 0.5 IW: CPE ratio (I_1), 0.75 IW: CPE ratio (I_2) and 1.0 IW: CPE ratio (I_3) (in sub plots); and mulched (M_1) (@ 5t/ha wheat straw mulch) and unmulched crop (in sub-sub plots), in a split-split plot design.

The first sowing date D_1 showed better results than by D_2 and D_3, respectively in terms of growth and yield. I_3 had more vigorous growth than I_2 and I_1, respectively. Mulched crop performed better than unmulched crop. The crop yielded was less during 2000 than 1999. This may be due to more vegetative growth during 2000 and infestation of whitefly, in which crop experienced more frequent rainfall than 1999.

Keywords: *Vigna Radiata* L. Wilczek; Plant Height; Sowing Dates; Irrigation; Straw Mulching; Grain Yield; Straw Yield; Harvest Index.

Introduction

Mungbean (*Vigna radiata* L. Wilczek) is an important pulse crop It is grown in an area of 3.42 million ha with production of 1.70 million tonnes in India (Anonymous, 2013). Mungbean is grown both in *Kharif* and summer seasons. Being a short duration crop (70 days), summer mungbean acts as a catch crop, therefore holds promise for increasing cropping intensity and improving soil productivity by fixing atmospheric nitrogen. The final yield of any crop is a continuous interaction of genetic variables and environmental factors to which crop is exposed.

Sowing dates plays an important role for optimum yield of this crop (Singh *et al.*, 2010). Summer/spring mungbean is sown from mid March to first week of April after the harvest of *Brassica* spp., lentil, potato, toria; and in the second fortnight of April after the harvest of wheat in most of the northern part of India. Sowing dates and irrigation regimes depict varied performance and productivity of summer mungbean due to changed environment plant interactions. Crop grown during April-June needs frequent irrigation due to higher evaporative demand and intense radiation. The recommended sowing window under Punjab is from 20 March to April 10 (Anonymous, 2015).

Supraoptimal temperature (> 35°C) and reduced water availability during pre-monsoon summer period restricts the growth of summer mungbean. The use of different types of mulches have been reported to lower evaporation losses and to reduce soil temperature fluctuation resulting into favourable modification of soil hydrothemal regimes. Straw mulch offers a mean of modifying supraoptimal temperature, conserving moisture and also increasing the crop productivity.

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Keeping this in view the present investigation was planned, to see the effect of modified hydrothermal regimes in ameliorating the harsh field climatic extremes and its consequences on the growth and yield of summer mungbean.

Material and Methods

The field experiment was carried out at the Research Farm, Department of Agricultural Meteorology, Punjab Agricultural University, Ludhiana, during summer season 1999 and 2000. Ludhiana in located at 30° 54'N latitude and 75° 56'E longitude, at an altitude of 247 m above mean sea level. The area is characterized by semi arid subtropical climate with very hot summer and cold winter during April - June and December - January, respectively. During summer maximum temperature ranges between 40-45°C and occasionally goes up to 47°C while during winter, the minimum air temperature ranges between 5-8°C and occasionally goes as low as 0°C. This region in dominated by hot dry westerly winds during summer season. The average annual rainfall of this region is 677 mm, more than 75 percent of it is received during July-Sept. Weekly average weather parameters has been shown in the Fig. 1a and 1b The treatments included three dates of sowing viz.12th April (D1), 19th April (D2) and 26th April (D3) (main plots); three irrigation levels viz. 0.5 IW/ CPE ratio (I1), 0.75 IW/CPE ratio (I2) and 1.0 IW/CPE ratio (I3) (sub plots); and mulched (M1) (@ 5t/ha wheat straw mulch) and unmulched crop (in sub-sub plots), in a split-split plot design. All the recommended practices were followed as per the Package and Practices, Punjab Agricultural University, Ludhiana.

The area is characterised by semi arid subtropical climate with very hot summer and cold winters during April - June and December - January, respectively. During summer maximum temperature ranges between 40-50°C and occasionally goes upto 47°C while during winter, the minimum air temperature ranges between 5-8°C and occasionally goes as low as 0°C. This region in dominated by hot dry westerly winds during summer season. The average annual rainfall of this region is 677 mm, more than 75% of which is received during the period from July to September. The weekly meteorological data of summer season 1999 and 2000 is presented in Table 3.1 and 3.2, respectively.

The experimental field was given a pre-sowing irrigation to maintain optimum soil moisture for germination. After two days, ploughing was done twice with disc harrow and cultivators, followed by planking. The crop was fertilized with 12.5 kg nitrogen/ha in the form of urea and 40 kg P_2O_5/ha in the form of single super phosphate, at the time of sowing. Summer mungbean (*Vigna radiata* L. Wilczek) was sown on 12th, 19th and 26th April in 1999 and 2000, by using the seed @25 kg/ha with '*Kera*' method, keeping row spacing of 22.5 cm and plant to plant spacing of 5 cm.First hoeing was done 25 days after sowing and second hoeing, as per requirement, about two weeks after the first hoeing.The irrigation schedule of the crop for 1999 and 2000 is given in the Table 3.3 and 3.4. The depth of each irrigation was 7.5 cm. Summer mungbean crop is mostly affect by

Table 1: Cropping history of the field

Year	Crop Grown	
	Kharif	*Rabi*
1996-97	Fallow	Winter maize
1997-98	*Dhaincha*	Sunflower
1998-99	Groundnut	Potato
1999-2000	Summer mungbean*	Potato
2000-2001	Summer mungbean*	Potato

Table 2: Physico-chemical properties of soil.

Depth (cm)	Bulk density (g/cc)	pH 1:2 soil water	EC (mmhos cm^{-1} at 25°C)	Field capacity (w/W)
0-15	1.48	7.9	0.3	12.5
15-30	1.50	7.9	0.2	14.5
30-45	1.52	7.8	0.2	16.5
45-60	1.52	7.9	0.2	17.0
60-90	1.54	7.6	0.2	17.2
90-120	1.55	7.5	0.2	17.4

Table 3: Dates of differential irrigations for first, second and third dates of sowing after a common irrigation at 25 DAS in 1999

Treatments	Dates of irrigation		
	I	II	III
$D_1 I_1 M_1$	25 May		
M_2	(43)*		
$D_1 I_2 M_1$	17 May	30 May	
M_2	(35)	(48)	
$D_1 I_3 M_1$	14 May	25 May	1 June
M_2	(32)	(43)	(50)
$D_2 I_1 M_1$	31 May		
M_2	(42)		
$D_2 I_2 M_1$	27 May	8 June	
M_2	(38)	(50)	
$D_2 I_3 M_1$	21 May	30 May	10 June
M_2	(32)	(41)	(52)
$D_3 I_1 M_1$	10 June		
M_2	(36)		
$D_3 I_2 M_1$	3 June	16 June	
M_2	(38)	(51)	
$D_3 I_3 M_1$	30 May	11 June	20 June
M_2	(34)	(46)	(55)

*Figures in parenthesis shows the days after sowing

Table 4: Dates of differential irrigations for first, second and third dates of sowing after a common irrigation at 25 DAS in 2000

Treatments	Dates of irrigation		
	I	II	III
$D_1 I_1 M_1$	22 May		
M_2	(40)*		
$D_1 I_2 M_1$	18 May	28 May	
M_2	(36)	(47)	
$D_1 I_3 M_1$	16 May	23 May	1 June
M_2	(34)	(41)	(50)
$D_2 I_1 M_1$	29 May		
M_2	(40)		
$D_2 I_2 M_1$	29 May	2 June	
M_2	(34)	(44)	
$D_2 I_3 M_1$	20 May	28 May	
M_2	(31)	(39)	
$D_3 I_1 M_1$	17 June		
M_2	(52)		
$D_3 I_2 M_1$	30 June		
M_2	(34)		
$D_3 I_3 M_1$	27 May	18 June	
M_2	(31)	(53)	

*Figures in parenthesis shows the days after sowing

Table 5: Effect of sowing dates, irrigation levels and mulching on periodic plant height (cm) of summer mungbean

Treatment	28 DAS		35 DAS		42 DAS		49 DAS		56 DAS		63 DAS		Harvest	
	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000
D_1	5.98	6.25	9.20	9.60	12.02	14.21	16.52	17.25	20.98	23.66	25.35	26.12	26.90	27.66
D_2	5.82	6.29	9.00	8.99	11.42	12.84	15.38	15.03	19.79	20.69	23.45	24.87	24.89	26.05
D_3	5.68	5.98	8.75	8.84	9.58	10.70	13.69	12.99	16.52	17.43	21.54	21.63	22.96	22.61
CD (P =0.05)	**NS**	**NS**	**0.43**	**NS**	**0.81**	**1.32**	**2.06**	**1.86**	**1.25**	**1.29**	**1.17**	**2.61**	**1.36**	**0.49**
I_1	5.68	5.97	7.59	8.84	10.04	11.49	14.58	13.83	17.62	19.05	20.87	20.73	22.15	21.96
I_2	5.89	6.20	8.92	8.88	10.64	12.66	14.09	15.20	18.61	20.06	22.85	24.90	24.50	26.07
I_3	5.92	6.27	9.45	9.61	12.34	13.60	16.86	16.20	21.06	22.67	26.62	26.99	28.17	28.29
CD (P =0.05)	**NS**	**NS**	**0.30**	**NS**	**0.62**	**1.29**	**1.39**	**0.78**	**0.73**	**0.81**	**0.58**	**1.91**	**0.67**	**0.58**
M_1	5.89	6.22	8.94	8.47	11.61	13.13	15.58	15.67	19.85	21.40	24.43	25.23	25.90	26.10
M_2	5.76	6.12	8.37	8.61	10.41	12.09	14.54	14.51	18.34	19.70	22.40	23.18	23.93	24.70
CD (P =0.05)	**NS**	**NS**	**0.14**	**NS**	**0.66**	**0.72**	**0.62**	**0.69**	**0.44**	**0.77**	**0.62**	**1.58**	**0.97**	**1.06**

CD (P = 0.05) for interaction: Non-significant

Table 6: Effect of sowing dates, irrigation levels and mulching on periodic dry matter accumulation (g/plant) in summer mungbean

Treatment	28 DAS		35 DAS		42 DAS		49 DAS		56 DAS		63 DAS		Harvest	
	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000
D_1	1.07	1.13	2.61	2.74	5.74	6.0	8.71	8.97	9.90	10.01	12.18	12.90	12.12	12.73
D_2	0.99	1.05	2.22	2.54	5.28	5.49	8.43	8.33	9.50	9.41	11.60	11.70	12.06	12.52
D_3	0.97	1.04	1.93	2.36	4.73	4.92	8.12	7.83	8.90	8.73	11.65	11.80	11.32	11.83
CD (P =0.05)	**NS**	**NS**	**0.14**	**6.09**	**0.52**	**0.51**	**0.33**	**0.33**	**0.59**	**0.70**	**0.80**	**0.71**	**0.23**	**0.70**
I_1	1.02	1.07	2.08	2.31	4.24	4.44	6.87	6.83	7.80	7.77	9.29	9.32	10.14	10.63
I_2	1.02	1.07	2.24	2.56	5.59	5.90	9.07	8.83	9.50	10.04	12.22	12.40	12.44	13.04
I_3	1.01	1.09	2.47	2.77	5.92	6.07	9.32	9.42	11.0	10.34	12.92	12.70	12.90	13.41
CD (P =0.05)	**NS**	**NS**	**0.04**	**0.11**	**0.24**	**0.28**	**0.28**	**0.27**	**0.24**	**0.43**	**0.50**	**0.42**	**0.38**	**0.64**
M_1	1.02	1.09	2.25	2.60	5.42	5.65	8.70	8.64	9.70	9.60	11.87	11.90	12.25	12.73
M_2	1.01	1.06	2.26	2.49	5.07	5.29	8.14	8.08	9.21	9.14	11.07	11.06	11.41	11.99
CD (P =0.05)	**NS**	**NS**	**NS**	**NS**	**0.20**	**0.32**	**0.33**	**0.34**	**0.17**	**0.38**	**0.48**	**0.53**	**0.45**	**0.42**

CD (P = 0.05) for interaction: Non-significant

Table 7: Effect of sowing dates, irrigation levels and mulching on grain yield (q/ha), straw yield (q/ha) and biological yield (q/ha) in summer mungbean

Treatment	Grain yield (q/ha)			Straw yield (q/ha)			Biological yield (q/ha)		
	1999	2000	Pooled	1999	2000	Pooled	1999	2000	Pooled
D_1	8.74	8.26	8.50	35.12	37.90	36.51	43.86	46.16	45.01
D_2	7.88	7.76	7.81	34.00	35.54	34.77	41.88	43.29	42.58
D_3	7.47	7.16	7.37	33.25	33.49	33.37	40.72	40.65	40.68
CD (P =0.05)	**0.74**	**0.65**	**0.41**	**0.98**	**1.33**	**0.68**	**1.01**	**1.88**	**0.88**
I_1	6.97	6.60	6.78	31.23	30.63	30.93	38.20	37.23	36.73
I_2	7.50	8.05	7.77	34.09	35.10	34.59	41.59	43.15	42.37
I_3	9.62	8.51	9.06	37.04	41.21	39.12	46.67	48.18	49.19
CD (P =0.05)	**0.98**	**0.74**	**0.58**	**1.03**	**1.12**	**0.72**	**1.40**	**1.43**	**0.94**
M_1	8.60	8.29	8.44	35.76	36.38	36.67	44.36	44.68	44.52
M_2	7.46	7.14	7.30	32.48	34.91	33.69	39.95	42.66	41.00
CD (P =0.05)	**0.68**	**0.82**	**0.51**	**1.03**	**1.16**	**0.75**	**1.44**	**1.65**	**1.06**

CD (P = 0.05) for interaction: Non-significant

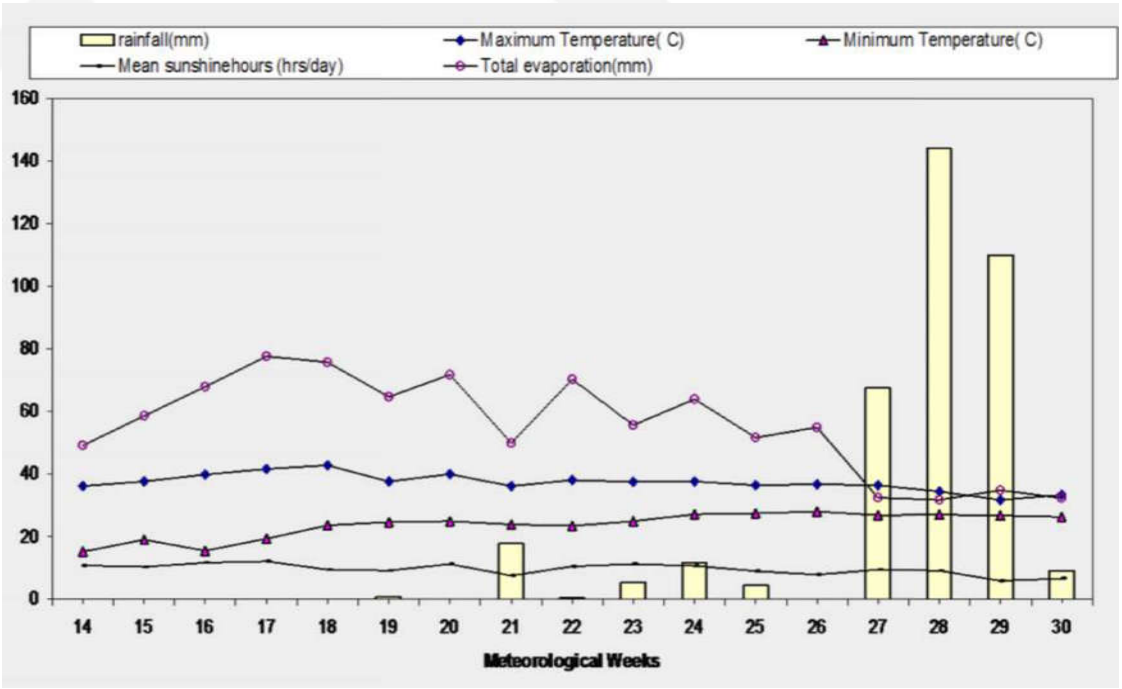

Fig. 1a: Weekly average weather parameter during crop growing period in 1999.

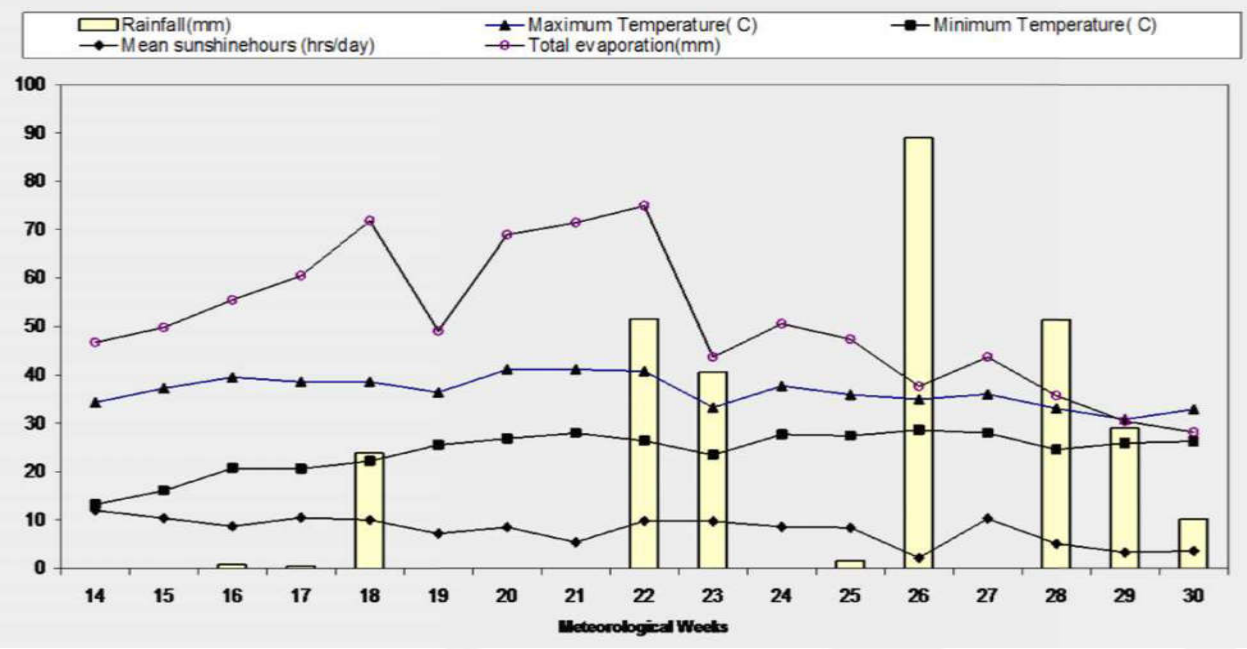

Fig. 1b: Weekly average weather parameter during crop growing period in 2000.

thrips, on flower causing flower drop, deformation of pods, deterioration of grain quality and ultimately high reduction in yield. To prevent the attack of thrips, the crop was sprayed with 120 ml metasystox 25 EC spray in 80-100 liter/ha. To prevent the attack of whites fly 375 ml malathion 50 EC spray in 80-100 liter/ha. The crop was harvested on 20th June, 23rd June and 25th June, 1999 and on 26th June, 26th June, 9th July, 2000, respectively, in case of D_1, D_2 and D_3. Sun dried pods were threshed and grain yield and straw yield was recorded and converted into q/ha.

Result and Discussion

Plant height

Plant height, an index of growth and development of plant, is an important physiological character to assess the vegetative growth. A persual of data in Table 4.6 shown that plant height increased progressively with the advancement of crop growth stages with different dates under different treatments. At initial stages of crop (28 DAS, 35 DAS), there was no significant differences within different treatments but later on it became significant due to advancement of crop and due to differential soil moisture regimes to which crop was exposed. The plant height was significantly different among sowing date treatments and was highest in D_1 (26.9 cm) followed by D_2 (24.8 cm) and D_3 (22.9 cm) during 1999 and D_1 (27.6 cm) followed by D_2 (26.0 cm) and D_3 (22.6 cm) during 2000, respectively. Plant height was higher in 2000 than 1999 due to more rainfall in 2000.

Irrigation treatments depict that differential irrigation significantly affected the plant height. Plant height was maximum in I_3 (28.1 cm) followed by I_2 (24.5 cm) and I_1 (22.1 cm), respectively, at harvest during 1999. During 2000, again the plant height was found maximum at I_3 (28.2 cm) followed by I_2 (26.0 cm) and I_1 (21.9 cm), respectively.

Mulched crop showed significantly higher plant height than that of unmulched crop, at each stage of the crop, 42 DAS onwards till harvest. The mulched crop had 25.9 cm, 23.9 cm plant height in 1999 and 26.1 cm, 24.7 cm in 2000, respectively.

Plant height was directly affected by the microclimate to which plant was exposed. Early sowing dates, highly irrigated and mulched treatments had higher plant height due to optimize growth conditions. In a study at New Delhi Gupta *et al.* (1994) also reported higher plant height in mulched summer mungbean than that of unmulched crop.

Dry Matter Accumulation

Dry matter accumulation is directly influenced by plant height and leaf area index. During both the years the dry matter accumulation differed significantly among the different dates of sowing, irrigation levels and mulching treatments and was found higher in

2000 than in 1999 due to higher rainfall in 2000. Peak dry matter accumulation was found at 63 DAS.

D_1 showed highest dry matter (in g/plant) at 63 DAS (12.18) followed by D_3 (11.65) and D_2 (11.60), respectively, during 1999. During 2000 the dry matter in D_1, D_2 and D_3 was 12.90, 11.70 and 11.80, respectively. Among irrigation treatments the dry matter (in g/plant) at 63 DAS in I_1, I_2 and I_3 was 9.29, 12.22 and 12.92 in 1999; and 9.32, 12.40 and 12.70, in 2000, respectively. The dry matter (in g/plant) in mulched and unmulched crop at 63 DAS was 11.87 and 11.07, during 1999; and 11.90 and 11.06, during 2000, respectively.

It is evident that leaf area plays important role in determining the total dry matter of plant. Due to higher leaf area, more photosynthate was synthesized and accumulated in plant resulting into the higher total dry matter accumulation. Similar results are also reported by Kundu (1988) and Gupta *et al.* (1994).

Grain Yield

Grain yield was found significantly different within the treatments during both the years. During 1999, the grain yield was highest in D_1 (8.74 q/ha) followed by D_2 (7.88 q/ha) and D_3 (7.47 q/ha), respectively. During 2000, the grain yield followed the same pattern and found highest in D_1 (8.26 q/ha) followed by D_2 (7.76 q/ha) and D_3 (7.16 q/ha), respectively. There was no significant difference in grain yield in D_2 and D_3 during both years.

Irrigation treatments also affected the grain yield, significantly. The yields were at par in I_2 and I_3 during both the years. Grain yield in I_1, I_2 and I_3 treatments were 6.97 q/ha, 7.50 q/ha and 9.62 q/ha during 1999 and 6.60 q/ha, 8.05 q/ha and 8.51 q/ha during 2000, respectively. Mulched crop was found to be higher yielding than that of unmulched crop. Grain yield in mulched and unmulched crop was found significantly different and was 8.60 q/ha and 7.46 q/ha during 1999; and 8.29 q/ha and 7.14 q/ha, during 2000, respectively. Higher grain yield was observed in 1999 than in 2000. This may be due to higher rainfall in 2000 that resulted into higher vegetative growth having an adverse effect on reproductive growth.

Straw Yield

The data reveal that straw yield was also found significantly different within the treatments, during both the years. The straw yield in D_1, D_2 and D_3 was 35.12 q/ha, 34.00 q/ha and 33.25 q/ha during 1999; and 37.90 q/ha, 35.54 q/ha and 33.49 q/ha during 2000, respectively. The straw yield in D_2 and D_3 in 1999 was statistically at par. Differential irrigation directly affected the straw yield during 1999 and 2000. The straw yield in I_1, I_2 and I_3 was found significantly different and was 31.23 q/ha, 34.09 q/ha and 37.04 q/ha during 1999; and 30.63 q/ha, 35.10 q/ha and 41.21 q/ha during 2000, respectively. Mulched crop had significantly higher straw yield than that of unmulched crop. The straw yield in mulched and unmulched crop was 35.76 q/ha and 32.48 q/ha during 1999 and 36.38 q/ha and 34.91 q/ha, during 2000, respectively.

Biological Yield

The data pertaining to the biological yield reveal that biological yield was found significantly different within treatments.

Among sowing dates, the biological yield in D_1, D_2 and D_3 was 43.86 q/ha, 41.88 q/ha and 40.72 q/ha during 1999; and 46.16 q/ha, 43.29 q/ha and 40.65 q/ha during 2000, respectively.

Irrigation levels showed significant effect on biological yield during both the years. The biological yield in I_1, I_2 and I_3 was 38.20 q/ha, 41.59 q/ha and 46.67 q/ha during 1999; and 37.23 q/ha, 43.15 q/ha and 49.19 q/ha, during 2000, respectively. The total biological yield (q/ha) in mulched and non mulched crop differed significantly and was 44.68 and 39.95 during 1999; and 44.68 and 42.66 during 2000, respectively.

These results also find support from the various research experiments conducted by research workers. Yadav *et al* (1992) also reported higher grain and straw yield with increased frequency of irrigation. Kumar *et al* (1995) reported higher total dry matter under straw mulched conditions in summer mungbean.

Conclusion

Grain yield and straw yield are the product of plant environmental interaction and genetic variables of the crop. This is directly affected by meteorological and micrometeorological conditions to which they are exposed. Early sowing of crop showed better results due to longer duration of crop. Frequently irrigated crop had better plant water status that ultimately affected the photosynthesis in a positive way and increased final yield. Mulching with wheat

straw reduced the excess heat load on the soil, therefore, reduced the temperature variation in soil and improved soil water status, that ultimately affected the nutrient absorption by plant, Therefore, the mulched crop had better yield than that of unmulched crop.

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Original Article

Inheritance of Diabetes Mellitus, Hearing Impairment and Epilepsy in Relation to Endogamy

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Abstract

Consanguineous marriage is an intra-familial relationship between two people who share a common ancestor. This type of practice is occurring in most populations, with varying percentages among all marriages in South India. The present study was carried out to estimate the prevalence of consanguinity, degree of consanguinity and patterns of abnormalities among the population.We interviewed 2673 families to determine the effect of consanguinity on common hereditary disorders such as diabetics, hearing impairment and epilepsy in Sivagangai population, Tamil Nadu between November 2015 and March 2016. The degree of consanguinity between each female and her spouse and the degree of consanguinity between their parents were recorded. Of the selected families approached, the rate of consanguinity was 29.62% and coefficient of inbreeding in the current generation was significantly coinciding with the global rate of 29.2%. The degrees of consanguinity among the population were 2nd degree in 279 families, 3rd in 306 and 4th in 207. All marriages were occurred in first cousins and double first cousins. All reported diseases were more frequent in consanguineous marriages. In order to avoid such hereditary related health problems in India, it is imperative to create awareness regarding the adverse effects of endogamous marriages in regions with high prevalence.

Keywords: Endogamy; Degree of Consanguinity; Diabetes; Hearing Impairment and Epilepsy.

Introduction

Consanguineous marriage is a relationship between two people who share a common ancestor. This type of marriage is one of the customary practice occurs in varying degree throughout the world. Consanguinity in Tamil Nadu is a deeply rooted cultural trend in all communities irrespective of religion. The Tamil culture is an assimilation of diverse conventions, customs, rituals, and ideas harmonizing in one central core of seal entity. They believe that consanguinity strengthens family ties and enforces family solidarity. The cousin marriages provide excellent opportunities for the transmission of cultural values and cultural continuity. Wife's parents prefer to have their daughter living near them and to enjoy the presence of their grandchildren. Moreover, wealthy land lords may prefer to keep their property within the family. The National and family health survey statistics reported that South India has the highest degree consanguinity of 20 -60%, when compare to other regions of India [1]. The phenomenon of inbreeding or endogamy increases the level of autosomal genetic disorders known as inbreeding depression [2]. This type of inbreeding has been known to increase the chance of lethal identical genes disorders derived from a common ancestor. The transmission of hereditary disorders increases with degree of genetic relationship of their parents. The risk is higher in offspring whose parents are close relatives cousin than distant relatives, such as second cousins [3]. In view of the above, the current study

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was carried out to determine the extent and nature of consanguinity among population, Sivagangai District and its effect on health disorders such as diabetes mellitus, hearing impairment and epilepsy.

Method

The study was conducted in the population of Sivagangai District between November 2016 and December 2017. The total samples of 2673 families were approached for study. The degree of consanguinity between each female and her spouse and the degree of consanguinity between their parents were recorded. The hereditary health disorder such as diabetes, hearing impairment and epilepsy among the families in the study area in relation to endogamy were assessed. The family members were interviewed separately without having the possible interactions with other sample respondents. Average time limits of 5 to 8 minutes were taken for each sampling.

Result

In consanguineous marriage, it was customary to exclude marital unions beyond second cousins. Second cousins inherit 1/32 of their genes from a common ancestor, which means that their offspring inherits identical genes at 1/64 (1.56%) of all loci. In numerical terms, this was conventionally expressed as coefficient of inbreeding, which for second-cousin progeny was 0.0156. A total of 792 consanguineous (29.62%) and 1881 non consanguineous (70.38%) marriage were recorded in the present study. Among the total populations, 180 individuals had diabetes (72 non-consanguineous and 108 consanguineous), 171 had hearing impairment (36 non-consanguineous and 135 consanguineous) and 315 epilepsy (126 non-consanguineous and 189 consanguineous). Out of 792 consanguineous population, 522 (65.9%) were affected with any one of the respective recessive hereditary disorder (Figure 1). While among 1881 non-consanguineous population, only 234 (12.44%) were affected with the same disorders (Figure 2). Percentages of affected individuals with various degrees of consanguinity were 35.22% in 2nd degree,

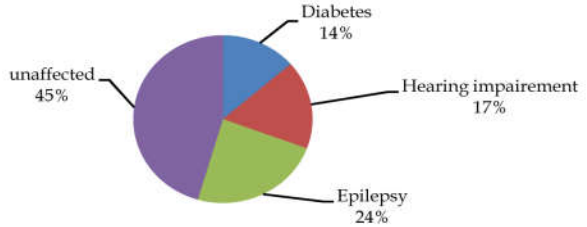

Fig. 1: Percentage of affected and unaffected individuals among the consanguineous population

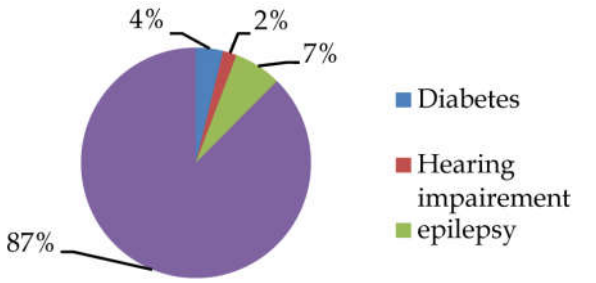

Fig. 2: Percentage of affected and unaffected individuals among the non consanguineous population

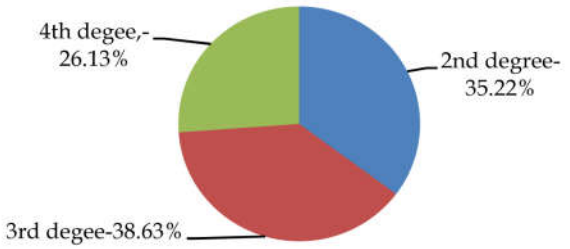

Fig. 3: Percentage of affected individuals with various degrees of consanguinity

38.63% in 3rd degree and 26.13% in 4th degree (shown in Figure 3).

Discussion

Consanguinity is an interbreeding between close people with a common grandparent or people who share another recent ancestor. This type of marriage practice is common among emigrant communities from Pakistan, Turkey, North Africa and Lebanon, North America, Australia and India [4,5]. The previous studies on consanguinity showed that the prevalence of this cultural aspect in South India was ranged from 20 to 60% (Rasathi), which is in accordance to the findings of present study (29.62%). According to Bhaskar, the pattern and prevalence of consanguinity in Mangalore, India was more among Muslims than Hindus and Christians [6]. The same reports were recorded in Belgaum [7]. These studies supported and substantiated the findings of present study that consanguinity is one of the commonest phenomenon amongst all communities of world. The degrees of inbreeding revealed that the endogamy in Sivagangai is an irreplaceable socio cultural aspect of ancient to current generation. The occurrence of consanguinity is more predominant among socioeconomically disadvantageous groups in rural populations. The pattern of cognate marriages in the study area is a cultural based aspect irrespective of religion and considered as the Hot Spot of Uncle-

Niece Consanguinity. Consanguinity has been attributed to low socio-economic status and knowledge about the consequences of consanguinity, can be held directly proportional to Education status of an individual. This trend is prevalent all over the world, especially in Middle Eastern and African Muslim countries.

The present study comprehensively examined the occurrence of blood related marriages and their association with adverse hereditary disorder among Sivagangai populations The rate of congenital malformation was lower among neonates from non-consanguineous marriages and higher from consanguineous marriages [10]. It is believed that high rates of inbreeding over multiple generations lead to elimination of deleterious recessive genes from the gene pool [11]. Among the total population studied, 180 diabetes, 171 hearing impairment and 315 epilepsy. The rates of hereditary abnormalities were higher in consanguine (Figure 1 & 2). Among the total affected population (666), 432 were consanguineous. It is well known that inbreeding leads to an increase in homozygosis by expression of some of the lethal recessive genes and results in an increase in genetic anomalies that cause congenital malformations, polygenic or multifactorial diseases, spontaneous abortions, stillbirths, infant death and sterility [8,9]. The genes responsible for hearing loss are GJB3, GJB6, TECTA and POU3F4, for Diabetes mellitus are HLA, INS, CTS, CTSSA, PPARY, ABCC8, KCNJ11and CALPN10, and for Epilepsy are MDR3, MRP1. Any point mutation in the genes can cause recessiveness and lead to lethal anomalies among individuals. The same multifactorial impairment may be transmitted with change generation after generation though endogamy.

Even though the rural populations are highly affected with hereditary disorder generation after generation, the removal of such consequences from the Tamil culture is a very difficult task at present. Further in-depth studies at gene level are needed to determine the impact of consanguinity in relation to lethal anomalies in this population. In order to avoid hereditary such health problems in South India, it is imperative to create awareness among people about the high risk of homozygous recessive disorders due to blood related marriages. However, the present study may serve as primary platform for further gene imbalance studies among blood related marriages.

Informed Conset

Informed consent was obtained for this study.

Compliance with Ethical Standard

This article does not contain any studies with human participants or animals performed by any of the author.

Financial Disclosure

The author declared that this study has received no financial support.

Cometing Interests

We have no financial interest to declare.

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Original Article

$NaBH_4$ and APS as Novel Inducers of Seed Germination in Green Gram (*Vigna radiata*) and Black Gram (*Vigna mungo*)

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Abstract

igna radiata (mogumah) and *Vigna mungo* (matimah) seeds loose vigour due to high temperature, if the moisture content of the seed is high as it is in state of Assam. Therefore, vigor loss due to heat and moisture is a common phenomenon in the region. Germination and vigour index of seed treated with $NaBH_4$ and APS were found to be higher (38-60%) than that of the seeds without chemical treatment. APS (1mM) was optimum for the germination of black gram as well as green gram. In case of $NaBH_4$, 8mM was optimum for the germination of black gram and 1mM was optimum for the germination of green gram. We believe that the above two chemicals might be useful for stimulating germination of other seeds as well.

Keywords: Heat Stress; Vigour Index; Germination; Sodium Borohydride; Ammonium Persulfate; Synchronized Germination.

Introduction

Researchers reports about seed germination stimulators like karrikins, cyanohydrins etc.[1]. Mehanna et al. (1985) reported the role of hormones and chemicals on seed germinations [2]. One of the common methods of colloidal silver nanoparticle synthesis is using $NaBH_4$ as reducing agent [3]. The excess of $NaBH_4$ in the colloidal solution acts as stabilizing agent [3]. Recently we have observed that colloidal silver nanoparticle solutions stimulate germination and vigor of black gram seeds. Silver nanoparticle has shown some beneficiary effects on plant as reviewed by Nair et al. [4]. Therefore we thought that the above stimulatory effect on seed germination might be due to either silver and/or $NaBH_4$. There are reports of both stimulatory and inhibitory effect of colloidal silver nanoparticle on seed germination [5,6,7]. It has been reviewed that silver nanoparticles with stabilizer might be less toxic compared to that of silver nanoparticles without stabilizer [4,5,6,7]. Colloidal silver nanoparticles often have problems related to stability [8]. Dissociated silver from silver nanoparticles might also form the precursor $AgNO_3$ [3]. Silver nitrate enhanced the abscisic acid sensitivity in embryo and thereby might inhibit germination [9]. Considering all the above different studies relating to the role of silver on seed germination, we got curious to study the effect of $NaBH_4$ on seed germination with an aim to eliminate any possible role of $NaBH_4$ in it. Accordingly when we did the experiment to evaluate the role of $NaBH_4$, we found its stimulatory impact on green gram and black gram seed germination. Based on these interpretations a hypothesis is prepared that $NaBH_4$ might lead to increase in seed vigor. $NaBH_4$ is a strong reducing agent. Therefore, we tested the impact of a strong oxidizing agent like ammonium persulphate (APS) on seed germination. Interestingly, APS also found to be a germination stimulator for heat stressed seeds.

Vigna radiata and *Vigna mungo* are most common staple food in India and other countries of the world. In 2003-04 pulses produced were 635 kg/ha in an area of 23.46 million hectare that slightly decreased to 597 kg/ha grown in an area of 24.54 million hectare

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in 2008-09 as per report of Agriculture Ministry, Govt. of India [10]. As reported by Dubey for a high yield of pulses, instead of considerable improvement has been made in developing techniques, their production per hectare has remained the same for the last two centuries [10]. In India, 12 major different pulse crops are grown and *V. radiata* (green gram) and *V. mungo* (black gram) are among them. Therefore, studying seed germination of these two crops *V. radiata* (green gram) and *V. mungo* is going to be of significant importance. Murthy *et al.* reported the aging of *V. radiata* seed in terms of decrease in the vigour index [11]. They suggested that moisture content of the seed is directly proportional to vigour index loss due to heat stress. In their experiment, they have reported complete loss of vigour index due to loss of germination percentage within 30 days of treatment at 33^{O}C when water content was 0.222 g/g dry seeds weight. This suggests that even moderate temperature can deteriorate the seeds if stored under high humid conditions. Unfortunately, the relative humidity of North Eastern India is very high (60-80%) throughout the year [12]. Further, a vast majority of the population is rural (>80%) and below poverty line (36%) [12].The climate change is increasing the average temperature and North East India along with rest of the Indian subcontinent is experiencing 3-5^{O}C warming [13]. Another problem with heat stress is loss of synchronized germination. This becomes major problem with respect to fertilizer application, pesticide application and harvesting due to non-uniform developmental stages of plants. These environmental and socioeconomic conditions ensures that a huge part of farmers seeds lose their vigour index during storage. There are many chemicals used for stimulating germination of seed. Gibberellic acid stimulate embryo and promote germination [9]. Literature on use of germination stimulator for countering heat stress based loss of vigour index in mung and black gram are scanty [14]. Further, gibberelic acid being a plant hormone is not user friendly with respect to farmers use, storage, cost etc.

To address the above said problem and to find a new germination stimulator to counter heat stress based loss of vigour index in mung and black gram, a research is designed with an objective to evaluate the potency of sodium borohydride and ammonium persulfate.

Materials and methods

Materials and Chemicals

V. radiata (VR) and *V. mungo* (VM) seeds were obtained from local market. Sodium borohydride ($NaBH_4$) and ammonium per sulfate ($(NH_4)_2S_2O_8$) were of Merck, India. 2, 3, 5 Triphenyl Tetrazolium Chloride of AR grade were procured from Merck, India.

Imbibitions

Both seeds (*V. radiata and V. mungo*) of 25 seeds were washed and surface sterilized. These lots were subjected to soaking in sterile distilled water (25 ml) for different hrs from 0-24h with interval of 1h. The weight (g) of water absorbed per g of dry seed weight were measured in an hour wise manner from 1 to 24 hr. Average value of triplicate is presented graphically.

Heat Stressed Seed Production

Heat stressed seed production was performed using the method described by Murthy *et al.* [9]. Briefly, seeds of both species were allowed to imbibe water of around 0.222 g/g dry seed weight and incubated at 37^{O}C incubator (Orbitek-LE) for 144h for decreasing vigor index. After 72 hrs, the seeds having about 90% (*V. mungo*) and 60% (*V. radiata*) vigor index as compared to control, as presented in Figure S1, are used for chemical treatment assay using APS and $NaBH_4$ to counter vigour loss due to heat stress.

Stimulating Germination in Heat Stressed Seeds

Tweenty five seeds of both species were soaked in 25 ml volume of different concentrations of APS and $NaBH_4$ for 24hr. The concentrations of APS and $NaBH_4$ used were 1, 2, 4, 6, and 8 and 10mM.

- *Vigor Index Calculation*

The vigor index of the germinating seeds was calculated after 7 days of incubation at 25^{O}C under dark condition using the formulae:

Vigor index= Average plantlet length (cm) x germination (%)

- *Viability Assay*

Viability of seeds receiving optimum concentration of chemicals to show maximum vigor (for *V. radiata* 1mM APS and $NaBH_4$; *V. mungo*, 1mM APS and 8mM $NaBH_4$) were examined using the method described by Grzybowski *et al.* [15]. Briefly, treated seeds were soaked in 250 ml volume of 0.01% (w/v) 2, 3, 5 Triphenyl Tetrazolium Chloride for 24 h in dark, then washed with sterile distilled water and put in 100 ml of 95% (v/v) ethanol for distaining. Optical density of red color of formazan was measured at 480 nm against blank.

Biochemical Assay

Different biochemical assays were performed for total protein, reducing sugar, soluble starch and total polyphenols present in the seeds of optimum chemical treated concentrations. The seeds were imbibed in different treatments for 24 h along with controls. Protein estimation is carried out using Lawry's method [16]. Reducing sugar estimation is carried out using the procedure described by DNS method [17]. Starch estimation was carried out following I2/KI method [18]. Polyphenol estimation is carried out following Folin Ciocalteu method [19].

Results and Discussions

Water Imbibition and Heat Stressed Seed Production

Water imbibition is performed to obtain seeds containing 0.222 g/ g of dry seed weight. *V. radiata* (Supplementary Figure 1) has a more water imbibition capacity than *V. mungo*. *V. radiata* seeds needed only 1h to absorb 0.222g water/g of dry seed weight. On the other hand, *V. mungo* seeds needed 19h to imbibe the same amount of water. Water imbibition is known to be dependent upon the nature as well as the thickness of the seed coat [20,21]. These seeds containing 0.222g water/g of dry seed weight were subjected to heat treatment at 37^{O}C for production of heat stressed seeds. The seed vigour losses due to heat treatment at 37^{0}C for 144 h were presented in Supplementary Figure S2. It was observed that *V. mungo* vigour loss is quite slow compared to *V. radiata*.

V. radiata seeds vigour index decrease with increasing time period of heat treatment was smooth unlike that of *V. mungo* (Supplementary Figure 2). All *V. mungo* seeds did not imbibe uniformly. That might be reason behind the increase in vigour index on 24 and 96 h (Supplementary Figure 2). Collection of seeds

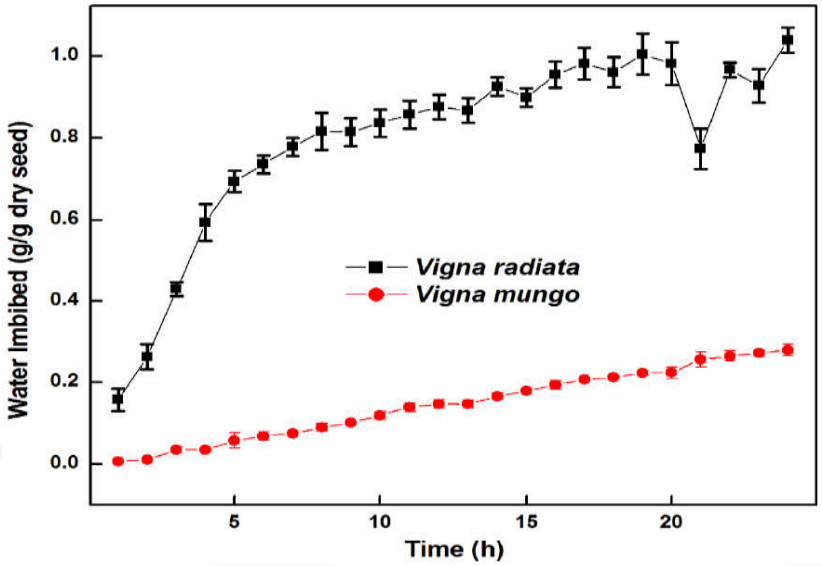

Fig. 1: Amount of water imbibed with reference to soaking for different time intervals

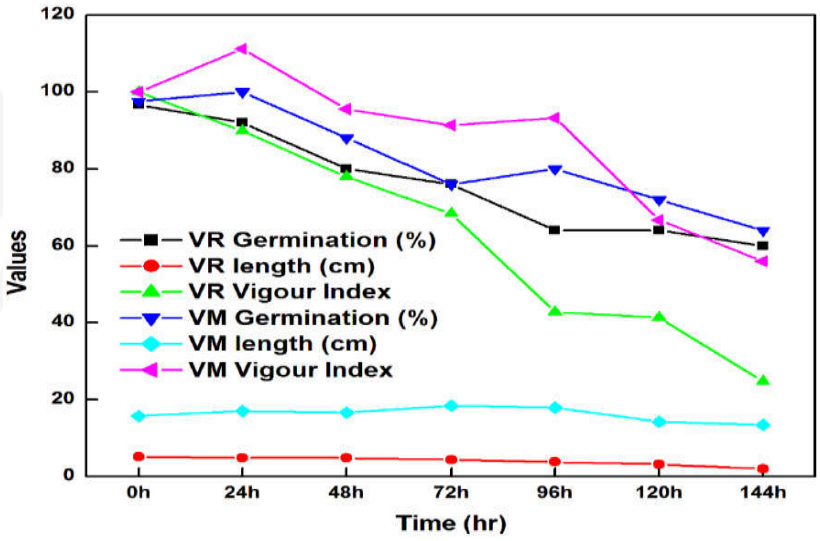

Fig. 2: Germination (%), vigor index and average plant length decrease due to heat treatment of seeds (0.222 g water/g dry seed) at 37^{0}C for different time intervals. VM: *V. mungo*; VR: *V. radiata*.

for chemical treatment was performed after 72h heat treatment, when vigour index *V. mungo* was 90% and that of *V. radiata* was 60% (section 2.3.) (Supplementary Figure 2). The above seeds were stored at 20°C for chemical treatment using APS and $NaBH_4$. During chemical treatment experiment performed later, vigour index for the same seeds of *V. mungo* was recorded around 70-80% (Figure 3b, cyan and pink colour) and that of *V. radiata* was around 40-60% (Figure 3b, yellow and brown colour). The decline in vigour index might happen during storage at 20°C after heat stress. Therefore, it might be suggested that, once heat stress was presented to the seeds with the experimental moisture condition, even storage in non heat stress condition (20°C) cannot stop the loss in vigour. The difference can be realized by comparing the vigour index of seeds without any heat treatment (controls) in Figure 3.a with that of heat stressed seeds (Figure 3b).

Countering Heat Stress and Germination Stimulation

The germination and vigour index calculations are related to each other. Vigour index is a product of germination (%) and average plantlet length (cm). Vigour index is a complete representation of germination percentage and length of germinated plantlet. Therefore it can be said that vigour index is summarization of germination and physique of the germinated seed.

- *V. mungo response to chemical treatment*

Maximum vigour index was recorded for *V. mungo* control seeds treated with 8mM $NaBH_4$ (Figure 3a, VMNABH4 vigour index, pink colour). The same concentration was showing best vigour in case of heat stressed *V. mungo* seeds (Figure 3b, VMHNABH4 vigour index, pink colour). The vigour index (%) of 8mM $NaBH_4$ treated heat stressed *V. mungo* seed was contributed by increase of average plantlet length from 19 to 27 cm (Figure 4b, VMHNABH4, red colour). We attribute the increase in vigour of *V. mungo* heat stressed seeds (treated with 8mM $NaBH_4$) to increase in average plantlet length, since the germination percentage does not differ prominently for $NaBH_4$ treatments from 1 to 8mM (Figure 3b, red colour). The same was true for controls receiving chemical treatment (Figure 1, a, red colour and Figure 4a, red colour). These observations suggest that 8mM NaBH4 treatments not only maintain the germination percentage attained during treatment with 1mM $NaBH_4$ but also stimulates the root-shoot elongation. Further, increase in concentration of $NaBH_4$ declines both germination (%) as well as average plantlet length leading to loss in vigour index (Figure 1, a, red colour and Figure 4 a, red colour).

Heat stressed *V. mungo* seeds when treated with APS, maximum vigour index (%) was recorded for

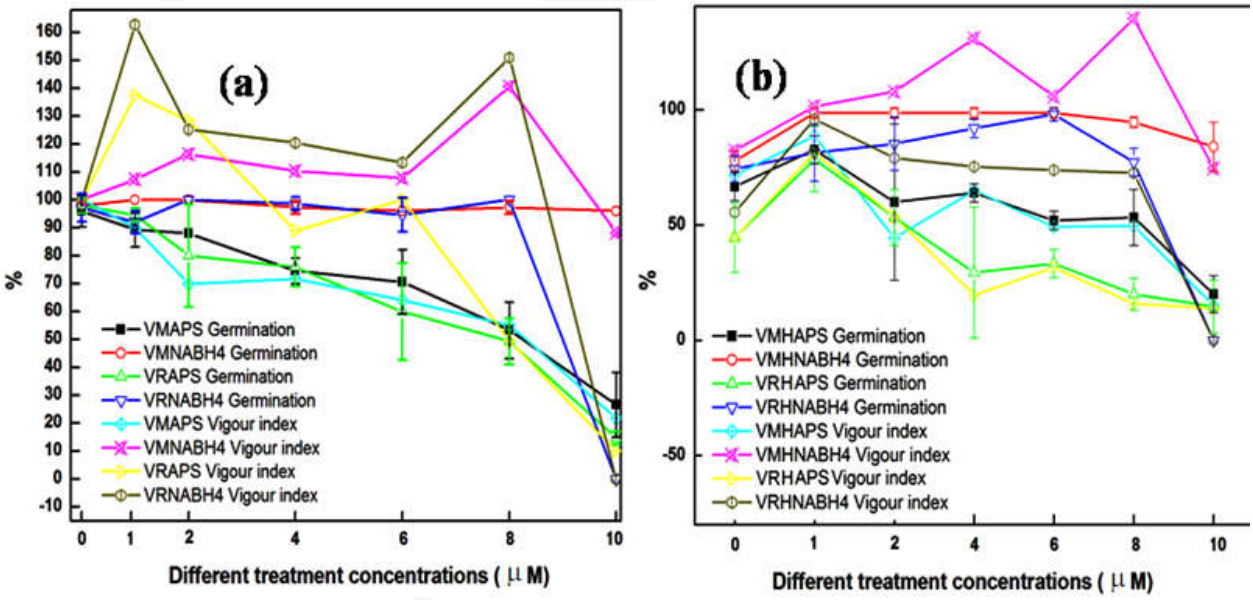

Fig. 3a: Control (without any heat stress) seeds of *V. mungo* and *V. radiata* after 7 days of germination, germination (%) and vigor index (%) data were presented with respect to different concentrations (0, 1, 2, 4, 6, 8 and 10 mM) of APS (ammonium persulphate) and $NaBH_4$ treatments on seeds. VMAPS, *V. mungo* seeds with APS treatment; VMNABH4, *V. mungo* seeds with $NaBH_4$ treatment; VRAPS, *V. radiata* seeds with APS treatment; VRNABH4, *V. radiata* seeds with $NaBH_4$ treatment.
Fig. 3b: Heat stressed seeds of *V. mungo* and *V. radiata* after 7 days of germination, germination (%) and vigor index (%) data were presented with respect to different concentrations (0, 1, 2, 4, 6, 8 and 10 mM) of APS and NaBH4 treatments on seeds. VMHAPS, heat stressed *V. mungo* seeds with APS treatment; VMHNABH4, heat stressed *V. mungo* seeds with $NaBH_4$ treatment; VRHAPS, heat stressed *V. radiata* seeds with APS treatment; VRHNABH4, heat stressed *V. radiata* seeds with $NaBH_4$ treatment.

seeds receiving 1mM treatment (Figure 3 b, VMHAPS, cyan colour). Further, treatment of seeds with increasing concentration of APS showed a decrease in the vigour index of the seeds. This decreasing germination with respect to increase in APS concentration might be attributed to decline in the percentage of germination (Figure 4 b, black colour).

The germination percentages of the plants were calculated on 7th day after sowing. If all the seeds germinate together on first day (synchronized germination) the standard deviation associated with average plantlet length will be less and *vice versa*. Therefore, the standard deviations associated with average plantlet length were also calculated as percentage (SD%). Lower the value obtained for SD (%), suggests more synchronized germination. The SD (%) for *V. mungo* optimum concentration (8mM $NaBH_4$) was lowest, suggesting that optimum vigour stimulating concentration was also synchronizing the germination (Figure 4a, pink colour). The same was also true for 1mM APS treatment (Figure 4a, yellow colour). It should be noted that unlike $NaBH_4$, APS optimum concentration treatment slightly reduce the vigour index of its control (Figure 3a, cyan colour). Summarizing all these, it can be concluded that $NaBH_4$ has a broad concentration range and safe than APS, in case of *V. mungo* seed vigour stimulation.

The optimum stimulation of vigour index was observed in case of heat stressed *V. radiata* seeds treated with 1mM $NaBH_4$ (Figure 3b, brown colour). Same concentration is also true for its controls (Figure 1, a, brown colour). With increasing concentration of $NaBH_4$ in case of heat stressed seeds, the vigour index decreases (Figure 3b, brown colour). The same was also true for control seeds except for treatment using 8mM $NaBH_4$ (Figure 1, a, brown colour). It should be noted that the vigour index of *V. radiata* control seeds treated with 1mM $NaBH_4$ was increased by 60% (Figure 3a, brown colour). The same for *V. mungo* control seeds treated with 8mM $NaBH_4$ was only 40% (Figure 3a, pink colour). This suggested that without heat stress *V. radiata* response to $NaBH_4$ is better than *V. mungo*. The 60 % increase in vigour index, due to treatment of *V. radiata* control seeds with 1mM $NaBH_4$ was due to average plantlet length increase (Figure 4a, blue colour) from 7 (without $NaBH_4$ treatment) to 12 cm (1mM $NaBH_4$). Unlike, other treatments referred above, in case of *V. radiata* seeds (control as well as heat stressed) treated with different concentrations of $NaBH_4$ the more synchronized germination is observed in seeds treated with 2mM NaBH4 rather than 1mM (Figure 3a and b, brown colour). It should also be noted in Figure 4(a and b, brown colour) the SD (%) lowest value was observed for 0.01M $NaBH_4$ treatment. It happens as per calculation because the no germination happened (Figure 3a, blue colour) and

- *V. radiata* response to chemical treatment

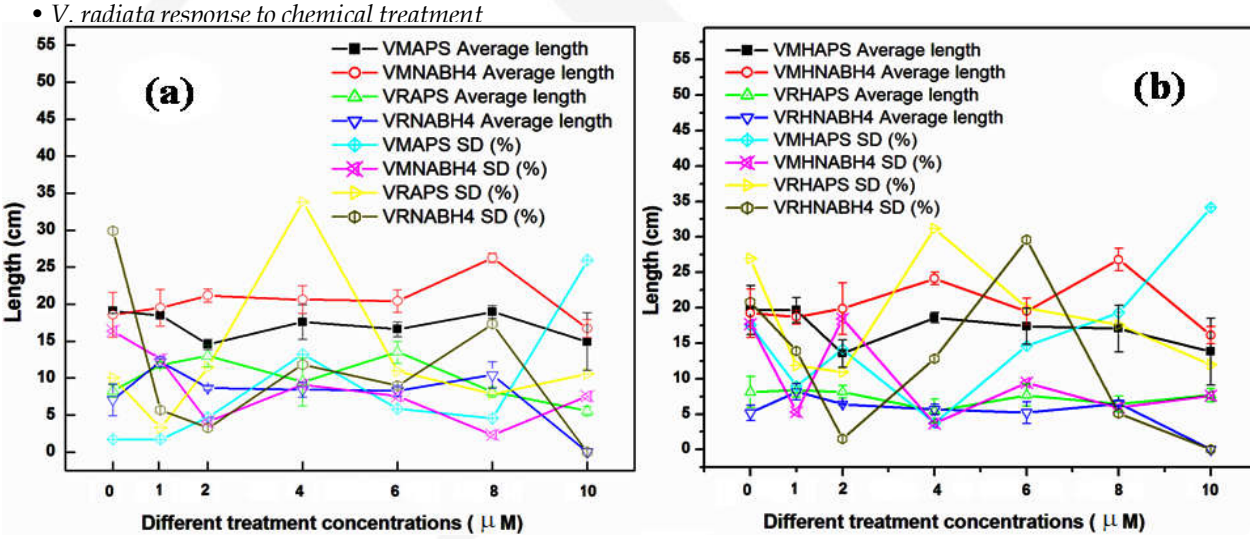

Fig. 4a: Control (without any heat stress) seeds of *V. mungo* and *V. radiata* after 7 days of germination, average plant length (cm) and SD (%) data were presented with respect to different concentrations (0, 1, 2, 4, 6, 8 and 10 mM) of APS (ammonium persulphate) and $NaBH_4$ treatments on seeds. VMAPS, *V. mungo* seeds with APS treatment; VMNABH4, *V. mungo* seeds with $NaBH_4$ treatment; VRAPS, *V. radiata* seeds with APS treatment; VRNABH4, *V. radiata* seeds with $NaBH_4$ treatment. SD (%), stands for standard deviation of length as percentage of original value.

Fig. 4b: Heat stressed seeds of *V. mungo* and *V. radiata* after 7 days of germination, germination (%) and vigor index (%) data were presented with respect to different concentrations (0, 1, 2, 4, 6, 8 and 10 mM) of APS and NaBH4 treatments on seeds. VMHAPS, heat stressed *V. mungo* seeds with APS treatment; VMHNABH4, heat stressed *V. mungo* seeds with $NaBH_4$ treatment; VRHAPS, heat stressed *V. radiata* seeds with APS treatment; VRHNABH4, heat stressed *V. radiata* seeds with $NaBH_4$ treatment. SD (%), stands for standard deviation of length as percentage of original value.

average plantlet length is zero (Figure 4a, blue colour). The same is also true for heat stressed seeds of V. radiata treated with 0.01M NaBH4 (Figure 3b and Figure 4b, blue colour).

In case of *V. radiata* seeds treated with APS, (Figure 3a and b, yellow colour) the results suggested that 1mM was the best concentration for countering the heat stressed seeds. After receiving 1mM APS treatment, the vigour index of heat stressed *V. radiata* seeds reached up to 82%, compared to 42% without APS treatment (Figure 3b, yellow colour). The same was also true for controls with 1mM APS treatment (vigour index 138%) compared to control (Figure 3a, yellow colour). Due to 1mM APS treatment in control seeds the vigour index of *V. mungo* decreases by 10% (Figure 3a, cyan colour) and the same for *V. radiata* increased by about 38% (Figure 1, a, yellow colour). Therefore, it might be suggested that *V. radiata* is more sensitive to APS and NaBH4 compared to *V. mungo*. After comparing Figure 3 (yellow colour) and Figure 4 (green colour), it can be concluded that vigour index difference among *V. radiata* seeds treated with different APS concentrations were not only because of average plantlet length difference but also due to difference in germination percentage (Figure 3a and b, green colour). The synchronized germination in case of *V. radiata* seeds treated with APS was observed for 1mM (Figure 4b, yellow colour).

Viability

The viability assays of the heat stressed seeds treated with chemicals were performed selectively (Figure 5). Only the seeds treated with optimum heat stress countering concentrations (for *V. radiata,* 1mM of both APS and $NaBH_4$; *V. mungo*, 1mM APS and 8mM $NaBH_4$) were subjected to viability assay. Figure 5 represents the viability with respect to formazan formation (represented as optical density at 480 nm) due to activity of mitochondrial dehydrogenase enzyme in seed. The optical density (OD) of *V. mungo* (about 1.0) C1 (control without heat stress and chemical treatment) decreased by about half (about 0.5) due to heat stress (C2) (Figure 5, red colour). The same trend is observed for C2 of *V. radiata*. Therefore, it suggests that 50% vigour index (Figure 3b, yellow colour) difference coincide with loss of mitochondrial dehydrogenase activity. On chemical treatment with 1mM $AgNO_3$ for 12 hr (known to stimulate dormancy by increasing abscisic acid receptor in embryo) and then 24hr APS treatment, OD decreases further below (compared to C2) to about 0.45 for both *V. mungo* (Figure 5, red colour) and *V. radiate* (Figure 3, blue colour). The same treatment when performed with $NaBH_4$ for both the seeds the OD increases to about 0.85 (Figure 5, green and black colour). This suggests that $NaBH_4$ might be a better agent to counter $AgNO_3$ induced dormancy than APS. On APS treatment the *V. mungo* seeds do not show much increase in OD (about 0.8) (Figure 5, T1, red colour)

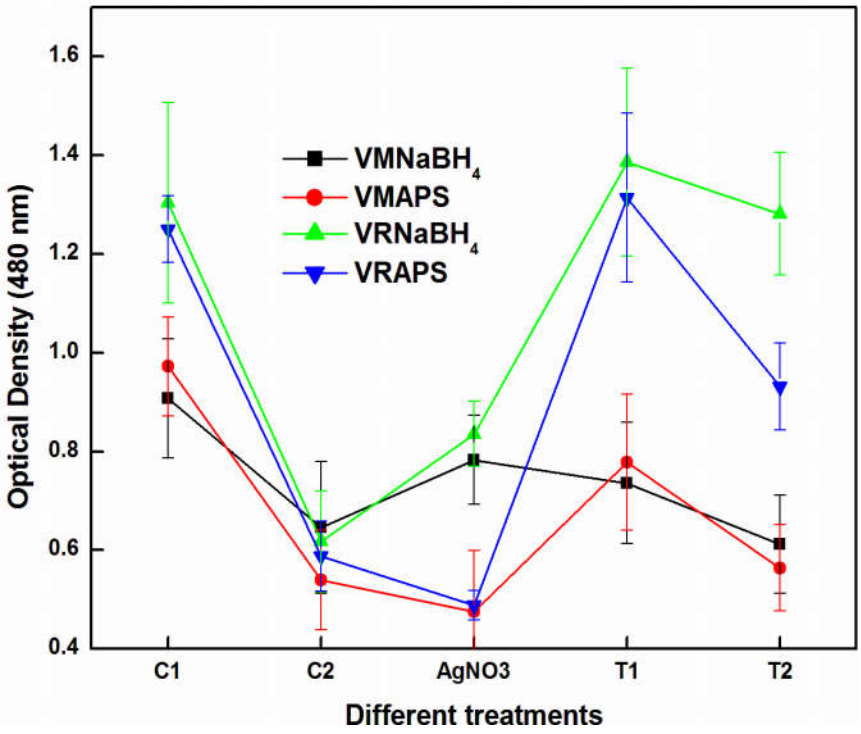

Fig. 5: Viability of chemically (APS and $NaBH_4$) stimulated seeds. $VMNaBH_4$, seeds of *V. mungo* after 8mM $NaBH_4$ treatment (except C1 and C2); VMAPS, seeds of *V. mungo* after 1 mM APS treatment (except C1 and C2); VRNaBH4, seeds of *V. radiate* after 1mM $NaBH_4$ treatment (except C1 and C2); VRAPS, seeds of *V. radiate* after 1mM APS treatment (except C1 and C2); C1, without heat stress and chemical treatment; C2, with heat stress and without chemical treatment; $AgNO_3$, 12h soaked in 1mM $AgNO_3$ solution, 24hr soaked in chemical treatments (APS and $NaBH_4$); T1, without heat stress and with chemical treatment; T2, chemical treated after heat stress.

and the T2 OD is comparable with C2. Therefore, in case of *V. mungo*, the chemical treatment did not affect the viability of the seeds tremendously (section 3.2.1.). Similarly the T1 of *V. radiata* with both chemical treatment show OD of about 1.2 compared to C1 with OD 1.3-1.4 (Figure 5, green and blue colour). On the other hand, in case of *V. radiata* T2 samples with both chemicals treatment show OD of about 0.9 (for APS treatment; Figure 5, blue colour) and 1.3 (for $NaBH_4$ treatment; Figure 5, green colour) which is significantly high compared to C2 OD (near 0.6). This coincided with our previous section result of vigour index that *V. radiata* seeds were more sensitive to the $NaBH_4$ and APS compared *to V. mungo* seeds.

Biochemical Profile of the Optimum Concentration Treated Seeds

A significant biochemical change in the seed happened with respect to germination. Out of these changes few are very important from germination prospect. Therefore to see a significant difference with respect to total protein, reducing sugar, total polyphenol and starch we analyzed only the seeds showing optimum germination due to chemical treatments along with controls. The set of samples is same as presented in viability test. The process of germination starts with imbibition of water, leading to drop of abscisic acid in embryo. Activated embryo secret gibberelic acid which activates the aleuron layer of cells to secret a-amylase; leading to hydrolysis of endosperm starch and production of reducing sugar. These soluble reducing sugars are used by the embryo for growth. The embryo and aleuron also secret minute quantities of protease and lipase depending on endosperm reserve. Therefore, increase in concentration of these chemical is a positive signal for germination in progress.

As presented in Figure 6 (black colour) the protein concentration was found highest in *V. radiata* seeds without heat stress with $NaBH_4$

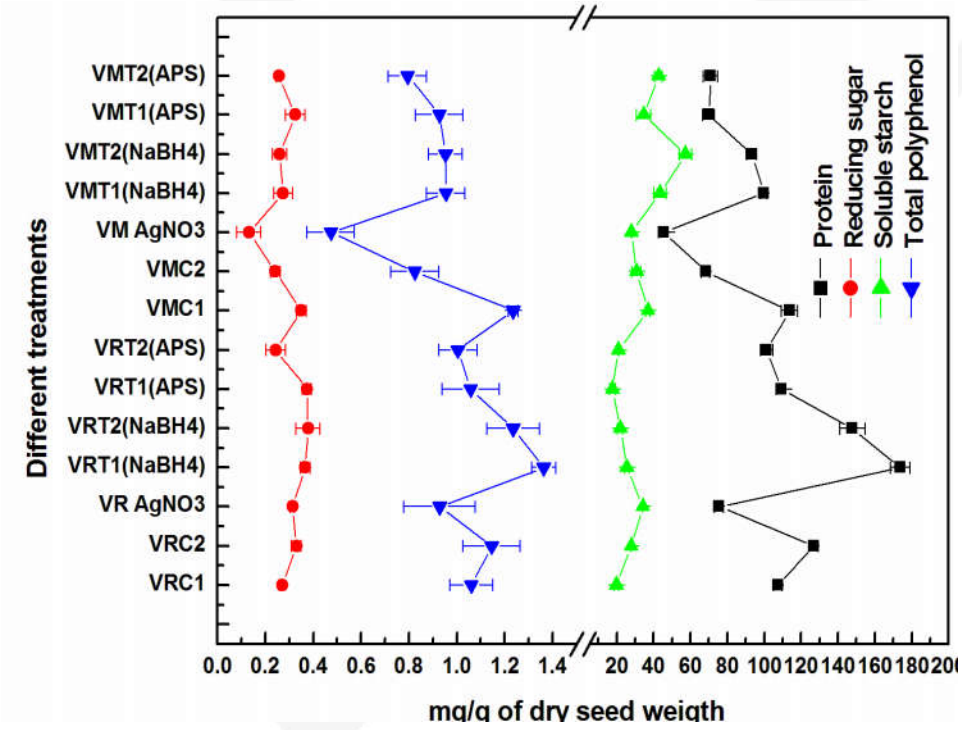

Fig. 6: Analysis of the optimum concentration chemically treated heat stressed seeds with respect to protein, reducing sugar, soluble starch and total polyphenol. VRC1, *V. radiata* seeds without heat stress and chemical treatment; VRC2, *V. radiata* seeds with heat stress and without chemical treatment; VR AgNO3, *V. radiata* seeds with 1mM $AgNO_3$ treatment for 24h; VRT1(NaBH4), *V. radiata* seeds without heat stress and with 1mM $NaBH_4$ treatment for 24 h; VRT2(NaBH4), *V. radiata* seeds with heat stress and 1mM $NaBH_4$ treatment for 24h; VRT1(APS), *V. radiata* seeds without heat stress and with 1mM APS treatment for 24h; VRT2(APS), *V. radiata* seeds with heat stress and with 1mM APS treatment for 24 h; VMC1, *V. mungo* seeds without heat stress and chemical treatment; VMC2, *V. mungo* seeds with heat stress and without chemical treatment; VM AgNO3, *V. mungo* seeds with 1mM $AgNO_3$ treatment for 24h; VMT1(NaBH4), *V. mungo* seeds without heat stress and with 8mM $NaBH_4$ treatment for 24h; VMT2(NaBH4), *V. mungo* seeds with heat stress and 01mM $NaBH_4$ treatment for 24 h; VMT1(APS), *V. mungo* seeds without heat stress and with 1mM APS treatment for 24h; VMT2(APS), *V. mungo* seeds with heat stress and with 1mM APS treatment for 24h.

treatment (VRT1 ($NaBH_4$)) followed by heat stressed seeds of *V. radiate* with $NaBH_4$ treatment (VRT2 ($NaBH_4$)). APS treated seeds of both species contain lower protein compared to $NaBH_4$. The lowest value in both *V. mungo* and *V. radiata* series is seen when treated with $AgNO_3$ only. These seeds were never germinated. The overall patter of protein content coincides with the vigor index pattern. As presented in Figure 6 the protein and polyphenol content varies several folds among germinated and non germinating seeds ($AgNO_3$ treated). Similar fold of variation is not observed for soluble starch and reducing sugar (Figure 6, red and green colour). Soluble proteins might be constant in all seeds, but once germination start the a-amylase production and cell division of embryo might leads to increase in protein content. Similarly, polyphenols content is also found to highly sensitive to germination process. Soluble starch may not change that contrastingly. Reducing sugar level might not be changing as the reducing sugars are utilized by embryo for growth and development but it follows the pattern of increasing and decreasing germination with exception in VRC2 (Figure 6, red colour).

V. radiata seed germination has been studied by several researchers in recent years [22,23,24]. Metal ion toxicity like Cd and Fe deficiency are reported for *V. radiata* [25]. The role of aleuron layer redox condition with respect to germination is reported by Saleh and Kebeish [26]. They also suggested that during germination aleuron layer cells undergo programme cell death due to production of huge amount of reactive oxygen species (ROS) [26]. APS might be stimulating the germination promoting the same by supplying exogenous ROS. Further experimentation is needed to establish the fact.

Comparing the treatment on two different seeds it can be concluded that $NaBH_4$ more effective for vigour stimulation of *V. radiata* compared to APS. APS is more effective on *V. mungo* than $NaBH_4$. Non optimal concentrations of APS (Figure 3a, black and green colour) are found to decrease germination percentage in both seeds, whereas the same of $NaBH_4$ have less detrimental effect on germination (Figure 3a, red and blue colour), except for 0.01M $NaBH_4$ treatment to *V. radiata* seeds.

From the biochemical analysis it can be concluded that soluble protein and total polyphenol content might be taken as marker for germination (Figure 6, black and blue colour respectively). The present study successfully able to stimulate increase the germination process of *V. mungo* and *V. radiata* seeds using $NaBH_4$ and APS and in future further study might be performed for looking into the productivity of the plants.

The 1mM APS concentration treatment to control *V. mungo* seed decreases the vigour (Figure 3a, cyan colour). Therefore, 1mM APS might be detrimental for *V. mungo* seeds, which does not received any heat stress. The present research opens our eye regarding plant seed treatment and suggests that there are huge possibilities of studying different toxic chemicals in low doses on many different species for other beneficial effects.

Hormones as seed germinator stimulator is reviewed by Miransari and Smith (2014) [27]. Non-hormone seed germination stimulators like reactive oxygen species (ROS) [28,29]; light [30] and testa rupture [31].

Conclusion

We hope that *V. mungo* and *V. radiata* cultivating farmers will get benefited by this technology and our recommendation are for *V. mungo* (black gram) $NaBH_4$ (8mM) or APS (1mM) should be used for soaking seeds for 24 h. Similarly for *V. radiata* (green gram) $NaBH_4$ (1mM) or APS (1mM) might be used. For getting synchronized germination in the field the above concentration treatment will work fine except for *V. radiata* (green gram) $NaBH_4$ stimulation concentration 2mM might give better result than 1mM. Future, investigation to evaluate mechanism is necessary to boost the simulation further. It is pertinent to note that the present work is aimed at demonstrating the role of $NaBH_4$ and APS as stimulator for seed germination. We are not excluding the possibility that silver nanoparticle might also stimulate germination, which will be a different study altogether.

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Author's Contributions: KS carried out the laboratory work and recorded the data. The origin of the concept, experimental design, data analysis and figure preparation was done by JPS. SKR and JPS prepared the manuscript. All authors reviewed the manuscript.

Competing Financial Interests

The authors declare no competing financial interests.

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Original Article

Biodeterioration of Cultural Heritage and Indigenous Methods Used for Preserving Cultural Heritage

Ravindra Goswami*, Anuradha Chauhan, Neha*****

Abstract

The world is full of cultural heritage of all kinds. A large number of monuments, artefacts and manuscripts spread all over the world are finest example of rich cultural heritage and a symbol of men's cultural identity and continuity. Cultural heritage is unique and irreplaceable, which places the responsibility of preservation on the present generation. The different types of deterioration of heritage collection are reflected in wear and tear, shrinkage, cracks, brittleness, warping, bio-infestation, discoloration, abrasion, holes, dust, and dirt accumulation etc. The ravages of time, and extreme climatic conditions such as changes in temperature, humidity, intensity of light or even ignorance and most important biological agents, often destroyed priceless cultural property and records. It is therefore imperative that measures be taken at the earliest and in time to save and preserve these culture and heritage for posterity. The research work undertaken for an understanding of morphological and physiological characteristics of biological agents, required to identify accurately the biological species that have established themselves on the surface or within the material. With the exact characterization of the organisms, it is also necessary to assess the cause-effect of biodeterioration action of a specific identified biological agent. The identification of the microorganisms on the materials and further understanding of their involvement and causes in biodeterioration of art objects and manuscripts have to be evaluated to find possible measure to prevent and successfully solve the associated problems and restore our Cultural Heritage. Traditional Indigenous methods for conserving cultural method is seem beneficial as it did not have any side effect on the materials and also the cheap and best way in this fields. During the experiments it is observed that traditional way gives wonderful results in increase resistance development and prevent the growth of microorganisms on the surface.

Keywords: Traditional; Indigenous; Cultural Heritage.

Introduction

Heritage exists at different levels. We can say that humanity as a whole has inherited as a culture which may be called human heritage. Every nation also inherits a culture which may be termed as national cultural heritage. Cultural heritage includes all those values of culture transmitted to human beings by their ancestors from generation to generation. These cultural heritages are cherished, protected and maintained by them with unbroken continuity and they feel proud of it. The Taj Mahal, Sun Temple Konarak, Jagannath Temple, Puri, Lingaraja Temple, Bhubaneswar, Red Fort of Agra, Delhi's Qutub Minar, Mysore Palace, Jain Temple of Dilwara (Rajasthan) etc., are all important places of our heritage and are to be protected by all means. Besides the architectural creations, monuments, material artefacts, the intellectual achievements, philosophy, treasures of knowledge, scientific inventions and discoveries are

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also the part of heritage. In India the contributions of Aryabhatta, Baudhayana, Bhaskaracharya in the field of Mathematics, Nagarjuna in the field of Chemistry, Astronomy and Astrology; Varahmihir in the field of Physics; Patanjali in the field of Yoga and Susruta and Charak in the field of Medicines are

Fig. 1: Biodeterioration in the (A) Madan Mohan temple (B) Gopinath temple (C) Govinddev Ji temple

profound treasures of Indian Cultural heritage. The Knowledge of these ancient saints firstly transfers orally through guru shishya parampar.

Later most of them recorded and preserved in the form of manuscripts. Culture is liable to change, but our heritage does not. As individuals, we belonging to a culture or a particular group sometime may be acquire or borrow certain cultural traits of other communities/cultures, but our belongingness to Indian cultural heritage will remain unchanged. Our Indian cultural heritage will bind us together e.g. Indian literature and scriptures namely Vedas, Upanishads Gita and Yoga System etc. have contributed a lot by way of providing right knowledge, right action, behavior and practices as complementary to the development of civilization.

Biodeterioration: The word biodeterioration has only been in used for few decades, but describes process affected humankind ever since we began the process and use the materials. It refers to the negative impact of live-organisms activity. Biodeterioration is usually concerned with the action of small organisms i.e. microorganisms (bacteria, fungi etc.). The development of these biological species on the materials determined by nature and properties of material (pH, salinity, moisture content, minerals and texture) and also depends upon the some environmental factors (relative humidity, light, temperature, gases, atmospheric pollution, rainfall and wind). While the biological process are the primary causes of deterioration, in biodeterioration, the chemical and physical processes are the primary causes related to biodeterioration. Deterioration is a loss of structural capacity with time by the action of the external agents or material leaching (Saiz and Liaz 2000). Biodeterioration in its widely accepted form of definition is: "any undesirable change in the properties of a material caused by the vital activities

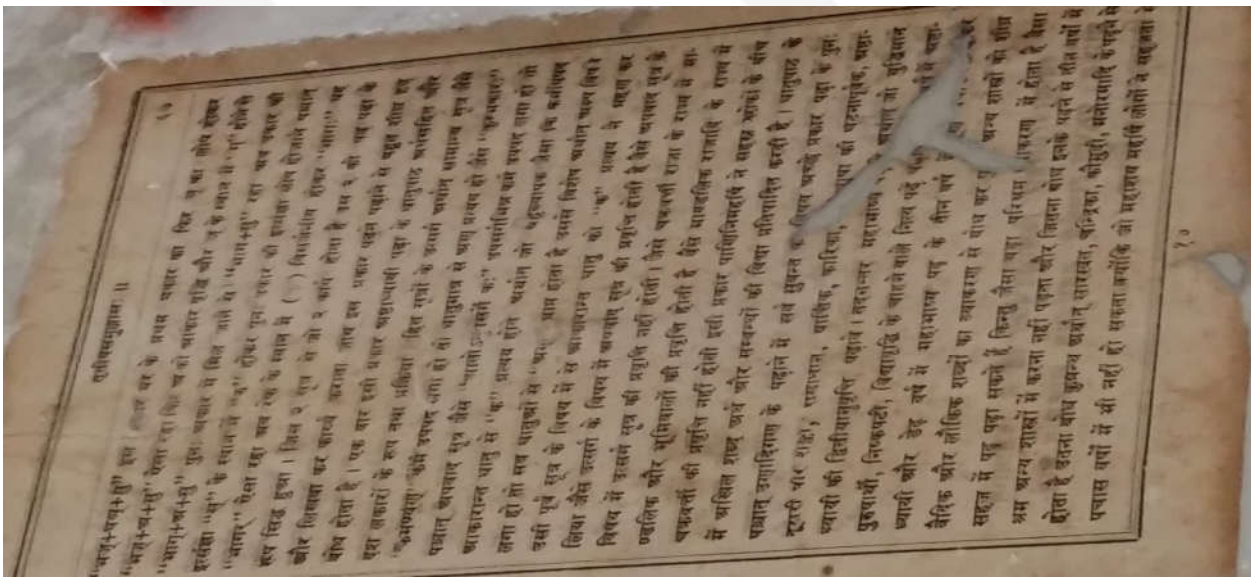

Fig. 2: Biodeterioration on paper manuscript

of organisms" (Hueck 1968). Rose defines "the process by which biological agents (i.e. live organisms) are the cause of the (structural) lowering in quality or value" (Rose 1981).

Biodeterioration can be classified into main three categories: (i) biophysical (ii) biochemical and (iii) aesthetic (Gaylarde *et al.* 2003). Biodeterioration depends up on biodeteriogens, the nature of material and environmental conditions, the above processes (biophysical, biochemical, aesthetic) may occur separately or simultaneously. Biophysical and biochemical deterioration directly affect the material and mechanical properties. This related to the process of growth or movement but not use the material as food. Biochemical deterioration divided in to (i) assimilatory and (ii) dissimilatory. In assimilatory process organisms use the component as food, thus modify the properties. However in dissimilatory process waste products react chemically with components and affecting the material. Aesthetic biodeterioration is caused by the presence of organisms, their excreta, metabolic products, dead bodies forming a layer on the surface known as 'biofilm', and may cause physiochemical damage to the material.

Biodeterioration is an alteration process of an object in which the interaction takes place between the object and factor of destruction (Agarwal 1993). The objects

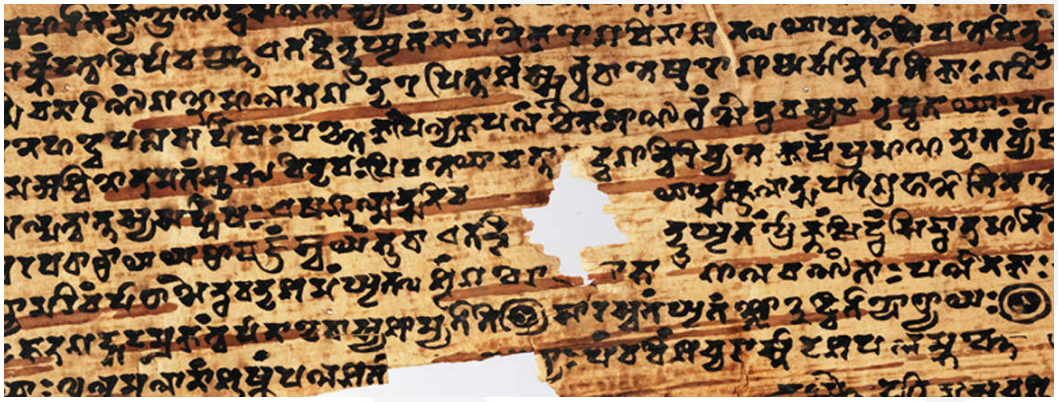

Fig. 3: Biodeterioration on birch bark manuscript

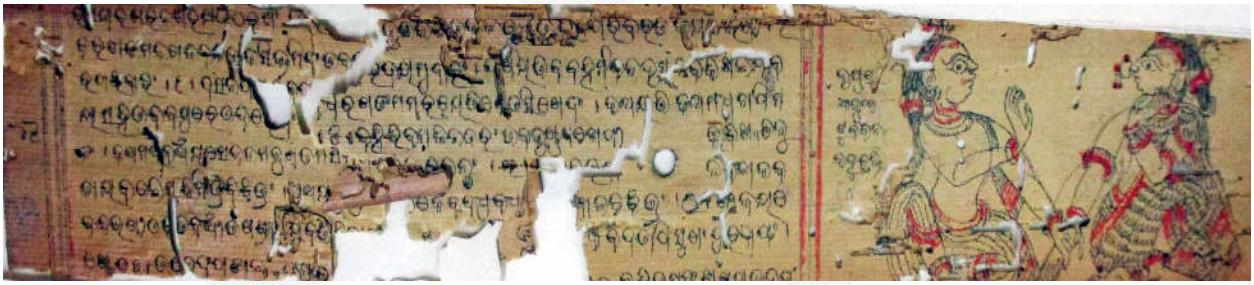

Fig. 4: Biodeterioration on palm leaf manuscript

made of organic substances are more susceptible to inevitable decay in due course of time. Therefore art objects made of organic substances need special care and preservation.

Material and Methods

Sample collection: The microorganisms' growth is collected from manuscripts sample by scrapping and direct plating method collected from the Vrindavan Research Institute, Ramanreti, Vrindavan, Mathura and K.M. Institute of Hindi and Linguistics, Dr. B.R. Ambedkar University, Agra. Sand stone sample are collected from different temples in Vrindavan, Mathura namely Radha Madan Mohan Temple, Radha Gopinath, Radha Govinddev ji. These temples devoted to lord Krishna situated in the bank of river Yamuna or near by the River Yamuna. According to temple authority the temples are more than 550 years old but renovated time to time by different people or authority. Mathura and Agra both come under the subtropical/semiarid climate and prone to extreme as high as 46^0 Celsius in the summer and 2^0 Celsius during winter season. Annual rainfall is about 27 inch. And the most important factor for the biodeterioration is relative humidity in the range 70-80% in dry season and 90-100% during wet season (rainy season).

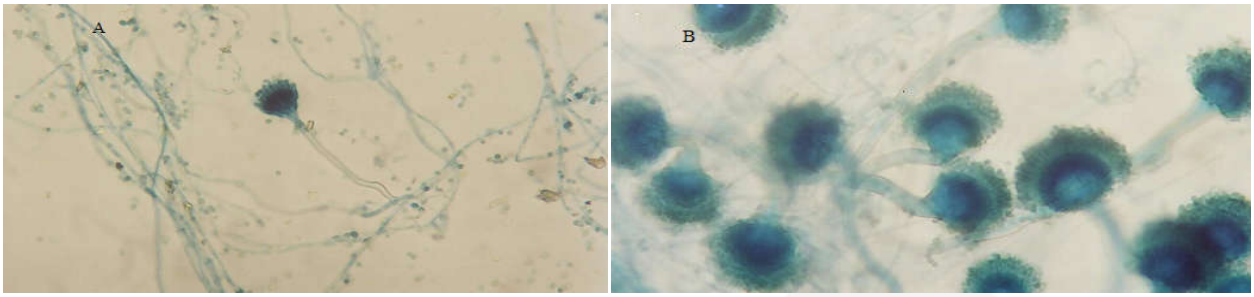

Fig. 5: Different Microorganisms found in abundant: A) *Penicillium Sp.* B) *Aspergillus Sp.*

Result and Discussion

Table 1: Occurrence of microorganisms on deteriorated pieces from different site

S. No.	Microorganisms	VRI	MM	RMT	RGT	RGVT
1.	*Achromobacter sp.*	+ +	+	+ +	+ +	+
2.	*Bacillus sp.*	+ + +	+ +	+ +	+ +	+ +
3.	*Chlorococcum sp.*	+ +	-	-	-	-
4.	*Flavobacterium sp.*	-	-	-	-	+ +
5.	*Pseudomonas sp.*	+ +	+	+	-	+ + +
6.	*Sarcina sp.*	-	-	-	-	+
7.	*Staphylococcus sp.*	+	+	+ +	+	+ +
8.	*Micrococcus sp.*	+ +	+ +	+	+ +	+ + +
9.	*Rhodobacterium sp.*	-	-	-	-	+ +
10.	*Micrococcus roseus*	-	-	-	-	+

+++ = Highly Abundant, ++ = Moderately abundant, + = Less abundant, - = Absent VRI= Vrindavan Research Institute, MM= Mathura Museum, RMT= Radha Madanmohan Temple RGT=Radha Gopinath Temple, RGVT= Radha Govind dev Temple

Table 2: Occurrence of microorganisms on deteriorated pieces from different site

S. No.	Microorganisms	Vrindavan Research Institute	K.M. Institute of Hindi & Linguistics
1.	*Alternaria alternate*	++	++
2.	*Aspergillus sp.*	++	+++
3.	*Clodosporium sp.*	-	+
4.	*Dematium sp.*	+	+
5.	*Fusarium sp.*	-	+
6.	*Mucor sp.*	+	-
7.	*Penicillium sp.*	++	++
8.	*Stemphylium sp.*	-	+
9.	*Trichoderma sp.*	-	-

+++ = Highly Abundant, ++ = Moderately abundant, + = Less abundant, - = Absent

Table 3: Changes in microflora of soil around the material at different duration

S. No.	Microorganisms	3		6		9		12	
		T.P.	%A	T.P.	%A	T.P.	%A	T.P.	%A
1.	*Alternaria alternate*	-	-	3	3.2	3	2.2	3	2.5
2.	*Aspergillus fumigatus*	8	8.8	2	2.1	4	2.8	8	6.6
3.	*Aspergillus flavus*	5	5.5	4	4.3	9	6.7	2	1.6
4.	*Aspergillus niger*	5	5.5	7	7.5	8	5.9	2	1.5
5.	*Botryotrichum*	-	-	6	6.5	8	5.4	6	.5
6.	*Curvularia lunata*	-	-	2	1.8	3	2.4	2	1.7
7.	*Clodosporium sp.*	-	-	6	6.5	8	5.9	6	5.0
8.	*Dematium sp.*	-	-	10	10	5	3.7	12	10
9.	*Fusarium oxysporum*	-	-	10	10	5	3.9	10	9.0
10.	*Geotrichum sp.*	-	-	-	-	1	0.7	-	-
11.	*Humicola sp.*	-	-	-	-	9	6.7	-	-
12.	*Mucor hiemalis*	10	11.1	5	5.4	10	7.4	1	0.8
13.	*Mucor globosum*	8	8.8	7	7.6	4	2.9	-	-
14.	*Penicillium chrysogenum*	15	16	6	6.5	12	8.9	18	15.1
15.	*Penicillium frequentans*	3	3.3	5	5.4	-	-	2	1.6
16.	*Rhizopus oryzae*	2	1.7	4	3.6	1	1.8	-	-
17.	*Streptomyces*	-	-	-	-	-	-	6.0	5.0
18.	*Trichoderma sp.*	-	-	2	2.1	2	2.1	-	-

T.P: Total Population, %A: Percentage Abundance

The samples collected have black patina Crust on the upper surface. Different methods used to the sample for observation such as SEM, Tropical Chamber test, material characterization by X-ray diffraction.

Why Indigenous Methods?

Boyaghchi (2009) has explained a scientific elucidation for Hanzal extracts used by Iranian artists in making old paper manuscripts. She finds the good results for Hanzal extracts to inhibit biodeterioration of paper manuscripts. Baruah *et al.,* (2008) reported that main sources of deterioration and degradation of library resources are the bacteria and fungi. Biodeterioration of library materials is a worldwide problem and it cause great damage especially to unique manuscripts and rare books that are stored in library. Bakkali *et al.,* (2008) observed essential oils are volatile, natural complex, secondary metabolites, characterized by a strong odour and have a generally lower density than that of water therefore can be used for the controlling the biodeterioration . At the present scenario there are no dearth of modern chemical pesticides and repellants for the safe upkeep of manuscripts. The advent of technology has also given rise to greater concerns of preservation of manuscripts by adopting modern technologies. Still beside the modern technology the traditional methods of preservation are in vogue, as these methods have their own merits:

- These methods are not hazardous for human health.
- The Indigenous methods do not have any adverse effect on the materials.
- The methods do not require much expertise, equipment and money.

In this context an attempt has been made to summarize the effectiveness of various traditional practices, Indian herbal pesticides and insect repellants which are being used by different organizations or could be used by the organizations to seize the growth of insect infestation in the manuscript repositories.

Traditional Preservation Methods

The art of preservation is not new to Indians. From the ancient times several indigenous methods have been used for preservation of manuscripts. The people were aware of the basic factors that cause deterioration of the manuscripts namely light, dust, heat and humidity. So in order to protect the manuscripts from these possible factors, the manuscripts were usually covered by clothes. Most of time it is observed that red colored clothes used for this purpose as it also worked as repellents. Nevertheless some traditional practices, which were adopted by the custodians of manuscripts and observed that still, being practiced, are enumerated below:

1. Wrapping the manuscripts in clothes, protect them from worms, dust as well as to a great extent from variation in atmospheric humidity and absorption of acidic fumes.
2. Palm leaves are wrapped in red or yellow color clothes. It is believed that red is a repelling color for the insects and yellow color if, produced by turmeric itself work as repellant and possess some germicidal power that can repel the insects from getting in contact with the manuscripts.
3. Manuscripts in olden days are also wrapped in silk clothes as silk is remarkably free from bookworms for which its extensive use has been seen.
4. Exposing palm leaves in the kitchen have the scientific fact that smoke particles have the capacity to repel the insects. Though the smoke deposits bring out undesired changes on the leaves yet this system is very effective for prevention of insect attack over the palm leaf manuscripts.
5. Manuscripts are generally exposed to the Sun in the Lunar month of Bhadraba i.e. in August as the rays of the Sun in that particular month are very favorable. By this the worms are killed under the Sun.
6. At some places underground cells are prepared for preservation purpose of manuscripts.

l. Herbals and Natural Products used in preservation purpose

Boyaghchi (2009) has explained a scientific elucidation for Hanzal extracts used by Iranian artists in making old paper manuscripts. She finds the good results for Hanzal extracts to inhibit biodeterioration of paper manuscripts. Some of the plants and their products, which have been recognized since ancient times for their germicidal properties and insect repellency potentialities, have been mentioned below: Dried and powdered leaves of Aswagandha in small packets are kept with the manuscripts covered in clothes to repel insect attack. Along with bundles of manuscripts pieces of Vasambu or dried ginger are kept to save these from insect attack. Coatings of lemon-grass oil are given to strengthen the leaves

of manuscripts and destroy the growths of micro-organisms. In some repositories people use vermillion or kumkum fruit powder (which is red in color) that act as a very good insect repellant. Powdered roots of dried sweet flag known as Bacha, filled in small bags are kept in cup-boards of manuscripts which has got very good medicinal value and insecticidal power. Oil extracts of some natural products like black pepper, sandal wood or clove facilitate in the restoration of flexibility to the palm leaf manuscripts. The use of fresh palm leaf extract has also the possibilities of imparting flexibility to the old and brittle leaves. Powdered Ajwain also acts as an insect killer and fungicide. Custard-apple seeds powder is used to kill the insects that thrive on manuscripts. The mixture of neem laves, karanja, nirgundi and citronella are known to have insecticidal properties for which it could be used in the manuscript libraries.

Neem oil contains limonoids, a class of compounds that acts as anti-feedants or growth regulators in insects; they don't kill instantly but wipe out a whole generation of insects by preventing the young ones from maturing and adults from reproducing. Dried Neem leaves and seeds are also useful in keeping away insects. So its use has been widely recognized since ancient times.

Another natural product – Camphor (Karpura) is commonly used in India to protect valuable documents. Filled in small cloth bags it is kept inside the storage of manuscripts. Besides, synthetic Camphor Oil is also used to protect palm leaf manuscripts against insect attack.

Small bags of a sort grass – Panadi by name (which is grown in Jaisalmir and used in making perfumes) are placed among the bundles of manuscripts to save them from white ants.

Application of turmeric paste to the seasoned palm leaves is well known for its dis-infecting effect.

Conclusion

The safe upkeep of manuscripts has also been inscribed by the authors of manuscripts, generally written in the colophon which is evident from the following lines:

"Jaladraksha Tailadraksha raksha man shlatha vandhanat

Ashubhya parahastebhya Ebam badati pustakam"

That means: "The book itself appeals the owners to protect it from water, oil, slack binding, rats and from the hands of other people who do not know proper handling". Some of the authors also request to the user to treat the manuscripts as their own sons.

"Yatnen likhitam shashtram, Putravat paripalayet"

Making of manuscripts was very difficult task on that time, It take a long time and lot of patience. The people who worked on it were special as they conserve the cultural and other important information for the next generation. And that's why there is a responsibility to us to preserve their documentary heritage for our next generation.

Our cultural heritage is our identity and they reflect our richness. So it is necessary to protect them from further harm and deterioration. Microorganisms' attack, Climatic factors and the most important factor that deteriorate our cultural heritage is human negotiation. Many of our cultural property and documentary heritage are deteriorate day by day due to several reasons.

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Original Article

Impact of Thyroid Hormone in Liver Collagen of *Duttaphrynus Melanostictus*

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Abstract

Thyroxine is important for both collagen synthesis and matrix metabolism (Yen, 2001, Oliva *et al.*, 2013). The thyroid hormones T_4 and T_3 are formed in a large prohormone molecule, thyroglobulin, the major component of the thyroid and more precisely of the colloid. Thyroid dysfunction may perturb the liver function and vice versa (N. Kumar, 2013). T_3 stimulates synthesis and post-translational modification of type I collagen (Varga, *et al.*, 2010), induces expression of alkaline phosphatase (Gouveia, *et al.*, 2006) and regulates synthesis and secretion of the bone matrix proteins i. e. osteopoetin and osteocalcin (Gouveia *et al.*, 2001, Varga *et al.*, 2010). Recent study demonstrated the changes in liver collagen in *Duttaphrynus melanostictus* by daily administration of thyroid hormones (both T_4 & T_3) at the dose of 0.5 µg/gm, for 7 days by the method of Newman & Logan (1950) as modified by Leach (1960). The salt soluble, acid soluble, insoluble, total collagen, % of salt solubility, % of acid solubility, salt soluble/ insoluble ratio, acid soluble/insoluble ratio of collagen were statistically found out at 0.05 P confidence level.

Keywords: Thyroid Hormone (T_4& T_3); Collagen; *Duttaphrynus Melanostictus.*

Introduction

Thyroid hormones (THs), T_3 and T_4, play an essential role in the development and metabolism of many tissues and organs, and exert profound metabolic effects in adult life, including changes in oxygen consumption, protein, carbohydrate, lipid, and vitamin metabolism (Oliva *et al.*, 2013). Most circulating T_3 is derived via metabolism of T_4, from which an outer-ring iodine atom is removed by activity of the type 1 iodothyronine deiodinase enzyme (Dio1) principally in liver and kidney (Bianco and Kim,2006, Williams, 2013). The thyroid hormones, triiodothyronine (T_3) and its prohormone, thyroxine (T_4), are regulated by TSH made by the thyrotropes of the anterior pituitary gland that are primarily responsible for regulation of metabolism (Pattnaik, 2014). Limited studies use T_3 and T_4 to specifically improve the functional properties of neocartilage engineered from articular chondrocytes, as existing studies largely focus on understanding hormone effects at the cellular level. For instance, T_3, when applied to salginate-embedded chondrocytes, enhanced the hydroxyproline content per cell (Randau *et al.*, 2013). In the presence of bone morphogenetic protein 2(BMP-2) and insulin, T_3 significantly increased collagen type II mRNA and reduced BMP-2/insulin-induced collagen type X expression (Liu *et al.*,2007). These studies demonstrate the beneficial effects of T_3 in eliciting increased collagen production in articular chondrocytes in three-dimensional culture. However, the effect of T_3 and T_4 hormones on increasing the functional properties of engineered neocartilage is understudied.

Collagen is one of the most abundant animal proteins, constituting approximately one-third of the total body protein of mammals. It is a major protein of the extracellular matrix and the most profuse protein in humans making up 30% of our skin, bone and connective tissues. During developmental

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growth, collagens are believed to be continuously deposited into an extracellular matrix which is increasingly stabilized by the formation of covalent crosslinks throughout life (Mays *et al.*, 1991). Collagen formation is an important function of liver parenchymal cells that may be relevant to the pathogenesis of hepatic fibrosis. The hepatic stellate cell (HSC) is the primary cell type in the liver responsible for excess collagen synthesis during hepatic fibrosis.According to the experiment of Tseng *et al.*, 1983 collagen IV along with collagen type I, III, and V was observed from normal rat liver hepatocytes culture. The mechanical properties, biocompatibility, and degradation rate of collagenous materials are profoundly influenced by the method and extent of collagen crosslinking. Crosslinking also further reduces collagen antigenicity (Meade and Silver, 1990). Collagen solubility in weak acids is indirectly related to the degree of cross linkage in the collagen of the tissue under study: a higher solubility index indicates a higher degree of cross linkage of the collagen molecules (Robins *et al.*,1973). Changes in collagen content and cross-linking occur in many organs with a variety of diseases, chronic injury, and aging (Peleg *et al.*,1993).

Materials & Methods

Collection

Animals of both sexes were collected from nature during evening time and were transferred to the laboratory in the next morning. They were maintained in laboratory conditions in wire-netted wooden cages (75× 40× 35 cm in size) containing a moist sand bed for about five days. They were forced-fed with about 1 gm of goat liver (composition mg/gm wet weight: 110±41 protein, 84±16 lipid, 2.3±1.1 glycogen) each on every alternate day and water was provided ad libitum).

Treatment

After laboratory acclimation animals of mixed sexes were divided into control and experimental groups. There were two treated groups named as experiment. One experimental group of toads were injected intramuscularly with thyroxine (T4) Na- salts (fluka AG), and the other group with triiodothyronine (T_3) Na-salts (Fluka AG) at a dose of 0.5 µg/gm dissolved in 0.65% NaCl solution, pH 8.3. The control animals received an equal volume of 0.65% NaCl solution, pH 8.3. These injection periods were maintained for 7 days. On the 8th day, the animals were sacrificed in batches for estimation of biochemical parameters after taking their final body-weight.

Collection of Tissue

The animals were pithed by piercing a pointed needle immediately posterior to the occipital region. The animals were quickly dissected out. The liver tissue was transferred to cold Amphibian ringer (KCl – 140 mg, NaCl – 6.5gm, $CaCl_2$ – 120 mg, $NaHCO_3$ – 100 mg per litre, pH – 7.4). The adherent tissues were cleaned, blotted off in Whatman filter paper. After soaking in filter paper, weighed quantities (25mg) of tissue were taken for extraction of different collagen fractions. Then the different collagen fractions were extracted and estimated following the method of Neuman and Logan (1950) as modified by Leach (1960).

These data were statistically analysed by the student t – test (Abramoff and Thomson, 1966, Bishop, 1966).

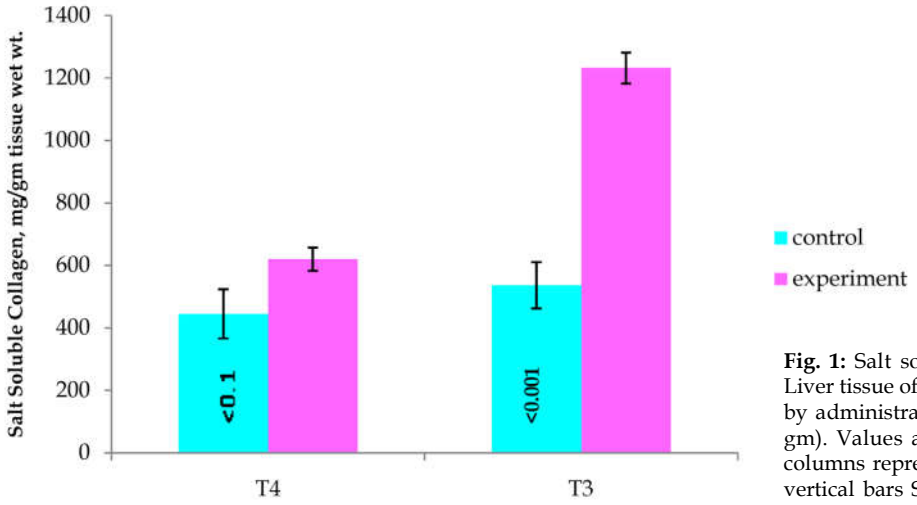

Fig. 1: Salt soluble collagen fraction in Liver tissue of *Duttaphrynus melanostictus* by administration of T_4 and T_3 (0.5 µg/gm). Values are µg/gm tissue wet wt., columns represent the mean values and vertical bars SEM

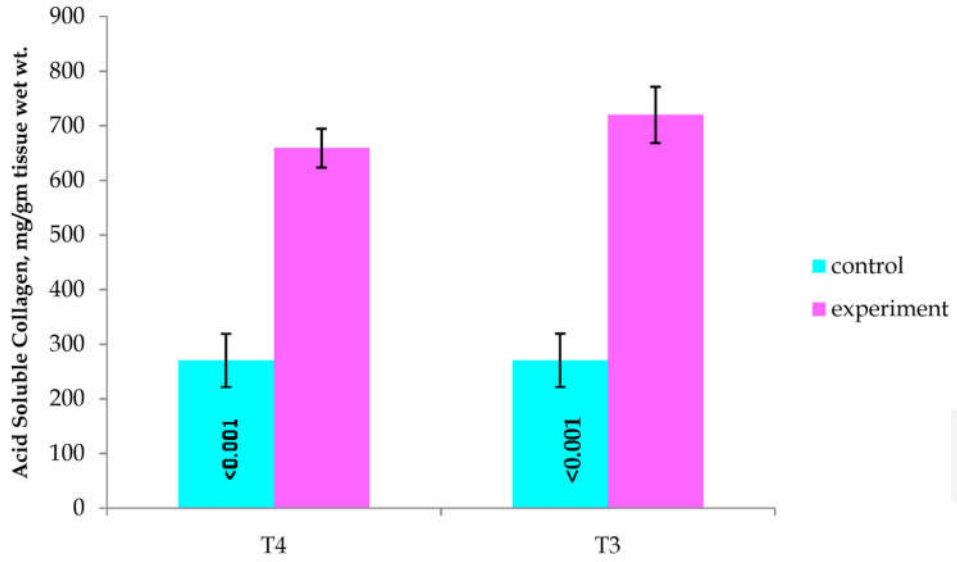

Fig. 2: Acid soluble collagen fraction in Liver tissue of *Duttaphrynus melanostictus* by administration of T_4 and T_3 (0.5 µg/gm). Values are µg/gm tissue wet wt., columns represent the mean values and vertical bars SEM.

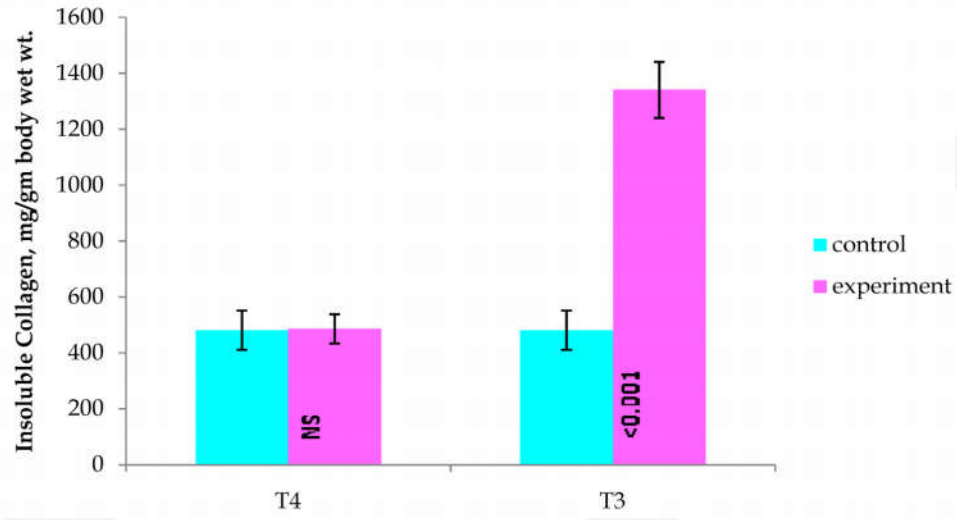

Fig. 3: Insoluble collagen fraction in Liver tissue of *Duttaphrynus melanostictus* by administration of T_4 and T_3 (0.5 µg/gm). Values are µg/gm tissue wet wt., columns represent the mean values and vertical bars SEM.

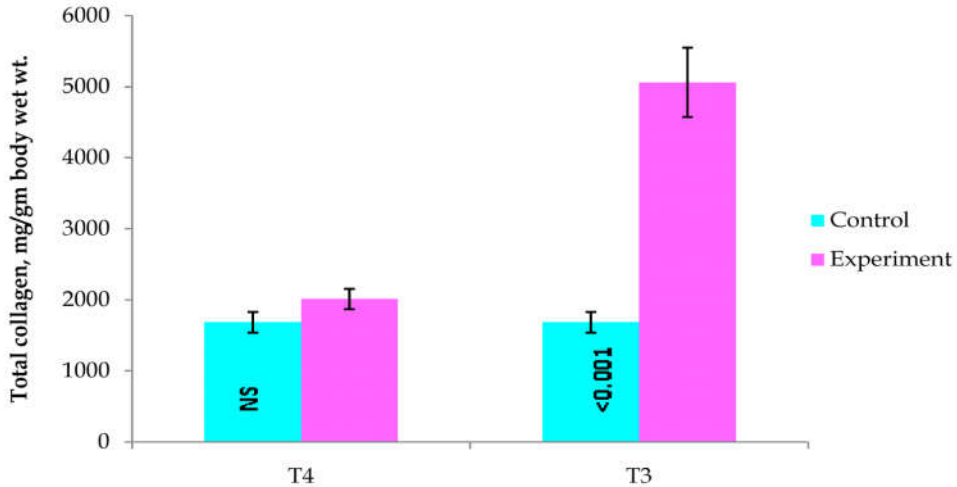

Fig. 4: Total collagen fraction in Liver tissue of *Duttaphrynus melanostictus* by administration of T_4 and T_3 (0.5 µg/gm). Values are µg/gm tissue wet wt., columns represent the mean values and vertical bars SEM.

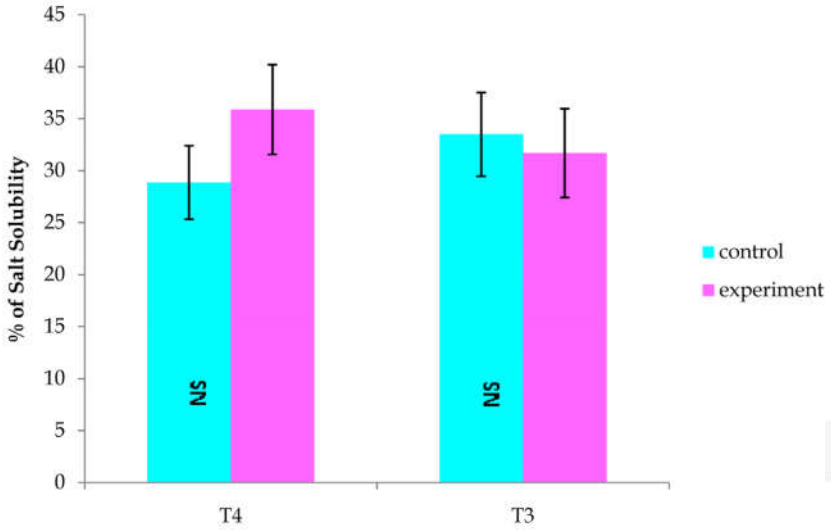

Fig. 5: % of solubility collagen fraction in Liver tissue of *Duttaphrynus melanostictus* by administration of T_4 and T_3(0.5 µg/gm). Values are µg/gm tissue wet wt., columns represent the mean values and vertical bars SEM

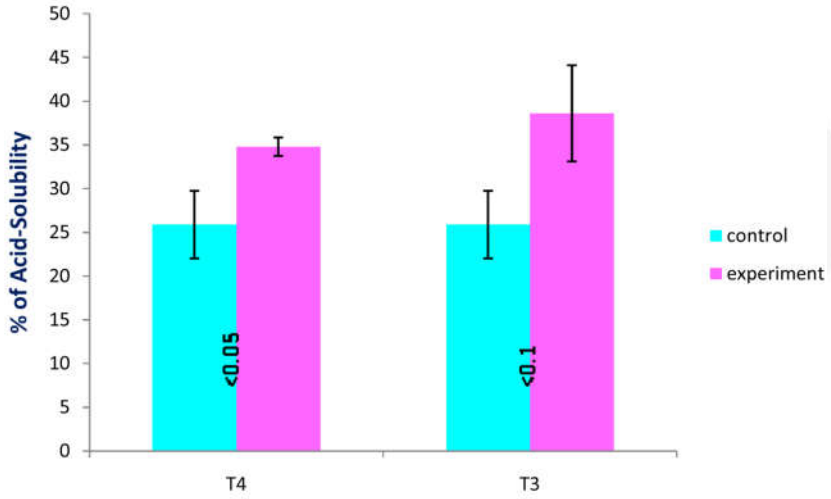

Fig. 6: % of acid solubility collagen fraction in Liver tissue of *Duttaphrynus melanostictus* by administration of T_4 and T_3(0.5 µg/gm). Values are µg/gm tissue wet wt., columns represent the mean values and vertical bars SEM.

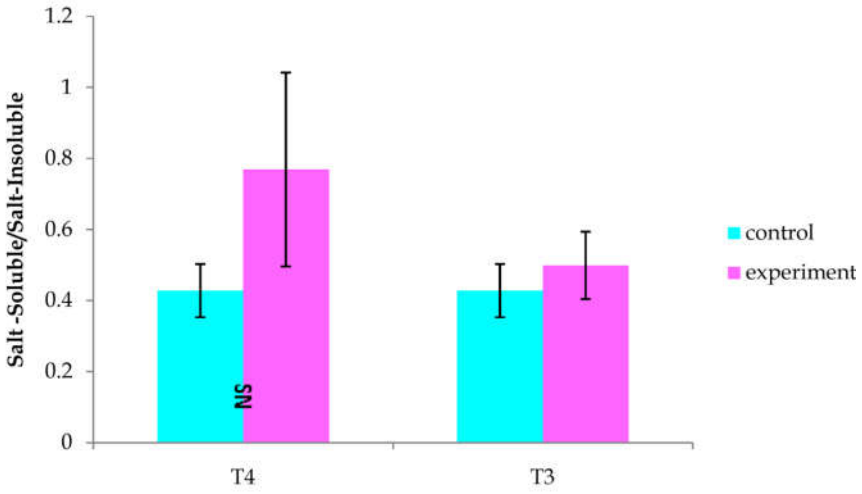

Fig. 7: Salt soluble/ salt insoluble collagen fraction in Liver tissue of *Duttaphrynus melanostictus* by administration ofT_4 and T_3 (0.5 µg/gm). Values are µg/gm tissue wet wt., columns represent the mean values and vertical bars SEM.

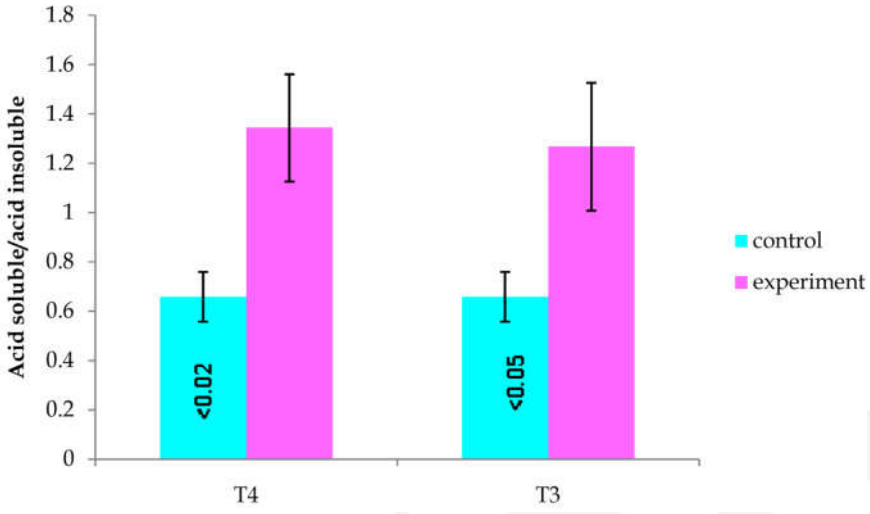

Fig. 8: Acid soluble/ acid insoluble collagen fraction in Liver tissue of *Duttaphrynus melanostictus* by administration of T_4 and T_3(0.5 µg/gm). Values are µg/gm tissue wet wt., columns represent the mean values and vertical bars SEM

Table 1: Effect of thyroxine (T_4) 0.5µg/gm on collagen characteristics of liver tissue of common toad.Values for soluble, insoluble and total collagen are µg/gm tissue wet-weight (Mean ± SEM), numbers in parentheses indicate sample size, NS, not significant at 0.05 P confidence level

Experimental Condition	Salt Soluble	Acid Soluble	Insoluble	Total	% of Salt Solubility	% of Acid Solubility	Salt Soluble/Salt Insoluble	Acid Soluble/Acid Insoluble
Control	445.069 ± 78.695 (7)	270.673 ± 48.837 (7)	481.880 ± 70.120 (7)	1684.964 ± 146.006 (7)	28.875 ± 3.541 (7)	25.890 ± 3.867 (7)	0.428 ± 0.075 (7)	0.659 ± 0.101 (7)
P	P<0.1	P<0.001	NS	NS	NS	P<0.05	NS	P<0.02
Experiment	620.076 ± 37.154 (7)	659.144 ± 35.621 (7)	486.543 ± 52.108 (7)	2011.625 ± 144.643 (7)	35.892 ± 4.316 (7)	34.794 ± 1.051 (7)	0.769 ± 0.273 (7)	1.344 ± 0.218 (7)

Table 2: Effect of Triiodothyronine(T_3) on collagen characteristics of Liver tissue of common toad. Values of soluble, insoluble and total collagen are µg/gm tissue wet-wt (Mean±SEM), numbers in parentheses indicate sample size, NS, not significant at 0.05 P confidence level

Experimental Condition	Salt-Soluble	Acid-Soluble	Insoluble	Total	% of Salt Solubility	% of Acid Solubility	Salt Soluble/Salt Insoluble	Acid Soluble/Acid Insoluble
Control	536.720 ± 74.270 (7)	270.673 ± 48.837 (7)	481.880 ± 70.120 (7)	1684.964 ± 146.006 (7)	33.512 ± 4.029 (7)	25.890 ± 3.867 (7)	0.428 ± 0.075 (7)	0.659 ± 0.101 (7)
P	P<0.001	P<0.001	P<0.001	P<0.001	NS	P<0.1	NS	P<0.05
Experiment	1232.097 ± 49.725 (7)	720.324 ± 51.551 (7)	1341.164 ± 100.316 (7)	5061.016 ± 488.234 (7)	31.703 ± 4.280 (7)	38.985 ± 5.498 (7)	0.499 ± 0.095 (7)	1.268 ± 0.259 (7)

Results & Discussion

L-Thyroxine accelerated the conversion of soluble to insoluble collagen in adjuvant induced arthritic rats more effectively than prednisolone but was less effective with regard to the inhibition of enhanced catabolism of collagen. However, the synthesis of collagen in adjuvant induced arthritis was improved by both prednisolone and L-thyroxine (Kuberasampath and Bose, 1979). By the administration of T_4, there was a significant increase in salt soluble collagen, acid soluble collagen, in-soluble collagen, % of acid solubility, acid soluble/acid insoluble ratio. The insoluble collagen, total collagen, % of salt solubility, salt soluble/ salt insoluble ratio increased insignificantly.

Salt soluble, acid-soluble, insoluble, total collagen, % of acid-solubility, acid soluble/acid insoluble ratio of collagen increased significantly by the administration of T_3. The salt soluble/salt- insoluble ratio of collagen increased insignificantly. In contrast, the % of salt soluble collagen decreased insignificantly.

L-Thyroxine accelerates the conversion of soluble to insoluble collagen in adjuvant induced arthritic rat more effectively (Kuberasampath and Bose, 1979). Liu *et al.,* 2007 found that in presence of bone morphogenetic protein- 2(BMP-2) and Insulin, T_3 significantly increased collagen type-II mRNA.

The total collagen reflects a balance between a synthesis and degradation. By the administration of T_4 and T_3, the total collagen is increased, more significantly in T3 in comparison to T4. In the preceding sections we have seen that the concentration of salt-soluble collagen increases significantly in liver tissue of *Duttaphrynus melanostictus.* The acid soluble collagen concentration also increased significantly in liver tissue. Salt soluble collagen refers to newly synthesized collagen. Acetic acid extract a form of collagen cross linked into fibers by aldimine bond. The insoluble collagen concentration also increased in both T_4 and T_3. The insoluble collagen are due to the stabilization of the collagen fibrous by inter and intramolecular cross linking. The percentage of salt-solubility, percentage of acid-solubility, salt soluble /salt-insoluble, acid soluble/acid insoluble ratio of collagen increased in both T_4 and T_3 in contrast to % of salt solubility in T_3 which is decreased. The changes in solubility and soluble/ insoluble collagen ration are indirect indicators of alterations in the degree of cross linkages of collagen molecules. This is an indication of impact of thyroxine hormone on the synthesis of collagen. However, the result on % of salt solubility showing a contradiction which is decreased.

Conclusion

Collagen forms a small components of the total protein of normal liver. Liver in turn metabolizes the collagen protein. Crosslinking participates in the increased stability of collagen towards proteolytic degradation. The degree of cross-link formation in collagen affects the physiological functions of the concerned tissue. Thyroxine plays an important role in liver remodelling during metamorphosis. From the findings of the present study, it is concluded that thyroxine (both T_4 and T_3) administration accelerated the collagen synthesis and also accelerated the conversion of soluble to insoluble collagen differing to a small degree of fraction and is showing a tissue specific action.

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Original Article

Morphometric, Meristic and Comparative Studies of *Mystus* Three Species (Family: Bagridae) from Two Different Habitats of Andhra Pradesh, India

Satyanarayana Murthy CH.V.*, T. Baby Ratnakumari**

Abstract

The present study concerned with three Mystus species, Mystusvittatus, Mystusgulio, Mystuscavasius. Mystus species are small indigenous fresh water fishes with high nutritional value. Morphology of fishes has been the primary source of information for taxonomic and evolutionary studies. In the present study total 16 morphometric external characters were analysed and the correlation of body parts with total length and head length were analysed. The differences in morphometric characters is due to geographical and environmental variations are also observed. The mean value of the meristic counts are also studied in three species

Keywords: Mystusvittatus; Mystusgulio; Mystuscavasius; Morphometric Characters; Meristic Counts.

Introduction

Historically the morphology of fishes has been the primary source of information for taxonomic and evolutionary studies. Despite the value and availability of genetic, physiological, behavioural and ecological data for such studies, systematic ichthyologists continue to depend heavily on morphology for taxonomic characters. Species have characteristic shapes, sizes, pigmentation patterns, disposition of fins and other external features that aid in recognition, identification and classification.

Moreover, morphometric analyses can be a tool in assessing habitat – specific differentiation of populations, such as differentiation related to predation pressures, salinity, temperature, food availability etc. Differences in morphometric and meristic characters among populations of a species are thought to be the result of genetic differences or environmental factors or their interactions.

Morphometric analyses have been very useful for separating species, populations and races in the past and have been widely used for the identification of different fish stocks (Turan et al., 2004,2005). Such morphometric studies of fish populations are very important for understanding the interactive effect of environment, selection and heredity on the body shapes and sizes within a species (Cadrin 2000). Several studies on the comparative morphometrics of different fish populations have been conducted Nakamura 2003; Turan et al., 2005; Ibanez Aguirre et al., 2006, Negi Ramakrishna and NegiTaran 2010, Muhammadzafari et al., 2002. Morphometrics is very important in biology because it allows quantitative descriptions of organisms. Quantitative approach allowed scientists to compare the shapes of different organisms much better.

The objectives of the present study are to assess and describe geographic variation in morphological and meristic characters of three Mystus species. *Mystusvittatus, M. gulio and M. cavasus*from two different places Visakhapatnam and Srikakulam is to identity the best set of characters for group separation and relate the observed variations with the specific ecological constraints of each population.

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Material and Methods

Very limited information is available on the morphometric measurements and meristic counts of Mystus species from the freshwater bodies of Andhra Pradesh. Further recognition or identification of a species is necessary and must be done in all types of biological studies, where morphological systematics is used for quick identification and conformation. Therefore the present study is designed to generate data on morphometry and geographical variations in three Mystus species, *Mystusvittatus, M. gulio, M. cavasius*.

The materials for the present study were collected from two different aquatic habitats namely Nagavali river at Srikakulam, Meghadrigeddain Visakhapatnam of Andhra Pradesh.

Nagavaliriver is one of the major rivers in Srikakulam. The river rises on the eastern slopes of the eastern ghats near Lakhbahai in the Kalahandi district of Odisha. Geographically the river located between 18^0 10″to 19^0 44″ north latitudes and 82^0 53″ to 84^0 05″ east longitudes.

The total length of the river is about 256km, of which 161km are in Odessa and the rest in Andhra Pradesh. The catchment area of the basin is 9,510 square km. Nagavali is an interstate river with 5048km^2 river basin area in Andhra Pradesh.

Mehadrigedda is a major drinking water source of Visakhapatnam city, a perennial freshwater stream stretching between latitude $17^0$44"N and longitudes $83^0$1′54"E and located 15 km South of Visakhapatnam. The reservoir has a maximum water spread area of 360 Sq. km.

The samples collected during the period from January 2010 to December 2010. Total 150 species were used for the study of morphometric and meristic counts in each species. Different types of fishing methods were employed for the collection of specimens. Gill nets, Drag nets and other traditional traps were used for the collection of fishes, with the help of local fishermen.

The fish samples were collected and preserved in 10% formalin in the field itself and brought to the laboratory for further systematic studies. Details of the coloration were recorded in the fresh specimens itself.

The fishes were identified up to the species level with the help of authentic keys such as Day (1878), Talwar and Jhingran (1991). Morphometric and meristic counts were done by following Standard measure ments of jayaram.

Morphometric measurements were recorded with a dial-reading caliper with an accuracy of 0.02 mm. In the present study the data of maximum and minimum values presented in Centimetres and the ratios of body parts in percentages. Analysis of variance (ANOVA) was carried out to test the significance of morphological differences.

Table 1: Definitions and Acronyms of morphometric measurements and meristic counts of Mystus species used in this study

Character	Description	Acronym
Total length	Distance from the tip of the snout to the longest caudal fin ray	TL
Standard length	Distance from the tip of the snout to the tail base	SL
Body Weight,gm	Weight of the fis in grams	BWT
Body Width	The greatest width just posterior to the gill opening	BW
Body Height or depth	Maximum depth measured from the base of the dorsal spine	BH
Head length	Distance from the tip of the snout to the posterior margin of the opercula	HL
Head length excluding snout	from the anteror edge of the orbit to the posterior margin of the opercula	HLExS
Widh of head	It was a straight measurement of the distance between the two eyes	WH
Snout length	The front of the upper lip to the fleshy anterior edge of the orbit	SNL
Eye diameter	The greatest bony diameter of the orbit	ED
Caudal peduncale length	From base of the last anal fin ray to middle of caudal fin fold	CPL
Anal fin length	Base length, greatest distance measured in a straight line between the anterior most and posterior point of junction with the body	AFL
Pelvic fin length	Base length, greatest distance measured in a straight line between the anterior most and posterior point of junction with the body	PVFL
Pectoral fin length	Base length, greatest distance measured in a straight line between the anterior most and posterior point of junction with the body	PFL
Caudal peduncle height	The depth of the tail base	CPH
Dorsalfin length	Base length, greatest distance measured in a straight line between the anterior most and posterior point of junction with the body	DFL
Caudal fin length	From tail base to tip of the caudal fin	CFL

Results

Mystus Vittatus

The morphometric measurements of M. vittatus from two different places were shown in the Table no 2. Total length varies between 11.09 to 16.05cm with a mean value of 13.87±1.53 and Standard length 8.52 to 12.33cm with a mean value of 10.65±1.17 in Visakhapatnam. T.L and S.L in Srikakulam varies 9.36 – 14.53 and 7.39 – 11.48cm with a mean value of 12.18±1.74, 9.62±1.37 respectively. Comparison of the mean of morphometric ratios was shown in the Table 10.

In the present study coefficient correlation between the morphometric characters were analyzed to determine the relationship. Correlation between the various body parts with a total length of *Mystusvittatus* were shown in Table 8, SL, HL, BH, PFL, PVFL, AFL, CFL shows high correlation with TL. Correlation analysis of various body parts with head length are shown in Table 9, SL, SNL, ED, BW shows high correlation (r>0.9) with head length (HL).

Linear regression is used to analyze the relationship between two individual variables. In the present study by using Linear regression method the relationship between the morphometric characters of *M. vittatus* from two different places Visakhapatnam and Srikakulam were studied.

The morphometric characters *M. vittatus*shows that the R^2 value is 0.222. It shows that the model explains 22% of variations between Visakhapatnam and Srikakulam. Durbin- Watson static informs us whether the assumption of independent error is tenable. The value 0.883 is better when it is closed to 1.

The coefficients and Collinearity statistics when linear regression is applied. The two Collinearity statistics are T-test. The standardized coefficient value of Beta is 0.271 and unstandardised coefficient of B and std.error is 0.202 and 0.066. The statistic t-value is 3.058.Hence there is no problem of Collinearity among the variables used in the model and linear regression is appropriate.

The ANOVA tests the acceptability of the model from a statistical perspective. The Regression row displays information about the variation accounted for by the model. The Residual row displays information about the variation that has not been accounted by the model. The regression much is less

Table 2: Morphometric measurements of*Mystusvittatus*from two different placesVisakhapatnam and Srikakulam

Measurements (cm)	Visakhapatnam					Srikakulam				
	Minimum	Maximum	Mean ± SD		TL(%)/ Mean	Minimum	Maximum	Mean ± SD		TL (%) Mean
Total length (TL)	11.09	16.05	13.87	±1.53		9.36	14.53	12.18	±1.74	
Standard length (SL)	8.52	12.33	10.65	±1.17	76.79	7.39	11.48	9.62	±1.37	78.99
Body Weight,gm (BWT)	29.13	42.16	36.43	±4.02	262.67	17.14	26.60	22.30	±3.18	183.08
Body Width (BW)	1.64	2.38	2.05	±0.23	14.80	1.22	1.89	1.58	±0.23	13.00
Body depth (BD)	2.22	3.21	2.77	±0.31	20.00	1.71	2.66	2.23	±0.32	18.30
Head length (HL)	1.76	2.55	2.21	±0.24	15.90	1.53	2.37	1.99	±0.28	16.30
Head length excluding snout (HLExS)	1.30	1.88	1.62	±0.18	11.70	0.98	1.53	1.28	±0.18	10.50
Widh of head (WH)	1.47	2.13	1.84	±0.20	13.30	1.16	1.80	1.51	±0.22	12.40
Snout length (SNL)	0.47	0.67	0.58	±0.06	4.20	0.54	0.84	0.71	±0.10	5.80
Eye diameter (ED)	0.53	0.77	0.67	±0.07	4.80	0.37	0.57	0.47	±0.07	3.90
Caudal peduncale length (CPL)	1.64	2.38	2.05	±0.23	14.80	1.22	1.89	1.58	±0.23	13.00
Anal fin length (AFL)	1.42	2.05	1.78	±0.20	12.80	0.92	1.42	1.19	±0.17	9.80
Pelvic fin length (PVFL)	1.42	2.05	1.78	±0.26	12.80	1.22	1.89	1.58	±0.23	13.00
Pectoral fin length (PFL)	1.47	2.13	1.84	±0.19	13.30	1.10	1.71	1.44	±0.21	11.80
Caudal peduncle height (CPH)	1.12	1.62	1.40	±0.15	10.10	0.67	1.04	0.88	±0.13	7.19
Dorsalfin length (DL)	1.34	1.94	1.68	±0.18	12.10	1.13	1.76	1.47	±0.21	12.10
Caudal fin length (CFL)	1.51	2.18	1.89	±0.21	13.60	1.26	1.96	1.64	±0.23	13.50

Table 3: Meristic counts of the Mystusvittatus captured from Visakhapatnam and Srikakulam

Meristic data	Number			
	Visakhapatnam		Srikakulam	
	Range	Mean	Range	Mean
Dorsal fin rays	I, 6-7	I, 7	I, 5-7	I, 7
Pectoral fin rays	I, 8-10	I,9	I, 8-11	I,9
pelvic fin rays	5-7	5	5-7	5
Anal fin rays	9-13	12	9-14	12
Caudal fin rays	14 -18	17	14-19	17
No. of barbels	4 pairs	4 pairs	4 pairs	4 pairs

than residual sums of squares, which indicates that around 9% of the variation in *Mystusvittatus* at Visakhapatnam and Srikakulam is explained by the model. However, F statistic is found significant, since the p value (0.003) less than 0.05.

In the present study, meristic counts of all samples Table 4 ranged 6-7 fin rays and a single spine of the dorsal fin, 8-11 fin rays and a single spine for pectoral fin, 5-7 fin rays for pelvic fin, 9-14 fin rays for the anal fin, 14-19 fin rays of caudal fin. The mean numbers of above meristic characters are not significantly different. Generally the rayed dorsal fin equal to head in young specimens or less than head in adult specimens and the spine is serrated internally. Pectoral fin not reaching pelvic fin with a spine. Caudal fin forked with upper lobe longer. Barbells are 4 pairs, maxillary pair reaching pelvic fin base, outer mandibular pair extends to middle of pectoral fin, inner mandibular pair extends to pectoral fin base or to gill opening and nasal pair extends to the hind border of orbit.

Mystus Gulio

The morphometric measurements of *Mystusgulio* from two different places Visakhapatnam and Srikakulam were shown in the Table no 4. TL varies between 13.02-18.12cm with a mean value of 15.91±1.60 and 12.04-17.50 with a mean value of 11.77±1.45 from Visakhapatnam and Srikakulam. SL varies between 10.00–13.92cm (12.22±1.23) and 9.51–13.83cm (11.77±1.45) in two different stations. Comparison of the mean of morphometric ratios was shown in the Table 10.

Correlation between the morphometric characters with total length were analyzed and shown in Table 8. Almost all body parts show correlation with total length. SL, HL, DFL, PFL, PVFL, AFL, CFL shows high correlation (r>0.95) with total length. The correlation between head length and other body parts are shown in Table 9. SL, SNL, ED shows high correlation (r>0.95) with head length.

Linear regression is used to analyze the relationship between two individual variables. In the present study by using Linear regression method the relationship between the morphometric characters of *M. gulio* from two different places Visakhapatnam and Srikakulam were studied.

The model summary of *M. gulio* shows that the R^2 value is 0.075. It shows that the model explains 7% of variations between Visakhapatnam and Srikakulam. Durbin- Watson static informs us whether the assumption of independent error is tenable. The closer to 1 the value is the better and for the data it was 0.075.

The coefficients and Collinearity statistics when linear regression is applied. The two Collinearity

Table 4: Morphometric measurements of*Mystusgulio*from two different places Visakhapatnam and Srikakulam

Measurements (cm)	Visakhapatnam					Srikakulam				
	Minimum	Maximum	Mean ± SD		TL(%)/ Mean	Minimum	Maximum	Mean ± SD		TL(%)/ Mean
Total length (TL)	13.02	18.12	15.91	±1.60		12.04	17.50	14.90	±1.83	
Standard length (SL)	10.00	13.92	12.22	±1.23	76.79	9.51	13.83	11.77	±1.45	78.99
Body Weight,gm (BWT)	34.20	47.60	41.79	±4.19	262.67	22.05	32.04	27.28	±3.35	183.09
Body Width (BW)	1.93	2.68	2.35	±0.24	14.80	1.57	2.28	1.94	±0.24	13.00
Body depth (BD)	2.60	3.62	3.18	±0.32	20.00	2.20	3.20	2.73	±0.33	18.30
Head length (HL)	2.07	2.88	2.53	±0.25	15.90	1.96	2.85	2.43	±0.30	16.30
Head length excluding snout (HLExS)	1.52	2.12	1.86	±0.19	11.70	1.26	1.84	1.56	±0.19	10.50
Widh of head (WH)	1.73	2.41	2.12	±0.21	13.30	1.49	2.17	1.85	±0.23	12.40
Snout length (SNL)	0.55	0.76	0.67	±0.07	4.20	0.70	1.02	0.86	±0.11	5.80
Eye diameter (ED)	0.62	0.87	0.76	±0.09	4.80	0.47	0.68	0.58	±0.07	3.90
Caudal peduncale length (CPL)	1.93	2.68	2.35	±0.24	14.80	1.57	2.28	1.94	±0.24	13.00
Anal fin length (AFL)	1.67	2.32	2.04	±0.20	12.80	1.18	1.72	1.46	±0.18	9.80
Pelvic fin length (PVFL)	1.67	2.32	2.14	±0.23	13.45	1.57	2.28	1.94	±0.24	13.00
Pectoral fin length (PFL)	1.73	2.41	2.12	±0.21	13.30	1.42	2.07	1.76	±0.22	11.80
Caudal peduncle height (CPH)	1.32	1.83	1.61	±16	10.10	0.87	1.26	1.07	±0.13	7.19
Dorsalfin length (DFL)	1.58	2.19	1.92	±0.19	12.10	1.46	2.12	1.80	±0.22	12.10
Caudal fin length (CFL)	1.77	2.46	2.16	±0.22	13.60	1.61	2.35	2.00	±0.25	13.40

Table 5: Meristic counts of the Mystusgulio captured from Visakhapatnam and Srikakulam

Meristic data	Number			
	Visakhapatnam		Srikakulam	
	Range	Mean	Range	Mean
Dorsal fin rays	I, 6-7	I,7	I, 6-7	I,7
Pectoral fin rays	I, 8-10	I, 9	I,8-9	I, 9
pelvic fin rays	5-7	6	5-8	6
Anal fin rays	9 - 15	14	11 - 16	15
Caudal fin rays	15 - 18	16	15 -18	16
No. of barbels	4 pairs	4 pairs	4 pairs	4 pairs

Table 6: Morphometric measurements of*Mystuscavasius*from two different places Visakhapatnam and Srikakulam

Measurements (cm)	Visakhapatnam					Srikakulam				
	Minimum	Maximum	Mean ± SD		TL(%) Mean	Minimum	Maximum	Mean ± SD		TL(%) Mean
Total length (TL)	9.50	15.20	12.64	±1.83		10.50	16.40	13.62	±1.92	
Standard length (SL)	7.30	11.67	9.71	±1.41	76.81	8.30	12.96	10.76	±1.52	79.02
Body Weight,gm (BWT)	24.96	39.93	33.21	±4.81	262.73	19.23	30.03	24.94	±3.51	183.14
Body Width (BW)	1.41	2.25	1.87	±0.27	14.80	1.37	2.13	1.77	±0.25	13.00
Body depth (BD)	1.90	3.04	2.53	±0.37	20.00	1.92	3.00	2.49	±0.35	18.30
Head length (HL)	1.51	2.42	2.01	±0.29	15.90	1.71	2.67	2.22	±0.31	16.30
Head length excluding snout (HLExS)	1.11	1.78	1.48	±0.21	11.70	1.10	1.72	1.43	±0.20	10.50
Widh of head (WH)	1.26	2.02	1.68	±0.24	13.30	1.30	2.03	1.69	±0.24	12.40
Snout length (SNL)	0.40	0.64	0.53	±0.08	4.20	0.61	0.95	0.79	±0.11	5.80
Eye diameter (ED)	0.46	0.73	0.61	±0.09	4.80	0.41	0.64	0.53	±0.07	3.90
Caudal peduncale length (CPL)	1.41	2.25	1.87	±0.27	14.80	1.37	2.13	1.77	±0.25	13.00
Anal fin length (AFL)	1.22	1.95	1.56	±0.23	12.34	1.03	1.61	1.34	±0.19	9.80
Pelvic fin length (PVFL)	1.22	1.95	1.62	±0.23	12.80	1.37	2.13	1.67	±0.25	12.27
Pectoral fin length (PFL)	1.26	2.02	1.68	±0.25	13.30	1.24	1.94	1.61	±0.23	11.80
Caudal peduncle height (CPH)	0.96	1.54	1.28	±0.18	10.10	0.75	1.18	0.98	±0.14	7.19
Dorsalfin length (DFL)	1.15	1.84	1.53	±0.22	12.10	1.26	1.97	1.63	±0.23	12.00
Caudal fin length (CFL)	1.29	2.07	1.72	±0.25	13.60	1.41	2.20	1.83	±0.26	13.40

statistics are T-test. The standardized coefficient value of Beta is 0.276 and unstandardised coefficient of B and std-error is 0.061 and 0.019.The statistic t-value is 3.116.Hence there is no problem of Collinearity among the variables used in the model and linear regression is appropriate.

The ANOVA tests the acceptability of the model from a statistical perspective. The Regression row displays information about the variation accounted for by the model. The Residual row displays information about the variation that has not been accounted by the model. The regression much is less than residual sums of squares, which indicates that around 6% of the variation in MystusGulio is explained by the model. However, F statistic is found significant, since the p value (0.002) less than 0.05.

The range and the mean values of meristic counts of *Mystusgulio* from Visakhapatnam and Srikakulam are shown in Table 5. Meristic counts of all samples from two stations are ranged 6-7 (mean, 7) and a single spine of dorsal fin, 8-10 (m, 9) fin rays and a single spine for pectoral fin, 5-8 (m, 6) fin rays for pelvic fin, 9-16 (m, 14) fin rays for anal fin and 15-18 (m, 16) fin rays of caudal fin. Meristic counts from two different stations were compared; mean number of the meristic counts did not show significant variations. 4 pairs of barbels are observed, maxillary pair reaching the middle or end of the pelvic fin. Dorsal spine half as long as head, strong, serrated, pectoral spine strong, serrated as long as head without snout. Caudal fin forked, upper lobe longer.

Mystuscavasius

Maximum, minimum and mean values of morphometric measurements of *Mystuscavasius* are given in Table 6. TL in two different stations Visakhapatnam and Srikakulam varies between 9.50-15.20, 10.50-16.40 with mean values of 12.64±1.83, 13.62±1.92. SL varies between 7.30-11.67 (m 9.71±1.41), 10.50-16.40 (m 13.62±1.92). Mouth terminal, transverse, upper jaw longer.The median groove rather wide, extending to the base of occipital process. Occipital process narrow, 3 or 4 times as

Table 7: Meristic counts of the Mystuscavasius captured from Visakhapatnam and Srikakulam

Meristic data	Number			
	Visakhapatnam		Srikakulam	
	Range	Mean	Range	Mean
Dorsal fin rays	I, 6- 7	I,7	I, 6-7	I,7
Pectoral fin rays	I, 8-9	I,8	I,7-9	I,8
pelvic fin rays	5-7	6	6-8	6
Anal fin rays	10-12	11	9-12	11
Caudal fin rays	15-18	16	15-17	16
No. of barbels	4 pairs	4 pairs	4 pairs	4 pairs

long as wide at its base and reaching basal bone of dorsal fin.

The correlation of various morphometric measurements with total length are shown in Table 8. SL, HL, BW, DFL, PFL, PVFL, AFL, CFL shows high correlation (r>0.94) with total length. Correlation of various body parts with head length is shown in Table 9. SL, ED, SNL, BH, BW shows high correlation (r>0.9) with head length.

By using Linear regression method the relationship between the morphometric characters of *M. cavasius*from two different places Visakhapatnam and Srikakulam were studied.

The model summary of *M.cavasius* shows that the R^2 value is 0.396. It shows that the model explains 39% of variations between Visakhapatnam and Srikakulam. Durbin-Watson static informs us whether the assumption of independent error is tenable. The closer to 1 the value is the better and for the data it was 0.057.

The coefficients and Collinearity statistics when linear regression is applied. The two Collinearity statistics are T-test. The standardized coefficient value of Beta is 0.635 and unstandardised coefficient of B and std.error is 0.027 and 0.017.The statistic t-value is 2.517. Hence there is no problem of Collinearity among the variables used in the model and linear regression is appropriate.

The ANOVA tests the acceptability of the model from a statistical perspective. The Regression row displays information about the variation accounted for by the model. The Residual row displays information about the variation that has not been accounted by the model. The regression much is less than residual sums of squares, which indicates that around 6% of the variation in *Mystuscavasius* is explained by the model. However, F statistic is found significant, since the p value (0.004) less than 0.05.

Meristic counts of all samples from Visakhapatnam and Srikakulam (Table 7) ranged from 6-8 (mean 7) fin rays and a single spine for dorsal fin, 7-9 (m 8) fin rays and a single spine for pectoral fin, 5-8 (m 6) fin rays for pelvic fin , 9-12 (m 11) for anal fin rays, 15-18 (m 16) for caudal fin rays.

Meristic counts from two different places (Visakhapatnam and Srikakulam) were compared, the mean numbers of above meristic counts did not show significant differences. 4 pairs of barbels are observed, maxillary pair reaching beyond base of caudal fin. Dorsal fin with a weak finely serrated

Table 8: Correlation Analysis of various body parts with Total length in three Mystus species from two different places

Parameters	Coefficient of Correlation (r) values					
	Mystusvittatus		Mystusgulio		Mystuscavasius	
	Visakhapatnam	Srikakulam	Visakhapatnam	Srikakulam	Visakhapatnam	Srikakulam
SL	0.9765	0.9658	0.9768	0.9614	0.9721	0.9698
SNL	0.9497	0.9576	0.9452	0.9493	0.9521	0.9612
HL	0.9748	0.9872	0.9736	0.9859	0.9266	0.9595
ED	0.9654	0.9714	0.9597	0.9628	0.9711	0.9599
BW	0.9467	0.9582	0.9641	0.9218	0.9438	0.9507
BH	0.9727	0.9624	0.9708	0.8996	0.9463	0.9731
PFL	0.9687	0.9378	0.9724	0.9969	0.9711	0.9648
PVFL	0.9812	0.9769	0.9819	0.9788	0.9808	0.9795
AFL	0.9813	0.9844	0.9614	0.9782	0.9837	0.9195
DFL	0.9275	0.9138	0.9513	0.908	0.9431	0.9211
CFL	0.9912	0.9834	0.9729	0.9811	0.9799	0.9738
CPL	0.9599	0.8504	0.9604	0.8498	0.9421	0.8821

Table 9: Correlation Analysis of various body parts with head length in three Mystus species from two different places

Parameters	Coefficient of Correlation (r) values					
	Mystusvittatus		Mystusgulio		Mystuscavasius	
	Visakhapatnam	Srikakulam	Visakhapatnam	Srikakulam	Visakhapatnam	Srikakulam
TL	0.9748	0.9872	0.9736	0.9859	0.9266	0.9595
SL	0.9781	0.9737	0.9695	0.9865	0.9466	0.9354
SNL	0.9489	0.9289	0.9253	0.9824	0.9521	0.9359
ED	0.9156	0.9469	0.9043	0.9576	0.9635	0.9522
BW	0.9318	0.9599	0.9638	0.9408	0.9021	0.9282
BH	0.8175	0.9762	0.9623	0.9643	0.9775	0.9488
PFL	0.8511	0.8863	0.8835	0.9111	0.9017	0.8431
PVFL	0.8931	0.8573	0.8723	0.8169	0.8214	0.8181
AFL	0.9539	0.8726	0.9011	0.8627	0.7895	0.7989
DFL	0.8835	0.8664	0.8641	0.7965	0.8127	0.8081
CFL	0.8423	0.8391	0.9004	0.8571	0.8011	0.7916
CPL	0.8327	0.8469	0.8594	0.7821	0.7692	0.8279

Table 10: Comparison of mean of of morphometric ratios among three Mystus species from two different places with the mean of the mean values

% Ratio	Mystusvittatus				Mystusgulio				Mystuscavasius			
	Visakhapatnam	Srikakulam	M.M	±SD	Visakhapatnam	Srikakulam	M.M	±SD	Visakhapatnam	Srikakulam	M.M	±SD
HL/TL	15.93	16.34	16.14	0.29	15.90	16.31	16.11	0.29	15.90	16.30	16.10	0.28
HL/SL	20.75	20.69	20.72	0.05	20.70	20.65	20.67	0.04	20.70	20.63	20.67	0.05
WH/HL	83.26	75.88	79.57	5.22	83.79	76.13	79.96	5.42	83.58	76.13	79.85	5.27
ED/TL	4.83	3.86	4.34	0.69	4.78	3.89	4.33	0.63	4.83	3.89	4.36	0.66
ED/SL	6.29	4.89	5.59	0.99	6.22	4.93	5.57	0.91	6.28	4.93	5.60	0.96
ED/HL	30.32	23.62	26.97	4.74	30.04	23.87	26.95	4.36	30.35	23.87	27.11	4.58
BW/TL	14.78	12.97	13.88	1.28	14.77	13.02	13.90	1.24	14.79	13.00	13.89	1.27
BW/SL	19.25	16.42	17.84	2.00	19.23	16.48	17.86	1.94	19.26	16.45	17.85	1.99
BW/HL	92.76	79.40	86.08	9.45	92.89	79.84	86.36	9.23	93.03	79.73	86.38	9.41
BH/TL	19.97	18.31	19.14	1.18	19.99	18.32	19.15	1.18	20.02	18.28	19.15	1.23
BH/SL	26.01	23.18	24.60	2.00	26.02	23.19	24.61	2.00	26.06	23.14	24.60	2.06
BH/HL	125.34	112.06	118.70	9.39	125.69	112.35	119.02	9.44	125.87	112.16	119.02	9.69
BW/BH	74.01	70.85	72.43	2.23	73.90	71.06	72.48	2.01	73.91	71.08	72.50	2.00
DFL/TL	12.11	12.07	12.09	0.03	12.07	12.08	12.07	0.01	12.10	11.97	12.04	0.10
DFL/SL	15.77	15.28	15.53	0.35	15.71	15.29	15.50	0.30	15.76	15.15	15.45	0.43
DFL/HL	76.02	73.87	74.94	1.52	75.89	74.07	74.98	1.28	76.12	73.42	74.77	1.91
PFL/TL	13.27	11.82	12.54	1.02	13.32	11.81	12.57	1.07	13.29	11.82	12.56	1.04
PFL/SL	17.28	14.97	16.12	1.63	17.35	14.95	16.15	1.69	17.30	14.96	16.13	1.65
PFL/HL	83.26	72.36	77.81	7.70	83.79	72.43	78.11	8.04	83.58	72.52	78.05	7.82
PVFL/TL	12.83	12.97	12.90	0.10	13.45	13.02	13.24	0.30	12.82	12.26	12.54	0.39
PVFL/SL	16.71	16.42	16.57	0.20	17.51	16.48	17.00	0.73	16.68	15.52	16.10	0.82
VFL/HL	80.54	79.40	79.97	0.81	84.58	79.84	82.21	3.36	80.60	75.23	77.91	3.80
AFL/TL	12.83	9.77	11.30	2.17	12.82	9.80	11.31	2.14	12.34	9.84	11.09	1.77
AFL/SL	16.71	12.37	14.54	3.07	16.69	12.40	14.55	3.03	16.07	12.45	14.26	2.55
AFL/HL	80.54	59.80	70.17	14.67	80.63	60.08	70.36	14.53	77.61	60.36	68.99	12.20
CFL/TL	13.63	13.46	13.55	0.11	13.58	13.42	13.50	0.11	13.61	13.44	13.52	0.12
CFL/SL	17.75	17.05	17.40	0.49	17.68	16.99	17.33	0.48	17.71	17.01	17.36	0.50
CFL/HL	85.52	82.41	83.97	2.20	85.38	82.30	83.84	2.17	85.57	82.43	84.00	2.22
CPL/SL	19.25	16.42	17.84	2.00	19.23	16.48	17.86	1.94	19.26	16.45	17.85	1.99

spine, almost equal to head excluding snout. Adipose dorsal fin originates just behind the rayed dorsal fin. Caudal fin forked, pointed, upper lobe longer.

Discussion

The relative marphometric studies conducted in sixteen external characters were analyzed and significant differences were observed and the correlation of body parts with total length and head

length were analyzed. Almost all body parts shows high correlation with total length, eye diameter width of the head, snout length shows high correlation with Head length. The differences in morphometric characters is due to the geographically variations and environmental variations such as food abundance and temperature).

The mean values of the meristic counts studies in three Mystus species shows constant values but shows small differences among individuals this probably indicated identity in their parental stock.

We revealed significant differences in morphometrics between two populations of *Mystus* species populations from Srikakulam and Visakhapatnam. There is a clear morphological distinction between certain characters in both populations. It is often difficult to explain the causes of morphological differences between populations (Cadrin 2000). These differences may be genetically related differences (or) they might be associated with phenotypic plasticity in response to different environmental factors in each area (Murta 2000). Thus morphological variation can reflect genetic differences between stock and/or environmental differences between localities.

Morphometric comparisons of African catfish, *Clariasgariepinus* in different river systems in Turkey revealed a significant divergence (Turan et al., 2005). Similarly, both morphological and genetic methods have been used to characterize different populations of *Clariasgariepinus* and *Clariasanguillaris* (Agnese et al., 1997). Thus the possibility exists that the observed morphological variations in the present study might be because of genetic differences among the populations. Correlations between genetic variations and morphological variations has been confirmed in natural populations (Poulet et al., 2004) and both have been widely used to make assessments of population differentiation (Buth& Crabtree 1982; Agnese et al., 1997; Ibanez et al., 2006).

Genetic differentiations were observed among different populations of yellow catfish *Mystusnemurus* from Thailand (Leesa-Nga et al., 2000). Significant genetic diversity was observed among two different populations of Korean catfish, *Silurusasatus* (Yoon & Kim 2001). In Malaysian river catfish, (*Mystusnemurus*) genetic variations were observed among different rivers and tributaries of Malaysia (Chong et al., 2000). In the present study, the genetic basis of morphometric differences is not studied but the application of molecular markers would be a very useful method (Agnese et al., 1997; Delling et al., 2000; poulet et al., 2004) for confirming the observed phenotypic differences among different geographical regions and for facilitating the development management strategies. The information on morphometric measurements of fishes and statistical relationship between them are essential for taxonomic work (Narejo, 2008). To know the origin of stock; separation of stock or identification of fish species morphometric characters are frequently used (Lashari et al., 2004; Narejo et al., 2008).

The results of the present study Table 8, 9 shown that high co-efficient of correlation (r) values in all most all cases. From the co-efficient of correlation values it is evident that dorsal fin length, pectoral fin length, pelvic fin length, caudal fin length, standard length and head length are highly correlated with the total length (TL). Eye diameter, snout length, width of head is highly correlated with the head length (HL). The above relationship indicated that the body measurements are linear. The similar linear relationship was also obtained by Ganguly et al., (1959) in *Latescalcarifer*, Mehta and Bapat (1977) in *Ophiocephalusgachua*, Hoque and Rahman (1985) in *Gudusiachapra,* and Lashari et al., (2004) in *Cirrhinusreba.*

Morphometric differences among stocks are expected because they are geographically separated and may have originated from different ancestors. In the present study Meghadrigedda and the river Nagavali are two different habitats with wide environmental variations. Fishes are very sensitive to environmental changes and quickly adapt themselves by changing necessary morphometrics. Morphological characters can show high plasticity in response to differences in environmental conditions, such as food abundance and temperature (Allendorf and Phelps 1988; Swain et al., 1991; Wimberger 1992).

The phenotypic plasticity of fish is very high. Then adapt quickly by modifying their physiology and behavior to environmental changes. These modifications ultimately change their morphology (Stearns 1983).

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Original Article

Haematological Analysis of Leschenault's Leaf Toad Gecko, *Hemidactylus Leschenaultii* Dumeril and Bibron 1836

Sarbeswar Nayak*, Prafulla Kumar Mohanty**

Abstract

Haematology is useful in understanding the physiological features of lizards. The study was held to investigate the haemo profile of leschenault's leaf toad gecko in Odisha. The haematological parameters like Haemoglobin Concentration (HB), Packed Cell Volume (PCV), Total Erythrocyte Count (TEC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Total Leucocyte Count (TLC), Total Platelet Count (TPC) and Differential Leucocyte Count (DLC) were calculated using standard procedures. Statistical analysis like correlation and t-test were done. The study revealed that the TEC and percentage of lymphocytes show significant difference ($p<0.05$) between both sexes of lizard. The mean values of all other parameters are also showing difference between male and female of *Hemidactylus leschenaultii* Dumeril and Bibron 1836. The correlation coefficient varies in male and female with respect to the parameters analysed. Some parameters were positively correlated with each other and others were found to be negatively correlated. The data obtained could be a useful indicator for monitoring and managing the general health status of the species.

Keywords: Haematological Parameters; *Hemidactylus Leschenaultii*; Correlation; Significant Difference.

Introduction

The significant of haematological study for evaluating, analysing, examining and controlling the health status of animals is a prelude blueprint. The haematologic technique for assessment of body physiology has a great role being uncomplicated, serene, minimal invasive, productive first hand technique and economically sustainable.

Leschenault's leaf toad gecko is belonging to Class– Reptilia, Order– Squamata and Family Gekkonidae, found in warm climatic conditions throughout the world [1]. Haemocytological parameters are useful and widely used tools that assist in the diagnosis and monitoring of animal health [2]. The combinations of different haematological parameters detect the physiological conditions and clinical evaluation in reptiles [3,4]. The different external and internal factors also affect the haemoprofile of nonmammalian vertebrates [5,6]. Haematolgy of some saurians were studied [7,8,9]. The haemoglobin concentration, haematocrit value, mean cell volume, mean cell haemoglobin and mean cell haemoglobin concentration were studied in some reptiles [10,11,12]. Haematology in reptiles highly dependent on age as well as sex and it varies throughout life [13,14]. The blood cells were identified and different parameters were taken [15-20].

The data about haematology of reptiles is still challenging in comparison to other nonmammalian vertebrates. The literature available on haematology of this lizard specifically from the studied geographical region of Odisha is very less. The comparative scarcity of informations regarding the haematology of geckos provides a less evidences about

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family Gekkonidae. The purpose of this study was to determine the reference intervals of haematology and morphometry of blood cells of normal and apparently healthy lizards. The findings will serve as base line data for future health assessment of lizards as well as for conservation and protection.

Materials and Methods

Animals

Fifteen lizards of each sex were collected from the coastal area of Rajnagar block of Kendrapara- 754 225, Odisha, located in 20° 20′ N to 20° 37′ N latitude and 86° 14′ E to 87° 01′ E longitude. They were caught at day time from the crevices of trees and sometimes at wall of boundaries and homes adjacent to the fields. Lizards were clinically healthy and in good condition transferred to animal house. The investigation of haematological profiles on *Hemidactylus leschenaultii* Dumeril and Bibron 1836 was carried out from 2014 to 2017.

Blood Collection

The venipuncture site was prepared aseptically prior to blood collection. Blood was collected from the ventral tail vein of lizards by inserting an insulin syringe (BD Ultra – Fine ™ Needle 12.7 mm × 30G) at an angle of 45-60° between the scales on ventral midline [21,22]. Once blood appeared in the needle hub, held steady and a gentle negative pressure was applied to the syringe. The blood was kept in an EDTA vial and then transported in icebox to laboratory. The lizards were released to their natural habitat after collection of blood. Whole blood smear were obtained by push slide technique, air dried, fixed with methanol and stained with Giemsa as protocol cited by Lillie [23].

Haematological Analysis

The blood parameters were studied using the procedure [24,25,26,27]. The concentration of haemoglobin was estimated as oxyhaemoglobin by Sahalis haemometer and expressed in g %, Packed cell volume (PCV) was determined by microhaematocrit method with a spun of microhaematocrit tube at 2500 rpm for 15 minutes. The quantification of RBCs and WBCs was performed by manual methods using haemocytometer, with Hayem's diluting fluid for RBCs and Turk's diluting fluid for WBCs. Erythrocyte indices like MCV, MCH and MCHC were calculated using standard formulae [28]. The percentage of different leucocytes as well as total platelet count determined [29,30].

Statistical Analysis

The data presented as Mean ± SE (Standard Error) for both sexes and MS office Excel 2007 was used for statistical analysis. The correlation analysis between the parameters and the significant difference ($P<0.05$) was taken using Student's t-test (assuming equal variances) with the help of Paleontological Statistics (PAST) version 2.17 (Natural History Museum, University of Oslo).

Results

Haematological parameters were analysed in case of adult lizard (Table1). Assuming a confidence level of 95%, significant differences were found in two

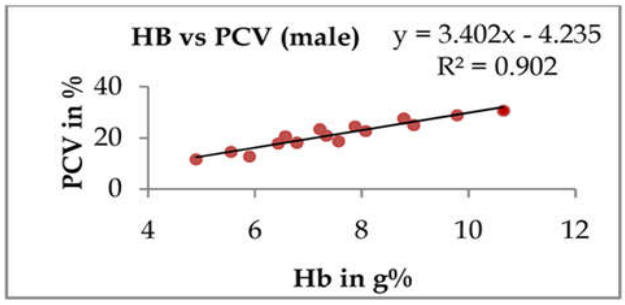
Table 1: Haematological parameters of *H. Leschenaultii* Dumeril and Bibron 1836

Sl. No.	Parameters	Unit	Male			Female			P value
			Range	Mean	SEM	Range	Mean	SEM	
1	Haemoglobin	g%	4.89 – 10.65	7.49	0.41	4.53 – 9.64	6.53	0.36	1.75
2	PCV	%	11.76 – 30.76	21.25	1.46	11.89 – 30.89	20.12	1.39	0.54
3	TEC	$10^6 mm^{-3}$	0.87 – 2.34	1.43	0.14	0.72 – 1.86	1.14	0.12	2.27*
4	MCV	fl	91.51 – 267.48	161.41	13.55	105.87 – 304.61	185.33	14.14	1.26
5	MCH	pg	34.24 – 92.61	56.59	4.14	47.59 – 64.11	59.77	3.18	0.64
6	MCHC	%	24.36 – 45.76	35.17	1.32	23.36 – 47.1	34.16	1.72	0.43
7	TLC	$10^3 mm^{-3}$	7600 - 14356	11362.13	493.92	8500 - 16540	12647.6	556.58	1.72
8	TPC	$10^3 mm^{-3}$	15800 - 44356	30049.8	2182.46	16783 - 48900	34178.93	2525.89	1.31
9	Heterophils	%	50 - 79	65	2.03	50 - 78	60.8	2.41	1.24
10	Lymphocytes	%	17 - 38	26.73	1.81	21 - 42	33.8	1.99	2.73*
11	Eosinophils	%	1 - 15	6.2	0.92	2 - 7	4.33	0.45	1.59
12	Monocytes	%	2 - 4	2.13	0.32	0 - 4	1.73	0.24	0.89
13	Basophils	%	0	0	0	0	0	0	0

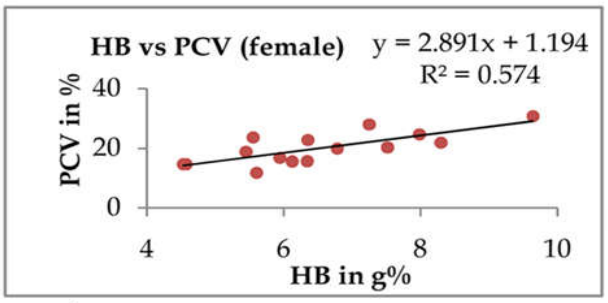
(Significant difference * $p<0.05$ for each haematological parameters)

parameters throughout the investigation. The total erythrocyte count was found to be highest in male and lowest in female ($p<0.05$). The percentage of lymphocytes is highest in female and lowest in male ($p<0.05$). The highest mean value of haemoglobin and packed cell volume and mean corpuscular haemoglobin concentration was found in male and lowest in female while the mean values of other parameters were found to be highest in female. The percentage of heterophils, eosinophils and monocytes was highest in male and lowest in female (Table 1). The correlation between haematological parameters of both male and female geckos was depicted (Figure 1). There is a positive correlation between Hb vs PCV (Fig.1a,b), HB vs TEC (Fig.1c,d) and MCV vs MCH (Fig.1 k,l) in both male and females, but HB vs MCV(Fig.1 e) is positively correlated in male and negatively correlated in female (Fig.1 f). A negative

a.



b.



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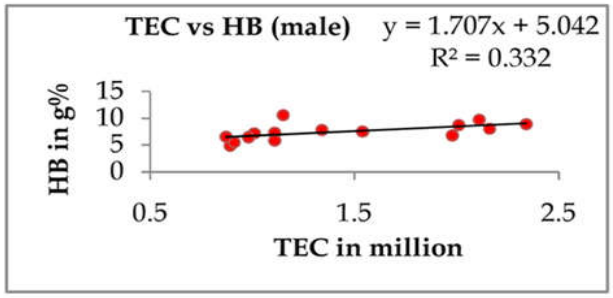

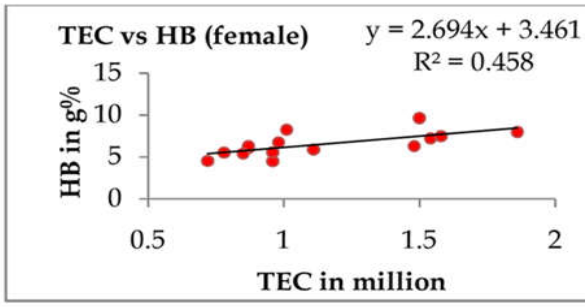

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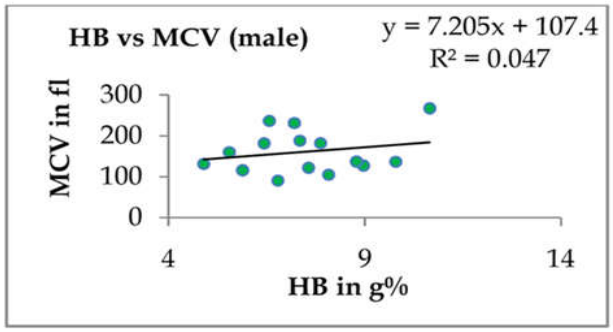

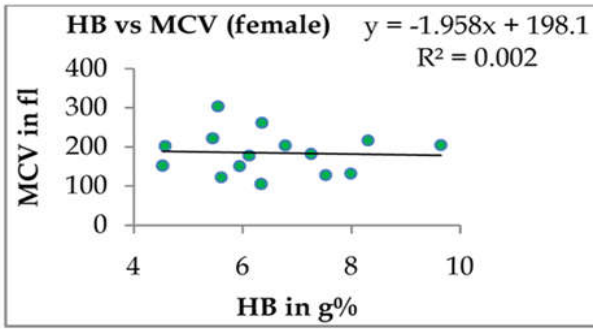

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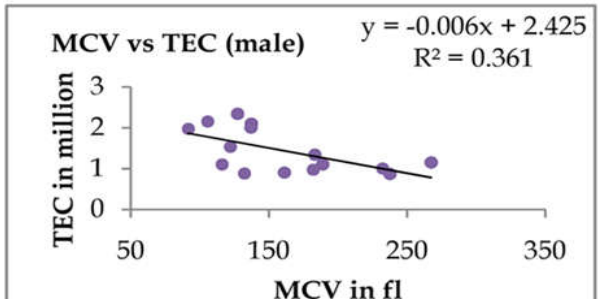

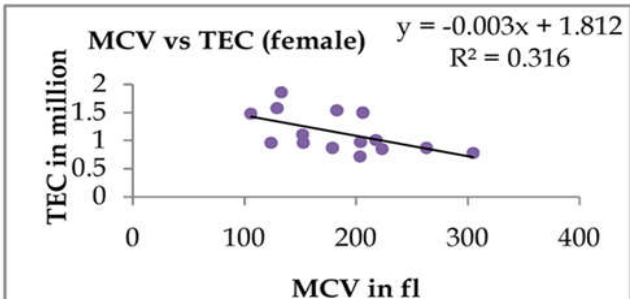

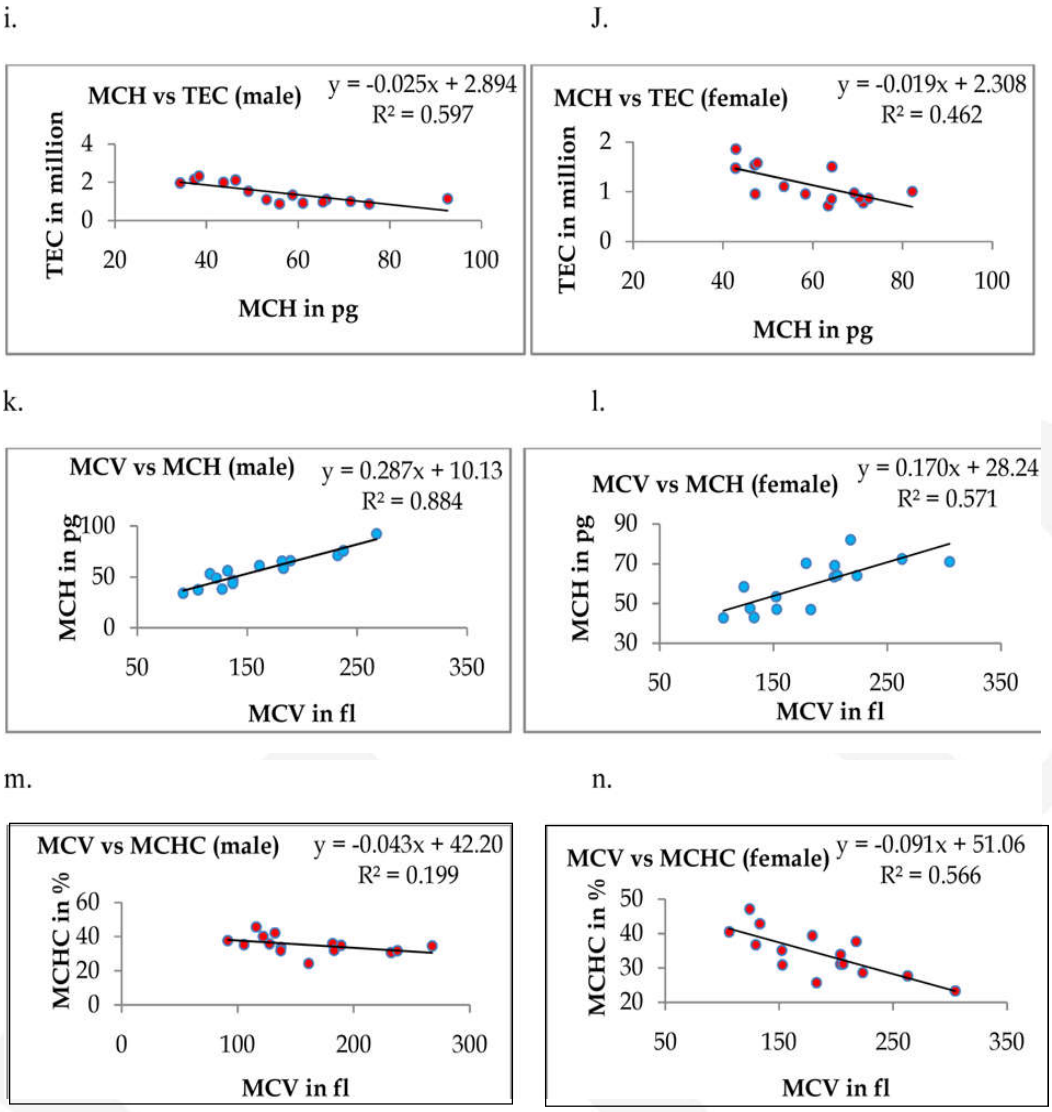

Fig. 1: Correlation between different haematological parameters (a-n) of *Hemidactylus leschenaultii* Dumeril and Bibron 1836

correlation was found in both sexes with respect to MCV vs TEC (Fig.1g,h), MCH vs TEC (Fig.1 i,j) and MCV vs MCHC (Fig.1 m,n).

Discussion

Haematological data are essential to correlate the health status of reptiles with their habitat. Haematology of reptiles provides a way to diagnose the animals [31]. There was no differences in total haemoglobin concentration in male and female lizards [32,33]. However the result of our investigation shows a difference in haemoglobin concentration. The difference in the value may be due to some seasonal factors in combination with age and sex of the individuals. According to [34] the PCV and TEC is higher in male of Free living Mediterranean Pond Turtle and TEC in other reptiles [9,24]. Our data regarding PCV and TEC corroborate with this. The PCV and TEC found in this study is lower than the report by [35] for prehensile tailed skink. The TLC was higher in male and MCV was higher in female Mediterranean Pond Turtle [34]. But in case of Leschenault's leaf toad gecko TLC and MCV was found higher in female only. There was difference in mean values of haematological parameters between sexes of the studied gecko which is also seen in some agamidae [13], geckos, *Hemidactylus frenatus* [36]. Our findings of TEC and TLC show closeness with the finding of some agamidae lizards [37,38,39,40]. The result obtained in this study regarding the haematological parameters also fall within the range as studied in iguana by [41,42]. Haematological

parameters in reptiles vary with age sex and seasons [31,43]. The TEC, in both male and female is lower in comparison to *Psammodromus algirus* [44].The MCV, MCH and MCHC in both male and female *Naja naja* were higher [45] in comparison to *Hemidactylus leschenaultii*.

The heterophil count, the lymphocyte count is higher in both male and female in this study in comparison to *Psammophilus blanfordans* and eosinophil and monocyte percentage falls within tha range [13]. The monocyte, eosinophil and lymphocyte percentage is highest and heterophil percentage is lowest in *Trapelus lessonae* [46]. Generally the heterophils and lymphocytes are the highest occurred leucocytes followed by eosinophils, monocytes and basophils are rare occurrence [47,31,48].

In Leschenault's leaf toad gecko, the PCV shows a positive correlation with increase in haemoglobin concentration in both male and female, this indicates that TEC also directly proportional to concentration of haemoglobin. With increase in TEC, the PCV and concentration of haemoglobin, both increased in different sexes and are correlated positively. With increase in MCV, the MCH is increasing in male and female.

In case of male the MCV is increasing with increase in concentration of haemoglobin where as in female it is negatively correlated. This is an indication of poor health status like anaemia in case of female lizards. TEC shows a negative correlation with MCV and MCH in case of both male and female and the MCV is also negatively correlated with MCHC in both sexes. The relation between all the haematological parameters may be due to influences of different external like seasonal variations, diurnal variations, temperature and intrinsic factors including age, sex and body physiology.

Conclusion

Haematology is highly dependent on body physiology of lizards with respect to their surroundings. However, it provides the fact that the geckos showed different haemoprofile with respect to their sex. The most important thing is that, the less population and low body mass of geckos limit the data interpretation and further validation is necessarily required. The present study provides a base line reference value for the haematological parameters of *Hemidactylus leschenaultii*. This data may be useful for further study relating to impact of climate, environmental conditions, use of microhabitat and seasonal fluctuations on haematology of lizards.

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Original Article

Perceived Impact of Light Quality on Seed Germination and Photosynthetic Pigments in Rice (*Oryza sativa* L.)

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Abstract

The present investigations attempted to study the effects of different colour of light on seed germination, hypocotyl growth, mobilization efficiency (ME), vigor index (VI), biomass production and content of photosynthetic pigments (Chl a, Chl b and Carotenoids) in Rice (*Oryza sativa* L.) cv. Sugandha 5, an important staple food crop of the world. Germination rate was found maximum in red light (98%), followed by blue (94%) and natural light (93%) at 84 hours while, almost no germination was shown in green light even after 96 hours. Yellow light caused significant reduction in % germination (85). The seedlings obtained under different lights revealed variation in biomass production (fresh weight of root and shoot). Root and shoot growth were observed highest in red light and the order of biomass production was red > natural > yellow > blue > green. ME and VI of rice seedlings recorded maximum (128.22 and 43.26 respectively) in red and minimum in yellow light (116.09 and 1.20 respectively). Both, ME and VI could not be estimated under green light due to absence of well-marked hypocotyl. The contents of photosynthetic pigments in seedlings indicate synthesis of photosynthetic pigments highly dependent on light quality. Chlorophyll b and carotenoids were recorded highest in natural light whereas total chlorophyll and chlorophyll a were highest in red light in comparison to other light treatments. These findings indicate possibility for exploring light quality for manipulation of germination and seedling health of crop plants in general and *O. sativa* in particular.

Keywords: Germination; Light Wavelengths; *Oryza Sativa*; Photosynthetic Pigments; Rice; Seedling Vigour.

Introduction

Light is an indispensable factor for plants, being the energy source for their growth and development, however, besides being essential to photosynthesis, it also serves as an environmental signal which, when perceived, triggers changes in plant metabolism and development (Jiao *et al.* 2007). The effects of light on a plant community, especially in terms of environmental signaling, are not only related to the magnitude of the photosynthetic photon flux i.e. the amount of light, but also to the direction, duration, and particularly, the quality of light available to plants (Majerowicz and Peres, 2004). Besides importance for growth, development, and environmental perception, experiments have shown that light has a connection with a number of other plant processes, including related to biotic and abiotic stress tolerance (Svyatyna and Riemann, 2012). In this regard, light and other environmental stimuli often work together to trigger the development of specific responses in plants (Jiao *et al.* 2007). The light quality reaching the soil/plants and the absorbing organs varies according to many factors which include the time of the day, season, geographic location, atmospheric gases and moisture, clouds, smoke, dust, and other pollutants in the air, topography, presence of barriers including plants, plant architecture, and location of absorbing plant organs within the canopy. Nature has produced a

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number of light absorbing molecules that enable organisms to respond to changes in the natural light environment. The changes in the light signal/quality (wavelength) influences various physiological processes (i.e. intra- and inter-cellular differentiation, seed germination and seedling growth, photosynthesis, flowering etc.), depending on the developmental stage and plant species or studied plant part (He *et al.* 2017). Green light, in the process of seed germination of *Arabidopsis*, stimulates the early elongation of the stems, antagonizing the growth inhibition by light whereas the *white* and *red* light, in ferns can cause delay of the chlorophyll loss due to senescence (Burescu *et al.* 2015). It has been mentioned earlier that light is absolute factor regulating the seed germination process in numerous plant species (Jala, 2011; Lal and Sachan, 2017).

Over a study period of 4 weeks, Jala (2011) found that the seeds of *Nepenthes mirabilis placed* under white and red light germinated first and those placed under green light were the last once to germinate and the highest average speed of emergence was also recorded highest for seedlings under red light. Pigments are biomolecules that absorb light usually in the range of 320 to 760 nm and their biosynthesis in seedlings is highly dependent on light quality they perceive.

Burescu *et al.* (2015) studied the effect of different wavelengths LED lights on the growth of Spruce (*Picea abies* L.) plantlets and observed increased biosynthesis of chl a, chl b under blue and yellow light, respectively. Carotenoid synthesis was also significantly enhanced in yellow light treated plantlets. However, all pigments analyzed were found lower in plants raised under green light than in other light treatments.

Studies on effect of light quality on early development of rice cultivars under laboratory and green house environment have shown that red light has promontory effect where as green light turns to be inhibitory, and interaction between light x temperatures proved role of red light in promotion of cold tolerance (Venske *et al.* 2013).

However, the information on effect of light quality (wavelengths) on seed germination, seedling health and photosynthetic pigment is not available in literature for most of the crop plants including rice. Considering these diverse effects of light, the present study was carried to investigate the effect of different colours of light (natural, red, blue, yellow, and green) on seed germination, hypocotyl growth, biomass production, mobilization efficiency (ME), vigor index (VI) and photosynthetic pigments in Rice (*Oryza sativa* L., Family- Poaceae), a monocotyledonous angiosperm and most important staple food crop of the world, mainly cultivated and consumed in Asian countries.

Materials and Methods

Rice (*Oryza sativa*) cv. Sugandha 5 was used as experimental material for germination using Knop's nutrient solution. Knop's solution (10X) was prepared by dissolving 0.8 mg of $Ca(NO_3)_2.4H_2O$, 0.2 mg of KNO_3, 0.2 mg of K_2HPO_4, 0.2 mg of $MgSO_4.7H_2O$ and traces of $FeSO_4$ in 50 ml of distilled water (DW). This stock solution was further diluted with DW for the preparation of 250 ml nutrient solution for germination of seeds.

Hundred surface sterilized seeds of *O. sativa* were grown in a series of 5 petri dishes, each containing equal amount of sand and moistened with 30 ml of nutrient solution. These petri dishes were exposed to light of different wavelength (i.e. natural, red, yellow, green and blue light) provided using with different color LEDs of Philips Company (different wavelengths) for the duration of 96 hours. The experiment was designed to assess the effect of different types of light: (white (fluorescent), red light with peak emission of 660 nm, green light with peak emission of 550 nm, blue light with peak emission of 490 nm, and yellow light with peak emission of 600 nm light). The adopted photoperiod in the experiments was 16 hrs light/24 hrs.

Mobilization efficiency (ME) in germinating seedlings of each light treatment was estimated by the method of Mohan *et al.* (1996) with the following formula:

ME = Dry weight of seedlings/ Dry weight of cotyledon X100

Vigor index (VI) of germinating seedlings against each light treatment was estimated by the method of Abdul-Baki and Anderson (1973) with the following formula:

VI = % germination X average hypocotyl length

The amounts of chlorophyll a, chlorophyll b, total chlorophyll and carotenoid were quantified in mg/ gm fresh weight according to method of Arnon (1949) with sight modification (Bansal *et al.* 1976). On 5th day 80% (v/V) acetone homogenate of plants was incubated at 4°C for 24 hours, and then the homogenate was centrifuged at 5000 revolution per minute for 15 minutes. The supernatant was used to determine OD of each sample/treatment at 480, 510, 630, 645, 652, 663 and 665 nm using Spectronic 20 Bousch and Lomb spectrophotometer. The pigment values were calculated as:

Total Chlorophyll = $O.D_{652}$ X 1000/ 34.5

Chlorophyll a = 15.6 X ($O.D_{665}$) – 2 X ($O.D_{645}$) – 0.8 X ($O.D_{630}$)

Chlorophyll b = Total Chl – Chl a

Carotenoids = 7.6 ($O.D_{480}$- 1.4 X $O.D_{510}$)

The actual pigment content (mg/g FW) was computed as pigment value X V/1000 X 1/W

Where

V - volume of acetone extract (in ml) and

W - weight of the leaf tissue used (in g).

Results and Discussion

The effects of different light treatments on germination of rice (*Oryza sativa*) seeds are summarized in Table 1. The extent/rate of germination has been found to depend on light quality (wavelength) and exposure period. The germination was recorded maximum (98%) in red light at 84 hours duration and this wavelength was found most suitable than other lights tested. In the study by Abdullateef and Osman (2011), red light (660 nm) had better influence on germination in *Stevia rebaudiana* Bertoni seeds than white light (400-700 nm) and present result are in conformity with germination in *Stevia rebaudiana*. Thereafter, in natural light the rate of germination was relatively much faster than other light treatments with maximum germination of 93% at 84 hours. Sharma and Sen (1975) also recorded highest percentage of germination in *Merremia* species with red light treatment. Blue light resulted in germination (94%) comparable to natural light and yellow light showed moderate germination (85%) at 84 hours. Almost no germination was noticed under green light even after 96 hours. The germination process did stop at 84 hours in all the light treatments. It has been observed previously in case of *Nepenthes mirabilis* and *Vigna unguiculata* that seeds placed under white and red light germinated first and those placed under green light were the last ones to germinate (Jala, 2011; Lal and Sachan, 2017). However, upon completion of germination, Jala (2011) recorded highest % germination in yellow light followed by red and natural light. The present results on germination show deviation from Jala (2011) but conform to findings of Colbach *et al.* (2002) on *Alopecurus myosuroides* and Ambika (2007) on *Chromolaena odorata* seeds.

The effect of different light wavelengths on biomass production in rice seedlings is summarized in Table 2 in terms of root fresh weight (FW), shoot fresh weight (FW), seedling dry weight, cotyledon dry weight and hypocotyl length. Seedlings under red light showed highest biomass yield in terms of root, shoot and cotyledon fresh weight. The order of biomass production under different light qualities was red > natural > yellow > blue > green. Irradiation with blue was not suitable for biomass growth (particularly root) in *O. sativa* although it turned superior over yellow light in respect of % germination. Blue light

Table 1: Germination (%) of rice (*O. sativa*) cv. Sugandha 5 in different light wavelengths

Treatments	% Germination							
	12 h	24 h	36 h	48 h	60 h	72 h	84 h	96 h
Natural Light	0	14	32	61	92	93	93	93
Red Light	0	23	81	91	95	98	98	98
Blue Light	0	21	36	75	84	90	94	94
Yellow Light	0	02	51	65	77	85	85	85
Green Light	*	*	*	*	*	*	*	*

*No germination

Table 2: Growth response of root, shoot and hypocotyls of germinating seedlings of rice (*O. sativa*) cv. Sugandha 5 in different light wavelengths

Treatments	Root Length (cm)	Root FW (g)	Shoot Length(cm)	Shoot FW (g)	Seedling Dry Wt. (g)	Cotyledon Dry Wt. (g)	Hypocotyl length (cm)
Natural light	1.72± 0.22	0.008±0.004	2.92± 0.17	0.146± 0.017	0.011± 0.004	0.013± 0.006	1.20± 0.19
Red light	1.92± 0.29	0.011± 0.002	3.76± 1.07	0.150± 0.013	0.012± 0.005	0.017± 0.009	1.58± 0.23
Blue light	0.40± 0.10	0.006± 0.003	0.32± 0.07	0.102± 0.016	0.004± 0.001	0.007± 0.003	0.66± 0.29
Yellow light	1.56± 0.23	0.012± 0.004	3.20± 0.64	0.129± 0.013	0.007± 0.002	0.012± 0.006	1.34± 0.17
Green light	*	*	*	*	*	*	*

*Parameters not measurable, Data on FW and DW is shown up to third place after decimal to reveal the differences

resulted in smaller cotyledons and leaves with relatively low FW and Seedling dry weight and caused significant reduction in hypocotyls length. Horizontal and vertical expansion of shoot, particularly leaves is genetically controlled developmental process (Tsukaya, 1998) and irradiation with blue light seems to cause imbalance in expression of concerned genes leading to inhibition of leaf expansion. For the lettuce crop, the fresh and dry weight accumulations were higher under the RB (red-blue) treatment (Mickens, 2012). Snowden (2015) also observed significant reduction in dry biomass in radish under green light at the high level among the comparable treatments. In contrast to present findings, green light is reported to stimulate the spruce (*Picea abies* L.) seed germination and plant growth whereas the blue light inhibits hypocotyl elongation (Burescu *et al.* 2015).

Variation was recorded in mobilization efficiency (ME) and vigor index (VI) in seedlings of rice (*O. sativa*) obtained in different light treatments and both ME and VI were highest (128.22 and 3.26, respectively) under red light followed by blue light (Table 3). Under green light, both ME and VI could not be determined due to lack of well differentiated hypocotyl. Contrary to these findings, Jala (2011) reported that seedling vigor index and germination index were highest under yellow light, followed by red light. In another similar study on *V. unguiculata* (a dicot plant), Lal and Sachan (2017) recorded highest total chlorophyll, chl b and carotenoids in natural light whereas chl a was highest in red light. These findings indicate pigment biosynthesis/content in response to different light wave lengths as a species/genotype specific trait.

The seedlings formed under different lights revealed differences in the quantity of photosynthetic pigments (Table 4). Chlorophyll b and carotenoids were recorded highest in natural light whereas total chlorophyll and chlorophyll a was recorded highest in red light in comparison to other treatments. Natural light also recorded maximum synthesis of carotenoids followed by red, yellow and blue light, respectively. Green light had no measurable pigments because of insufficient growth and development of seedlings. In some instances, green light may function by informing the plant of photosynthetically unfavorable conditions, allowing plants to adjust their compositions and physiology to the available light quality. The chlorophyll a : b ratio also varied in different light treatments. In red light, chlorophyll a:b ratio was found maximum (2.07) whereas total chlorophyll : carotenoid ratio was highest (2.72) in blue light in comparison to other lights. Saebo *et al.* (1995) reported that red light is important for the development of the photosynthetic apparatus (plastid differentiation) of plants and a combination of red and blue light is important in the biosynthesis of chlorophyll. The use of red-LED light to drive photosynthesis has been widely accepted due to fact that red wavelengths (600–700 nm) are efficiently absorbed by photosynthetic pigments (Sager and McFarlane, 1977) and the same is evident from the present results. Likewise, the highest inhibition of all the assimilating pigments in spruce (*Picea abies* L) plantlets was observed when exposed to green LEDs (Burescu *et al.* 2015). In a recent study, He *et al.* (2017) explained that the suitable combination of red- and blue-LED light enhances plant growth and

Table 3: Mobilization efficiency and Vigour index of seedlings of Rice (*Oryza sativa*) cv. Sugandha 5 grown in different light wavelengths

Treatments	Mobilization Efficiency (ME)	Vigour Index (VI)
Natural Light	117.03±2.184	2.58
Red Light	128.22±4.344	3.26
Blue Light	120.29±3.245	3.04
Yellow Light	116.09±2.135	1.20
Green Light	*	*

* ME and VI could not be determined because no differential/well-marked hypocotyl present

Table 4: Photosynthetic pigment contents of rice seedling leaves in different light wavelengths

Treatments	Total chl (mg/g FW)	chl a (mg/g FW)	chl b (mg/g FW)	Carotenoids (mg/g FW)	chl a: b ratio	Total chl: carotenoids ratio
Natural Light	0.850	0.526	0.324	0.456	1.62	1.86
Red Light	0.936	0.632	0.304	0.368	2.07	2.54
Blue Light	0.638	0.419	0.219	0.234	1.91	2.72
Yellow light	0.736	0.375	0.361	0.296	1.03	2.48
Green Light	*	*	*	*	*	*

*Not measurable pigment values, Chl- Chlorophyll

photosynthetic capacities of *M. crystallinum* compared to red- or blue-LED alone. This observation indicates need for further experiments in range of crop plants using combination of two or more wavelengths to improve germination and seedling vigour.

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Original Article

Comparative Analysis in Gut Content of Three Fresh Water Teleosts (*Clarias Batrachus, Channa punctatus, and Anabas Testudineus*) during Different Season

Sanatan Singh*, G. Mishra, P.K. Dixit*****

Abstract

Aim of the present piece of work was to have a comparative analysis of the food composition and feeding habits among three Indian fresh water teleosts. The gut content of three fishes has been analyzed and are broadly classified into 8 categories i.e. crustacea, rotifer, insects, Chlorophyceae, baccillariophyceae, Myxophyceae, plant matters and decaying organic matters. It was also seen that there were considerable variations in the percentage of different food items in the gut of above fishes during different months of the year. The different food items are variable increases during the different seasons of a year among the three fresh water teleosts.

Keywords: Preponderance Index (I); *Clarias Batrachus*; *Channa Punctatus; Anabas Testudineus.*

Introduction

Food and feeding habit of fishes has a great significance in aquaculture practice. It helps to select such species of fishes for culture which will utilize all the available potential food of the water bodies without any competition with each other but will live in association with other fishes. This will allow the best utilization of the food sources of water body and will give an optimum yield. Food and feeding habits of fish vary with the time of day and season of the year. Food and feeding habits of fishes have been a field of interest to fisheries researchers since very long Sakhare and Chalak (2014).

Sakhare (2010) studied food and feeding of *Cyprinus carpio* from local markets, reservoirs and ponds around Ambajogai. However, analysis of stomach contents is a method for determining the food and feeding habits of fishes by which we can easily find what the fish take as food. The various forms of feeding found in fishes include filter feeding, carnivores, and herbivores.

Osman and Mohmoud (2009) reported that various methods have been developed for the quantitative estimation of diet composition in fishes. Among these, the estimation of abundance and occurrence of different food items are the most popular. Emmauel and Ajibola (2010) reported crustaceans, pisces and bivalves as the three major food of the frillfin goby *Bathygobius soporator*. This study is intended to provide baseline information on the food and feeding habit of the species, which could serve as guide for aqua culturists and fisheries resource managers.

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Objectives

- This work deals with the studies of the food composition of Indian major carps.
- Variations in food and feeding habits in relation to season.
- Compare the feeding habits among three fresh water fishes.
- Find out the preponderance index (I) of the *C.batrachus*, *C.punctatus* and *A.testudineus* .
- To find out the grading system of food items, which is preferred most by an organism.

Materials and Methods

A total number of 60 specimens of above fishes were collected from fish market nearby university campus, Berhampur, Ganjam, Odisha of different seasons of the year during the months Jan to Dec 2017. Gut of each specimen was dissected out, gut was stretched out and removed from adhering viscera and mesenteries by using brush and blunt forceps to prevent injury of the gut its content emptied into separate Petridis with food items identified as per methods of Dewan et al., (1991).

The entire gut contents of each specimen, preserved in 5% formalin, were taken into consideration in the analysis of the diet. The food items were subjected to higher degree of higher mutilation due to the action of digestive juice. Therefore, the gut content could be identified up to higher taxonomic group. The Index of preponderance (I) for each food items was worked out applying the formula recommended by Natrajan and Jhingran (1961). The different mean values were analysed using the statistical package for social science (SPSS software program, version 10). the result is considered significant if $p<0.05$.

Results

Analysis of the gut content of fishes was an important and direct way of investigating their food habits in different seasons. The identification of food items eaten by a particular species of fish in its habitat was the direct interlink between the tropic components in an ecosystem. In this work, the gut contents of *Clarias batrachus*, *Channa punctatus* and *Anabas testudineus* had been analysed and grading was assigned to different food items.

Gut Content Analysis of *C.batrachus*

The percentage composition of food items in the gut of ***C. batrachus*** as observed in different months has been summarized in the (Table 1). The gut content of *C. batrachus* have been grouped into 8 broad categories i.e. crustacea, rotifer, insects, Chlorophyceae, baccillariophyceae, Myxophyceae, plant matter and decay organic matters . It was seen that there were considerable variations in the percentage of different food items during different months of the year. Crustacea was the highest percentage (43.95%) occurrence in the Spring season (February and March) and that of lowest (31.07%) in the Winter season (December and January) as per Figure 1 and 2. Rotifers percentage varies from higher to lower as 33.33% in Autumn (October and November), 27.17 % in Winter season (December and January), 25.46 % in Summer (April, May and June), 23.15 % Spring (February and March) and 22.79% in Rainy season (July, August and September) respectively as per Figure 3 and 4. The highest percentage of Insect's was observed in Rainy season (July, August and September) in 24.07 and lowest in spring season (February and March) in 12.84%. Percentage occurrence of Chlorophyceae was highest to lowest as Spring (10.65 %), Autumn (10.07%), Summer (10.05%), Winter (7.26%) and Rainy (3.69) respectively. Bacillariophyceae is the other food items, which highest percentage is Winter (10.17) and lowest in Summer in (2.77).

The highest percentage of Myxophyceae occurred in Winter (4.14%) and lowest in Autumn (2.32%). The highest percentage of occurrence of plant matter in the gut content was in Rainy season (July, August and September) is (3.80%) and lowest in Autumn in (0.88). The intake of dead and decaying matter of gut content analysis was highest in Winter (0.34%) and lowest in Autumn season (0.07%) as per Figure 5. Seasonal variation showed a slight variation in feeding main food items of *C. batrachus* were crustacea, rotifers and insects .

Gut Content Analysis of *C.punctatus*

The percentage composition of food items in the gut of ***C. punctatus*** as observed in different months has been summarized in the (Table 2). The gut content of *C. Punctatus* have been group into 8 broad categories i.e. crustacea, rotifer, insects, Chlorophyceae, baccillariophyceae, Myxophyceae, plant matter and decay organic matters. It was seen that there were considerable variations in the percentage of different food items during different months of the year. crustacea was the highest percentage (35.07%) occurrence in the Spring season (February and March) and that of lowest (13.30%) in the Rainy season (July, August and September) as per Figure 6,7 and 8. Rotifers percentage varies from 32.91% in Rainy season (July, August and September) to 13.05% in Winter season (December and January) . The highest percentage of Insect's was observed in Winter season (December and January) and lowest in spring season (February and March) in 10.90%. Percentage occurrence of Chlorophyceae was highest to lowest as summer (15.19%), Rainy (12.12%), Spring (10.72%), Autumn (10.13%) and in Winter (7.11%) respectively as per Figure 9 and 10. The highest percent of baccillariophyceae was seen in Summer

Table 1: Gut content and grading of various food items of *Clarias batrachus*

Food items	% composition of items		V_1O_1	Preponderance Index (I)	Grading
	Volume (V_1)	Occurrence (O_1)			
			Spring		
Crustaccea	30.25	25.25	763.81	43.95	I.
Rotifer	22.22	18.11	402.40	23.15	II.
Insect	13.63	16.38	223.25	12.84	III.
Chlorophyceae	14.21	13.03	185.15	10.65	IV.
Bacillariophyceae	10.05	9.04	90.85	5.23	V.
Myxophyceae	4.32	11.30	48.81	2.82	VI.
Plant matter	4.31	5.12	22.06	1.27	VII.
Decay organic matter	1.12	1.24	1.38	0.09	VIII.
Total			$\sum V_1O_1$= 1737.71		
			Summer		
Crustaccea	27.32	25.60	699.39	39.62	I.
Rotifer	23.12	19.44	449.45	25.46	II.
Insect	19.56	16.05	313.94	17.78	III.
Chlorophyceae	14.61	12.15	177.51	10.05	IV.
Bacillariophyceae	5.14	9.52	48.93	2.77	V.
Myxophyceae	4.17	11.13	46.41	2.63	VI.
Plant matter	5.24	5.18	27.14	1.54	VII.
Decay organic matter	0.81	3.20	2.59	0.15	VIII.
Total			$\sum V_1O_1$= 1765.36		
			Rainy		
Crustaccea	24.61	23.03	566.77	37.18	I.
Rotifer	19.17	18.12	347.36	22.79	II.
Insect	18.33	20.02	366.97	24.07	III.
Chlorophyceae	7.62	7.38	56.23	3.69	IV.
Bacillariophyceae	7.83	10.91	85.42	5.60	V.
Myxophyceae	9.08	4.42	40.13	2.64	VI.
Plant matter	8.13	7.13	57.97	3.80	VII.
Decay organic matter	1.52	2.26	3.43	0.23	VIII.
Total			$\sum V_1O_1$= 1524.28		
			Autumn		
Crustaccea	21.32	22.08	505.85	29.54	II
Rotifer	24.91	22.91	570.69	33.33	I
Insect	18.18	17.60	319.97	18.68	III
Chlorophyceae	11.93	14.46	172.51	10.07	IV
Bacillariophyceae	8.55	10.21	87.29	5.11	V
Myxophyceae	9.72	4.09	39.75	2.32	VI
Plant matter	3.58	4.23	15.14	0.88	VII
Decay organic matter	1.17	0.97	1.13	0.07	VIII
Total			$\sum V_1O_1$= 1712.33		
			Winter		
Crustaccea	25.43	19.91	506.31	31.07	I
Rotifer	22.05	20.08	442.76	27.17	II
Insect	18.23	16.86	307.36	18.86	III
Chlorophyceae	11.02	10.74	118.35	7.26	V
Bacillariophyceae	12.43	13.32	165.57	10.17	IV
Myxophyceae	6.60	10.22	67.45	4.14	VI
Plant matter	3.06	5.28	16.16	0.99	VII
Decay organic matter	1.53	3.50	5.35	0.34	VIII
Total			$\sum V_1O_1$= 1629.31		

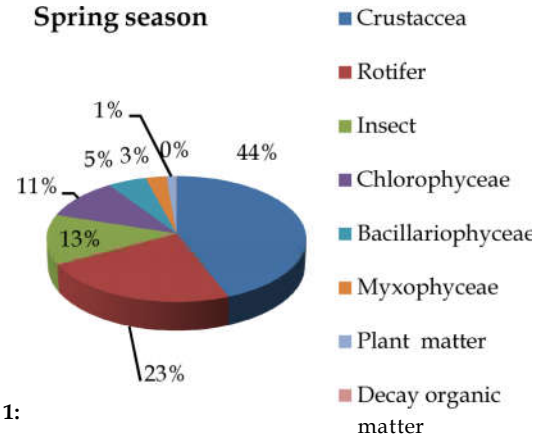


Fig. 1:

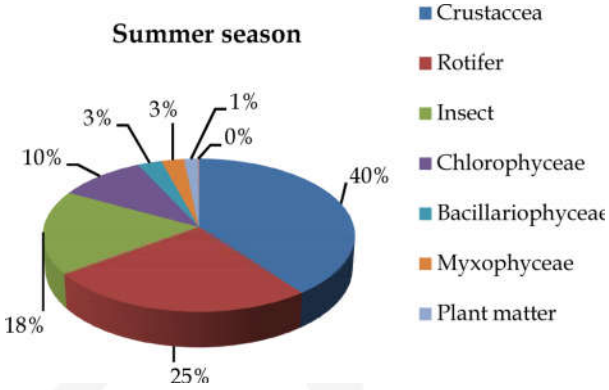


Fig. 2:

Fig. 1 & 2: Gut content and grading of food items of ***Clarias batrachus*** in **spring** & **summer** season.

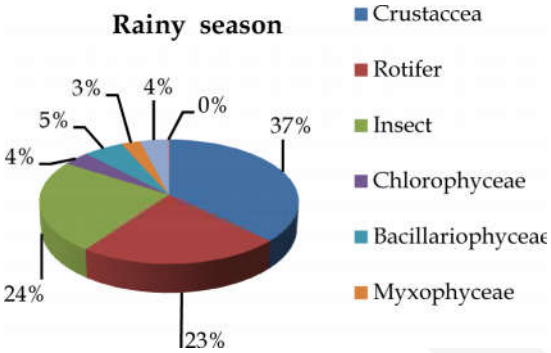


Fig. 3:

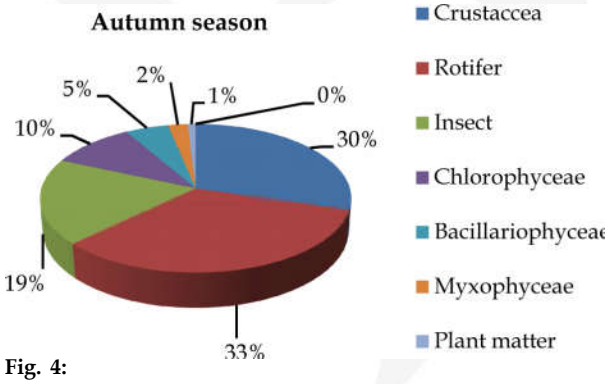


Fig. 4:

Fig. 3 & 4: Gut content and grading of food items of ***Clarias batrachus*** in **Rainy & Autumn** season

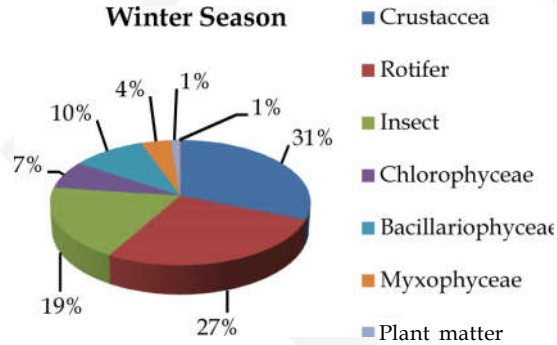


Fig. 5: Gut content and grading of food items of *Clarias batrachus* in Winter season

(11.32) and lowest was seen in Winter (4.88). The highest percentage of Myxophyceae occurred in Winter (5.82%) and lowest in Spring (2.28%). The highest percentage of occurrence of plant matter in the gut content was in Rainy season (July, August and September) is (4.05%) and lowest in Autumn (1.36). The intake of dead and decaying matter of gut content analysis was highest in Winter (4.97%) and lowest in Spring season (0.26%). Seasonal variation showed a slight variation in feeding main food items of *C. punctatus* were crustacea, rotifers and insects.

Gut Content Analysis of *A. Testudineus*

It was clearly seen that ***A.testudineus*** is a carnivorous fish because of they mostly select the crustacea, rotifers, insects and baccillariophyceae. The feeding of crustacea was highest in spring (62.92%) after then rainy (54%), autumn (47.99%), winter (46.26%) and lowest in summer (28.90%) respectively as per Table 3 and Figure 11 and 12. The highest percentage of rotifers are observed in summer season (28.90%) and lowest in spring (8.06%). Insects was the basic food for intake most of time i.e winter (20.30%), spring (18.30%), rainy (15.27%), autumn (8.50%) and least count was seen in summer (7.43%). The highest percentage of Chlorophyceae was accounted in the rainy season (9.18%) and lowest in the winter (0.17) as per Figure 13 and 1 4.

Similarly the baccillariophyceae was highest seen in summer season (20.27%) and least in the rainy season (2.54%). Myxophyceae was the another food items which was seen from highest to lowest are as follow winter (14.27%), autumn(10.26%), summer (9.70%) , rainy(6.66%) and spring(1.48%) respectively. Plant matter was negligible observed

in gut of *Anabas testudineus*. The highest of plant matter was seen in autumn (0.25%) and lowest in winter (0.01) as per Figure in 15 . Dead and decay organic matter was highest seen in summer (4.12%) and lowest in rainy (0.22%).

Table 2: Gut content and grading of various food items of *Channa punctatus*

Food items	% composition of items Volume (V_1)	Occurrence (O_1)	V_1O_1	Preponderance Index (I)	Grading
			Spring		
Crustaccea	20.87	23.36	487.52	35.07	I
Rotifer	22.78	19.65	447.63	32.21	II
Insect	11.03	13.74	151.55	10.90	III
Chlorophyceae	15.54	9.58	148.87	10.72	IV
Bacillariophyceae	14.52	6.34	92.05	6.62	V
Myxophyceae	5.24	6.04	31.65	2.28	VI
Plant matter	3.17	8.51	26.97	1.94	VII
Decay organic matter	2.21	1.65	3.64	0.26	VIII
Total			$\sum V_1O_1$= 1389.88		
			Summer		
Crustaccea	19.81	17.31	342.91	25.62	I
Rotifer	12.39	22.04	273.07	20.40	II
Insect	20.37	11.09	225.90	16.87	III
Chlorophyceae	12.20	16.67	203.37	15.19	IV
Bacillariophyceae	14.27	10.62	151.55	11.32	V
Myxophyceae	6.91	10.87	75.11	5.61	VI
Plant matter	9.68	2.92	28.26	2.11	VIII
Decay organic matter	4.47	8.63	38.57	2.88	VII
Total			$\sum V_1O_1$= 1338.74		
			Rainy		a.
Crustaccea	13.11	14.62	191.67	13.30	III
Rotifer	23.39	20.28	474.35	32.91	I
Insect	18.35	16.78	307.91	21.36	II
Chlorophyceae	8.6	20.32	174.75	12.12	IV
Bacillariophyceae	18.12	9.38	169.96	11.79	V
Myxophyceae	7.23	6.68	48.29	3.35	VII
Plant matter	8.06	7.23	58.27	4.05	VI
Decay organic matter	3.34	4.81	16.06	1.12	VIII
Total			$\sum V_1O_1$= 1441.26		
			Autumn		
Crustaccea	18.41	12.44	229.02	14.83	III
Rotifer	20.18	15.09	304.52	19.72	II
Insect	21.74	28.55	620.68	40.19	I
Chlorophyceae	11.88	13.18	156.58	10.13	IV
Bacillariophyceae	10.36	11.06	114.58	7.42	V
Myxophyceae	9.97	7.19	71.68	4.64	VI
Plant matter	3.8	5.53	21.01	1.36	VII
Decay organic matter	3.75	7.04	26.4	1.71	VIII
Total			$\sum V_1O_1$= 1544.47		
			Winter		
Crustaccea	17.31	13.69	236.97	16.22	II
Rotifer	16.24	11.74	190.66	13.05	III
Insect	18.72	34.81	651.64	44.59	I
Chlorophyceae	14.14	7.35	103.93	7.11	IV
Bacillariophyceae	8.51	8.38	71.31	4.88	VI
Myxophyceae	9.36	9.08	84.99	5.82	V
Plant matter	7.38	6.67	49.22	3.37	VIII
Decay organic matter	8.63	8.41	72.58	4.97	VII
Total			$\sum V_1O_1$=1461.3		

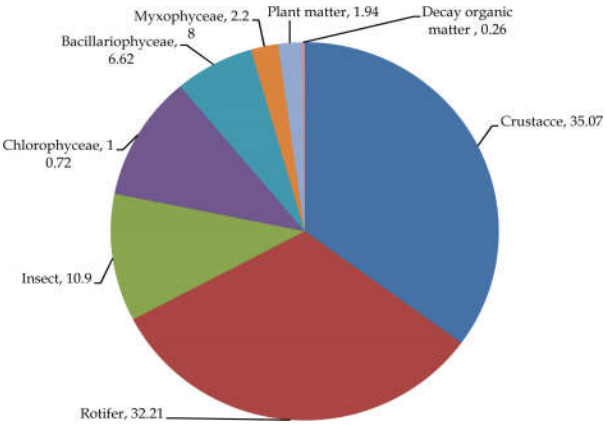


Fig. 6:

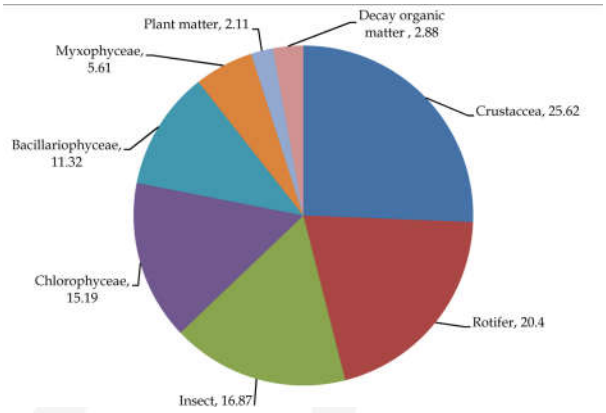
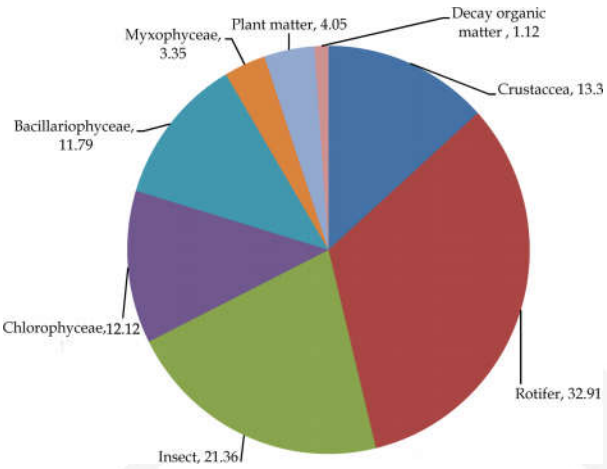


Fig. 7:

Fig. 6 & 7: Gut Content and grading of food items of ***Channa punctatus*** in **spring & Summer** Seasons



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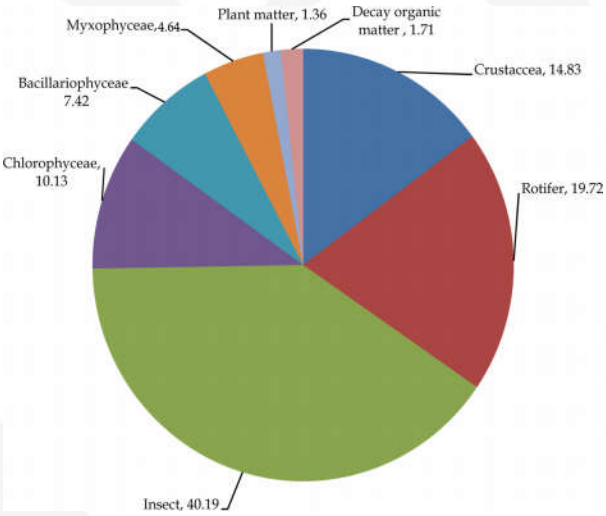

Fig. 9:

Fig. 8 & 9: Gut content and grading of food items of ***Channa punctatus*** in **Rainy &Autumn** Seasons

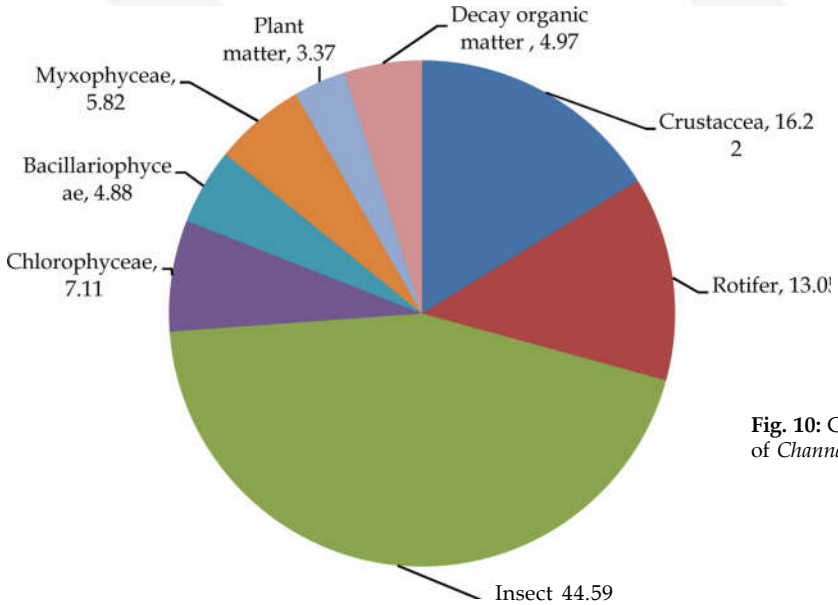

Fig. 10: Gut content and grading of food items of *Channa punctatus* in Winter

Table 3: Gut content and grading of various food items of *Anabas testudineus*

Food items	% composition of items		V_1O_1	Preponderance Index (I)	Grading
	Volume (V_1)	Occurrence (O_1)			
			Spring		
Crustaccea	22.44	53.21	1194.03	62.92	I
Rotifer	17.32	8.83	152.93	8.06	III
Insect	18.17	19.11	347.23	18.30	II
Chlorophyceae	3.38	5.47	18.49	0.97	VIII
Bacillariophyceae	21.54	6.61	142.38	7.50	IV
Myxophyceae	9.27	3.02	27.99	1.48	V
Plant matter	4.08	0.93	3.79	0.20	VI
Decay organic matter	3.81	2.84	10.82	0.57	VII
Total			$\sum V_1O_1$= 1897.66		
			Summer		
Crustaccea	13.65	29.93	408.54	28.90	I
Rotifer	17.54	16.48	289.06	20.45	II
Insect	7.83	13.41	105	7.43	VI
Chlorophyceae	18.21	7.08	128.93	9.12	V
Bacillariophyceae	16.42	17.45	286.53	20.27	III
Myxophyceae	14.77	9.28	137.06	9.70	IV
Plant matter	2.36	0.06	0.14	0.01	VIII
Decay organic matter	9.22	6.32	58.27	4.12	VII
Total			$\sum V_1O_1$=1413.53		
			Rainy		a.
Crustaccea	21.45	43.5	933.07	54	I
Rotifer	18.39	11.33	208.36	12.06	III
Insect	26.04	10.13	263.78	15.27	II
Chlorophyceae	11.28	14.06	158.60	9.18	IV
Bacillariophyceae	10.03	4.37	43.83	2.54	VI
Myxophyceae	9.58	12.01	115.05	6.66	V
Plant matter	0.39	3.29	1.28	0.07	VIII
Decay organic matter	2.87	1.32	3.79	0.22	VII
Total			$\sum V_1O_1$= 1727.76		
			Autumn		
Crustaccea	15.32	44.23	677.60	47.99	I
Rotifer	16.04	11.42	183.18	12.97	III
Insect	12.81	9.37	120.03	8.50	V
Chlorophyceae	9.70	1.5	14.55	1.03	VII
Bacillariophyceae	13.28	18.61	247.14	17.50	II
Myxophyceae	11.19	12.93	144.69	10.26	IV
Plant matter	6.04	0.58	3.50	0.25	VIII
Decay organic matter	5.63	1.36	21.25	1.50	VI
Total			$\sum V_1O_1$= 1411.94		
			Winter		
Crustaccea	18.34	42.8	784.95	46.26	I
Rotifer	16.32	10.54	172.01	10.14	IV
Insect	24.06	14.32	344.54	20.30	II
Chlorophyceae	2.42	1.21	2.93	0.17	VII
Bacillariophyceae	12.29	10.47	128.67	7.58	V
Myxophyceae	13.21	18.33	242.14	14.27	III
Plant matter	4	0.05	0.2	0.01	VIII
Decay organic matter	9.36	2.3	21.53	1.27	VI
Total			$\sum V_1O_1$=1696.97		

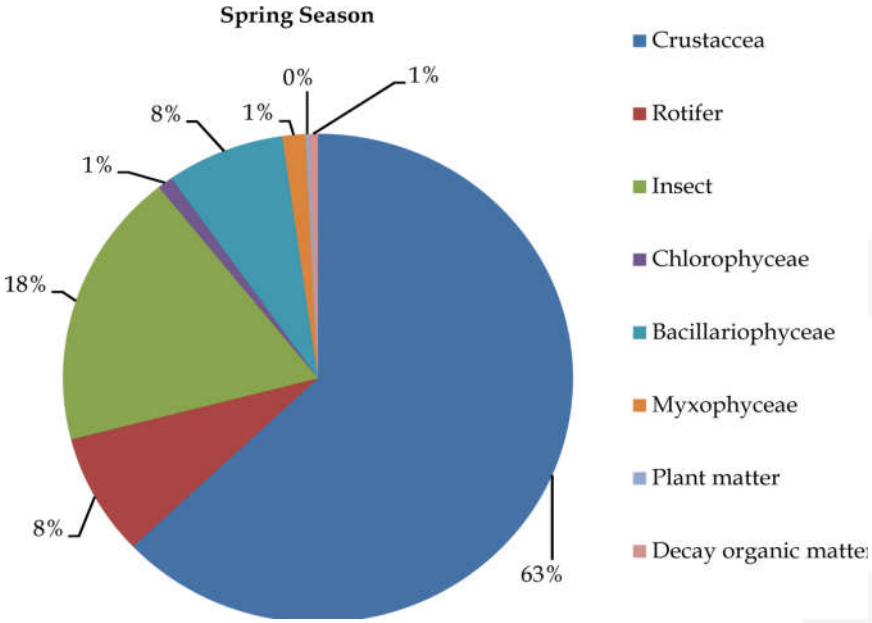

Fig. 11:

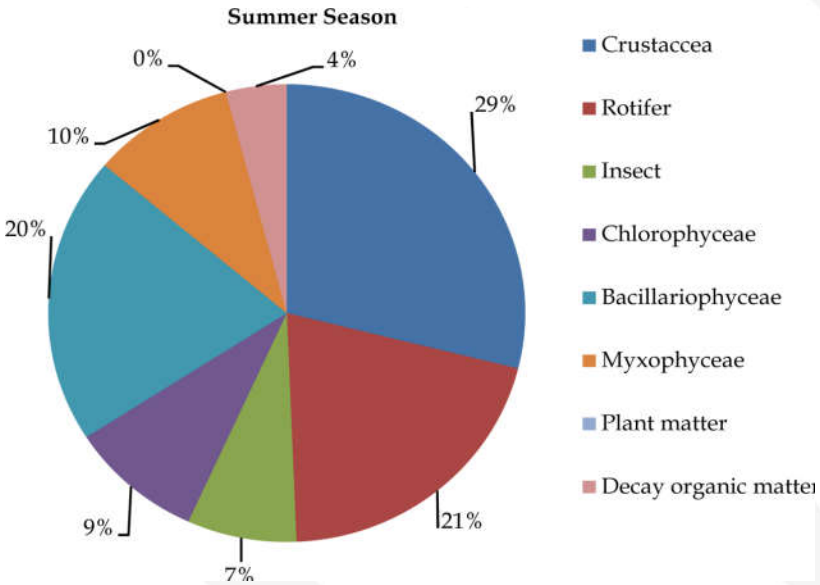

Fig. 12: Gut content and grading of food items of ***Anabas testudineus*** in **Spring & Summer**

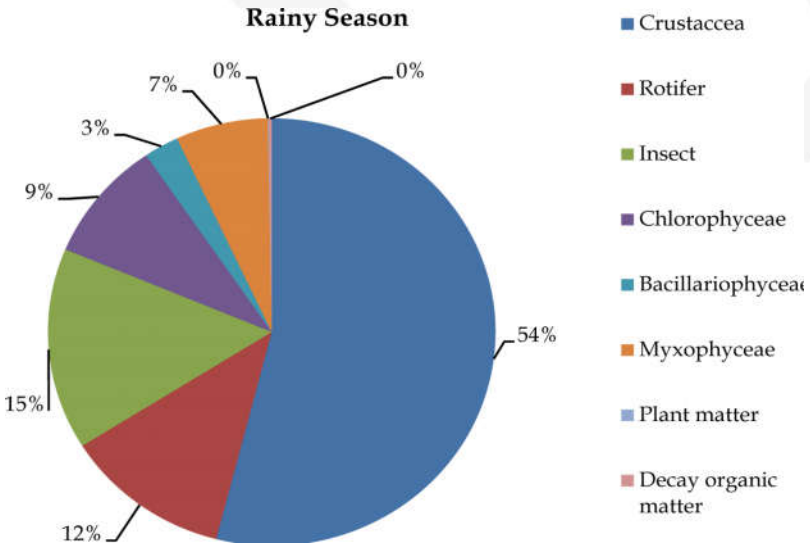

Fig. 13: Gut content and grading of food items of ***Anabas testudineus*** in **Rainy & Autumn**

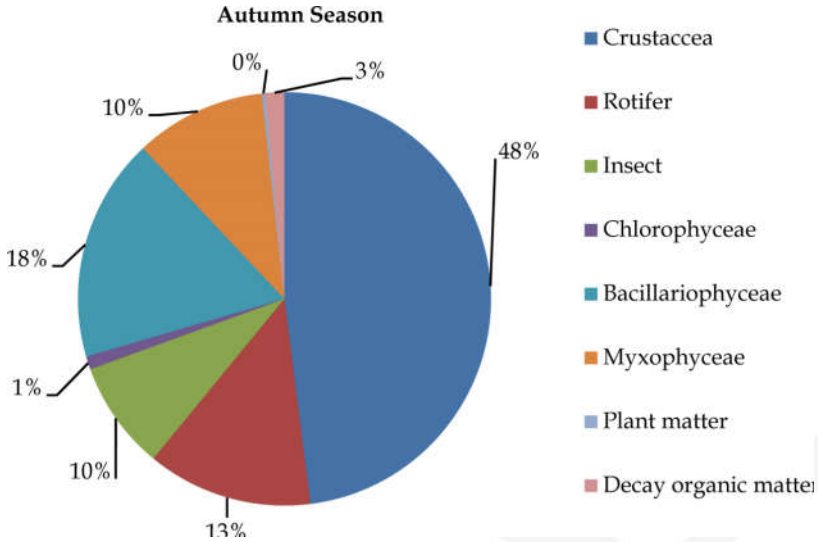

Fig. 14: Gut content and grading of food items of ***Anabas testudineus*** in **Rainy & Autumn**

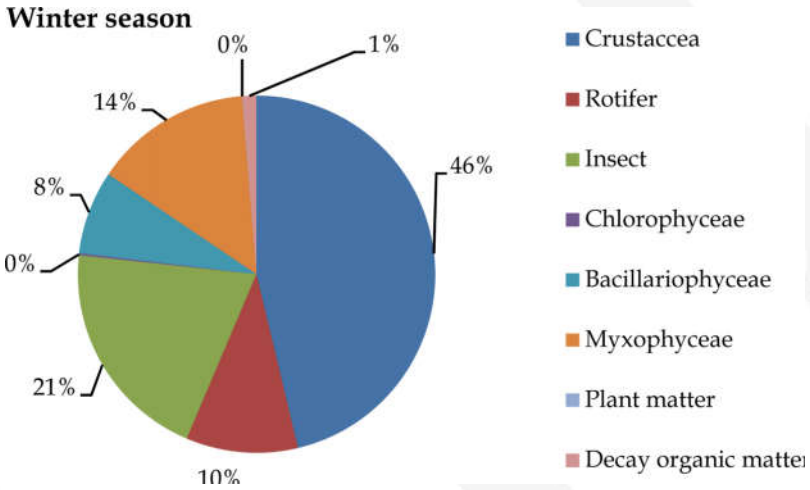

Fig. 15: Gut content and grading of food items of ***Anabas testudineus*** in **Winter**

Discussion

Hora and Pillay (1962) assigned *Clarias batrachus* as a plankton and detritus and reported that this fish consume primarily phytoplankton and zooplankton, *Clarias batrachus* feed mainly on crustacea, algae, rotifers and some insects and hence he categorized the same as plankton feeder. The present observations are in consonance with these food items as reported by earlier workers the *Claris batrachus* is planktophagus and feeds primarily on zooplanktons. David *et. al.*, (2010) observed that the presence of tiny unicuspid teeth in the mouth of the fish suggests that fish species feed on plants, leaves, buds and seeds of water lilies and are thus herbivorous feeders.

Smita *et. al.*,(2012) detailed discussed about feeding habits of *Channa punctatus* is mainly based on the plant matter. The fish is a bottom and water column feeder and less adapted to take zooplankton. Gut content analysis revealed by *Channa punctatus* depend on the vegetable matter. Unidentifiable plant matter formed the major items. Other items was green algae, filamentous algae, detritus and sand particles. The present finding is in agreement with that of earlier work Indranil *et. al.*, (2016) while *Anabas testudineus* feeds on detritus, phytoplankton as well as zooplankton with a narrow range of food varieties. It is a bottom feeder subsisting mainly on decayed vegetation. It is in the line with observation earlier made by Ramesh *et.al.*, (2016). *Channa punctatus* feed on higher percentage of

crustaceans, insects, molluscs, fishes and sand and mud particles and lowest percentage of plant material. Smaller fishes and their larvae were dominant food items thus making as carnivorous.

Similar results were also reported by Roy et.al (2013) in *Anabas testudineus.* Bhowmick (1965) on *Glossogobius guiris* also reported similar results. M. Nazrul Islam *et. al.*(2004)., revealed that the *Channa punctatus* feeds on animal foods (crustaceans, molluscs, insects and fishes).

Bhuiyan *et al.(*2006) reported very poor feeding intensity in mature species of *Channa punctatus* during May to July. Saikia *et al.* (2012) also reported low feeding intensity of *Channa punctatus* in June-July and November-January.The increase in number of empty stomachs during the rainy season could be attributed to the short feeding periods observed at this time due to the reduced low tide duration. This fish is found to be much active on the mud flats at low tide.

Conclusion

The results indicate the species fall in the carnivorous category. There is also variation in the percentage composition of different items of food in the gut in different months. It can be inferred that *C. punctatus* changes its food habit with the change in seasons.

Index of Preponderance of various food compositions in the gut of *C. punctatus* indicated that fish was the most dominant food item in the gut, followed by the insect, crustacean, plant matter mucks and unidentified materials, annelids and molluscs. The different type of food items such as protozoans, crustaceans, insects, algae etc. was very common in the month of October to December in *A. testudineus* while these food items was very common in the month of July to September. The young fishes were found to prefer insect pupae and these fishes fed during day time.

Acknowledgments

Authors are thankful to the Head, Department of Zoology, Berhampur University for providing the lab facilities for the research work. It is a great pleasure to acknowledge with most honoured and duly respected; we owe particular thanks to all those researchers and their works cited in this piece of work, and also most grateful to all persons who have helped or assisted in one way or the other in the course of carrying out this study successfully.

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Review Article

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Birds Around Mula River Right Bank Canal in Ahmednagar District of Maharashtra (India)

Prabhat Sunil Mhaske

Abstract

Ornithology is a Greek word: ornitha means chicken and logos mean a science. It is a branch of Zoology or Biology concerned with the scientific study of birds, creatures belonging to class Aves. (1) Aristotle was perhaps the first person who wrote on ornithology and mentions more than 170 birds. (2) Carolus Linnaeus (1758) was the pioneer in developing a classification system for birds and animals. His scientific classification system, with some modification is still being used. Dr Salim Ali (1896 - 1987) was India's most well-known Ornithologist and bird watcher. He is known as the "Birdman of India". (3) India is one of the best places in the world to see the birds. The extant and recently extinct species recorded within the political limits of the Republic of India as defined by the Indian government are known to have around 1266 species as of 2016.(4) I have taken the pictures of native Birds around Mula River Right Bank Canal in Ahmednagar district of Maharashtra (India). While doing these photographs I tried to study the scientific knowledge regarding them. One alarming signal came in my mind that these birds are not cared by anybody, they are totally ignored, there population and species may be in downhill. There are so many species of bird in same area, few of them I have mentioned in the study.

Keywords: Birds; Data; Mula River Right Bank; Population.

Introduction

Ornithology is a Greek word: ornitha means chicken and logos mean a science. It is a branch of Zoology or Biology concerned with the scientific study of birds, creatures belonging to class Aves [1]. Aristotle was perhaps the first person who wrote on ornithology and mentions more than 170 birds [2].

Carolus Linnaeus (1758) was the pioneer in developing a classification system for birds and animals. His scientific classification system, with some modification is still being used. Dr Salim Ali (1896 - 1987) was India's most well-known Ornithologist and bird watcher. He is known as the "Birdman of India" [3].

India is one of the best places in the world to see the birds. The extant and recently extinct species recorded within the political limits of the Republic of India as defined by the Indian government are known to have around 1266 species as of 2016 [4].

I have taken the pictures of native Birds around Mula River Right Bank Canal in Ahmednagar district of Maharashtra (India). While doing these photographs I tried to study the scientific knowledge regarding them. One alarming signal came in my mind that these birds are not cared by anybody, they are totally ignored, there population and species

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may be in downhill. There are so many species of bird in same area, few of them I have mentioned below:

Ashy Prinia (ashy wren) [5]

- *Scientific Classification*
  - ⇨ Kingdom: Animalia
  - ⇨ Phylum: Chordata
  - ⇨ Class: Aves
  - ⇨ Order: Passeriformes
  - ⇨ Family: Cisticolidae
  - ⇨ Genus: Prinia
  - ⇨ Species: P. socialis
- Small warbler.
- Resident breeder in the India, Nepal, Bangladesh, Bhutan, Sri Lanka and western Myanmar.
- Common bird in urban gardens and farmland.
- Small size, distinctive colors and upright tail.
- Distinct breeding and non-breeding plumage.
- 13–14 cm length.
- Short rounded wings
- Longish graduated cream tail tipped with black sub terminal spots.
- Gray crown, Strong legs.
- Found single or in pairs.
- Insectivorous.
- Call- repetitive tchup, tchup, tchup or zeet-zeet-zeet and nasal tee-tee-tee.
- Builds its nest close to the ground in a shrub or tall grass and lays 3–5 eggs.
- Eggs hatch in 12 days.
- Breeding season after the monsoons.
- Both the male and the female take part in incubation.

Pied myna (Asian pied starling) [6]

- Scientific classification-
  - ⇨ Kingdom: Animalia
  - ⇨ Phylum: Chordata
  - ⇨ Class: Aves
  - ⇨ Order: Passeriformes
  - ⇨ Family: Sturnidae
  - ⇨ Genus: Gracupica
  - ⇨ Species: Gracupica contra
- Found in the Indian subcontinent and Southeast Asia.
- Marked in black and white and has a yellowish bill with a reddish bill base.
- Have bare skin around eye is reddish.
- Upper body, throat and breast are black.
- Cheek, lores, wing coverts and rump are contrastingly white.
- Both sexes are similar in plumage
- Young birds have dark brown in place of black.
- Flight is slow and butterfly-like on round wings.
- Areas with access to open water.
- Found in small groups,
- Call -whistles, trills, buzzes, clicks, and warbling calls.

- Both sexes sing.
- Forage in fields, lawns and on open ground feeding on grains, fruit, insects, earthworms and mollusks.
- Breeding season in India is spread from March to September
- Nest in a large tree (banyan, mango, jackfruit, rosewood)
- Clutch: four to six glossy blue eggs.
- Eggs hatch after 15 days.

Baya weaver [7]

- Scientific classification-

  Name- Ploceus philippinus

  Kingdom: Animalia

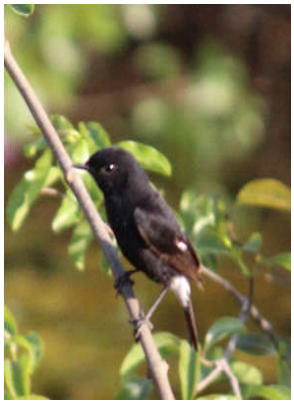
  Phylum: Chordata

  Class: Aves

  Order: Passeriformes

  Family: Ploceidae

  Genus: Ploceus
- Common, Weaver bird
- Found in Indian Subcontinent and Southeast Asia.
- Social and gregarious birds
- Known for their hanging retort shaped nests woven from leaves.
- Sparrow-sized (15 cm)
- Both males and females resemble female house sparrows.
- Have a stout conical bill and a short square tail.
- Non-breeding males and females- look alike, dark brown streaked fulvous buff above, plain (unstreaked) whitish fulvous below, eyebrow long and buff colored, bill is horn colored and no mask.
- Breeding males -bright yellow crown, dark brown mask, blackish brown bill, upper parts are dark brown streaked with yellow, with a yellow breast and cream buff below.
- Feed on wild grasses such as Guinea grass, insects, butterflies, small frogs, geckos and mollusks.
- Calls are a continuous chit-chit.
- Wheezy cheee-eee-ee - produced by males in a chorus
- breeding season of the baya -monsoons
- Nests-
  - ⇨ Nest colonies are usually found on thorny trees or palm fronds
  - ⇨ Nests are built near water or hanging over water where predators cannot reach easily.
  - ⇨ Nest in colonies up to 20-30, close to the source of food, nesting material and water.
  - ⇨ Baya weavers are woven nests constructed by the males.
  - ⇨ Are pendulous, retort-shaped
  - ⇨ With a central nesting chamber and a long vertical tube that leads to a side entrance to the chamber.
  - ⇨ Woven with long strips of paddy leaves, rough grasses and long strips torn from palm fronds
  - ⇨ Males take about 18 days to construct the complete nest.
  - ⇨ Partially built before the males begin to display to passing females by flapping their wings and calling while hanging from their nests
  - ⇨ Females inspect the nest and signal their acceptance of a male
  - ⇨ Once a male and a female are paired, the male goes on to complete the nest by adding the entrance tunnel.
  - ⇨ Males are solely in charge of nest building.
  - ⇨ Female partners may join in giving the finishing touches, particularly on the interiors.
  - ⇨ Females prefer nests high in trees, those over dry land, and those on thin branches.
  - ⇨ Males build many partial nests and begin courting females.
  - ⇨ Male finishes the nest only after finding a mate.
- Both males and females are polygamous.
- Female lays 2 to 4 white eggs and incubates them for 14 to 17 days
- Chicks leave the nest after 17 days.
- After mating with a female the male typically court other females at other partially constructed nests.
- Females are capable of breeding after a year while males take half a year longer
- Extremely intelligent, obedient and docile.

Oriental magpie-robin [8]

- Scientific classification-
  Kingdom: Animalia
  Phylum: Chordata
  Class: Aves
  Order: Passeriformes
  Family: Muscicapidae
  Genus: Copsychus
  Species- C. saularis
- National bird of Bangladesh.
- Small passerine bird
- Distinctive black and white birds with a long tail that is held upright as they forage on the ground or perch conspicuously.
- Occurs in Indian subcontinent and Southeast Asia
- Common birds in urban gardens as well as forests.
- Known for their songs.
- Long tail-held cocked upright.
- Male - black upperparts, head and throat apart from a white shoulder patch with White under parts and the sides of the long tail.
- Females - grayish black above and grayish white
- Young birds have scaly brown upperparts and head
- Breed -March to July in India
- Males sing from high perches during courtship.
- Display of the male -puffing up the feathers, raising the bill, fanning the tail and strutting.
- nest in tree hollows or niches in walls or building,
- Eggs are incubated by the female alone for 8 to 14 days.
- "Little concern" globally.

Siberian stonechat (Asian stonechat) [9]

- Scientific classification-
  Kingdom: Animalia
  Phylum: Chordata
  Class: Aves
  Order: Passeriformes
  Family: Muscicapidae
  Genus: Saxicola
  Species: Saxicola maurus
- widespread and common, found in Asia, Siberia south to the Himalaya, China, Turkey and Russia
- Five or six subspecies.
- Insectivorous.
- Darker above and paler below
- Male- during breeding plumage has black upperparts and head with white collar, scapular patch and rump, and a restricted area of orange on the throat
- Female: pale brown upperparts and head, white neck patches (not a full collar), and a pale, unstreaked pinkish-yellow rump.
- Male has a clicking call.

Indian roller [10]

- Scientific classification-

  Kingdom: Animalia

  Phylum: Chordata

  Class: Aves

  Order: Coraciiformes

  Family: Coraciidae

  Genus: Coracias

  Species: Coracias benghalensis
- Very common in the populated plains of India
- Hindi name is neelkanth-blue throat-a name associated with Shiva
- Found in Iraq, Arabia and India
- Used as caught and released during festivals such as Dussera
- State bird of Andhra Pradesh, Odisha, Karnataka and Telangana
- Member of the roller family of birds, not migratory,
- Subspecies – 1)C. b. benghalensis 2)Southern roller (C. b. indicus) 3) Burmese roller (C. b. affinis)
- Stocky bird about 26–27 cm long
- Breast is brownish, crown and vent are blue.
- Tail is sky blue with a terminal band of Prussian blue and the central feathers are dull green.
- Neck and throat -purplish lilac with white shaft streaks.
- Bare patch around the eye is ochre in colour.
- Long and compressed bill with a curved upper edge and a hooked tip
- Seen on bare trees or wires.
- Call of the Indian roller is a harsh crow-like chack sound.
- Capture their prey which may include insects, arachnids, small reptiles, small snakes and amphibians.
- Breeding season- March to June
- Clutch: 3-5 eggs.
- Both sexes incubate the eggs for 17 to 19 days.

Indian silverbill [11]


Indian silverbill or white-throated munia

- Scientific classification-

  Kingdom: Animalia

  Phylum: Chordata

  Class: Aves

  Order: Passeriformes

  Family: Estrildidae

  Genus: Euodice

  Species: Euodice malabarica
- Small passerine bird
- Occurs in Pakistan, Nepal, Bangladesh, India, Sri Lanka, Iran and Israel
- Adult Indian silverbill is 11–11.5 cm long
- Conical silver-grey bill, buff-brown upperparts, white underparts, buffy flanks and dark wings. tail is black and the wings are dark
- Sexes - similar
- Feeds on seeds, insects , nectar bearing flowers
- Frequents dry open scrub, fallow land and cultivation, near water
- Constantly utter a low cheeping or chirping contact call as they forage
- Nest in winter in southern India and after summer in northern India.
- Clutch -4 to 8 white eggs

Lark [12]


- Scientific classification-

  Kingdom: Animalia

  Phylum: Chordata

  Class: Aves

  Order: Passeriformes

  Superfamily: Passeroidea

  Family: Alaudidae Vigors,
- Passerine birds of family Alaudidae.
- Twenty-one extant genera in the family Alaudidae
- Small- to medium-sized birds, 12 to 24 cm in length and 15 to 75 g in weight.

- Ground birds, brown plumage
- Most species build nests on the ground
- Larks incubate for 11 to 16 days.
- Kept as pets in China.

Indian peafowl [13]

- Scientific classification
  - ⇨ Kingdom: Animalia
  - ⇨ Phylum: Chordata
  - ⇨ Class: Aves
  - ⇨ Order: Galliformes
  - ⇨ Family: Phasianidae
  - ⇨ Genus: Pavo
  - ⇨ Species: Pavo cristatus
- National bird of India in 1963.
- Celebrated in Indian and Greek mythology
- Krishna is depicted with a feather in his headband
- Kartikeya (also known as Skanda or Murugan).
- Resident breeder India.
- Large and brightly colored bird
- Capable of flight
- Lives mainly on the ground in open forest or on land under cultivation
- Feeds on berries, grains but also prey on snakes, lizards, and small rodents. loud calls
- Male:
  - ⇨ Blue with a fan-like crest of spatula-tipped wire-like feathers elongated upper-tail covert feathers which bear colorful eyespots.
  - ⇨ Metallic blue on the crown.
  - ⇨ Feathers of the head being short and curled.
  - ⇨ Fan-shaped crest on the head is made of feathers with bare black shafts and tipped with bluish-green webbing.
  - ⇨ White stripe above the eye
  - ⇨ Crescent shaped white patch below the eye are formed by bare white skin.
  - ⇨ Sides of the head have iridescent greenish blue feathers.
  - ⇨ Back has scaly bronze-green feathers with black and copper markings.
  - ⇨ Wings are buff and barred in black and tail is dark brown
  - ⇨ Feathers end with an elaborate eye-spot.
  - ⇨ Thighs are buff colored.
  - ⇨ Length: bill to tail -100 to 115 cm and weight: 4–6 kg

  Peahen:
  - ⇨ Smaller and lack the train
  - ⇨ Have a greenish lower neck
  - ⇨ duller brown plumage
  - ⇨ Lenghth: 95 cm and weight- 2.75–4 kg
  - ⇨ Rufous-brown head
  - ⇨ Tips are chestnut edged with green
  - ⇨ Upper body is brownish with pale mottling and tail are dark brown.
  - ⇨ Lower neck is metallic green
  - ⇨ Breast feathers are dark brown glossed with green
- Calls - loud pia-ow or may-awe.
- Frequency of calling increases before Monsoon
- Calls indicate the presence of predators such as tiger.
- Several color mutations of Indian peafowl.
- Peafowl forage on the ground in small groups- cock and 3 to 5 hens.
- Peafowl roost in groups during the night on tall trees
- Peacocks are polygamous
- Breeding season is spread out but appears to be dependent on the rains.
- Peafowl are omnivorous and eat seeds, insects, fruits, small mammals and reptiles, small snakes
- Lives for 23 years
- Listed as of Least Concern by the International Union for Conservation of Nature.

Kingfisher [14]

- Scientific classification-

  Kingdom: Animalia

  Phylum: Chordata

  Class: Aves

  Order: Coraciiformes

  Family: Alcedinidae

  Subfamily: Alcedininae
- Known as Eurasian kingfisher, and river kingfisher
- Widely distributed over Europe, Asia, and North Africa
- Important members of ecosystems and good indicators of freshwater community health.
- Seven subspecies -A. a. ispida Linnaeus, A. a. atthis, A. a. bengalensis Gmelin, A. a. taprobana Kleinschmidt, A. a. floresiana Sharpe, A. a. hispidoides Lesson A. a. solomonensis Rothschild and Hartert
- Sparrow-sized bird, short-tailed, large-head
- Blue upperparts, orange under parts and a long bill.
- Legs and feet are bright red.
- Feeds on fish, caught by diving
- Length- 16 centimeters and weight 34–46 grams.
- Female is identical in appearance to the male.
- Flight of the kingfisher is fast, direct and usually low over water.
- Has no song.
- Nest is in a burrow excavated by both birds of the pair in a low vertical riverbank.
- Lays two to ten glossy white eggs.

Purple heron [15]

- Wading bird in the heron family
- Total Population- 270,000 and 570,000 purple herons in the world
- Scientific classification-

  Kingdom: Animalia

  Phylum: Chordata

  Class: Aves

  Order: Pelecaniformes

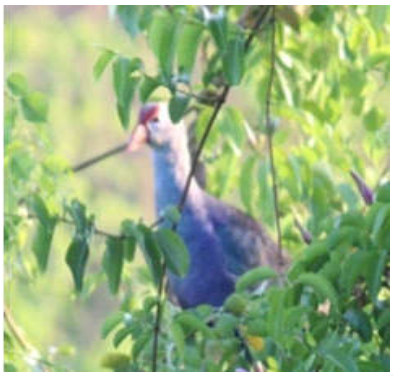
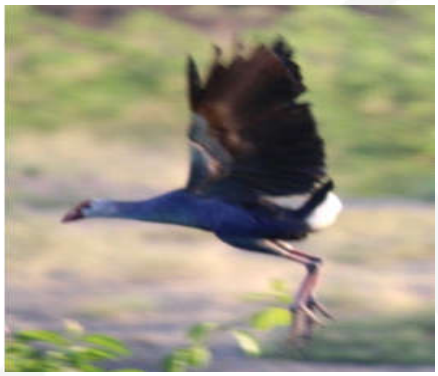
  Family: Ardeidae

  Genus: Ardea

  Species: A. purpurea
- Breeds in Africa, central and southern Europe, and southern and eastern Asia.
- Large bird.
- Length: 78–97 cm , height: 70 to 94 cm , wingspan : 120–152 cm , Weight: 0.5 to 1.35 kg
- Adults bird:
  - ⇨ Forehead and crown of the head is black
  - ⇨ Dark stripe down the back of the neck that terminates in a slender, dangling crest.
  - ⇨ Head and the neck are buffish chestnut with dark streaks and lines down either side of the whole the neck.
  - ⇨ Upper parts and tail is brownish grey.
  - ⇨ Breast: chestnut brown,
  - ⇨ Beak: brownish-yellow , long, straight and powerful
  - ⇨ Iris : yellow
  - ⇨ Legs :brown at the front and yellowish behind
- Call is a harsh "frarnk"- more high-pitched, less noisy bird,
- Migrates in August - October and returns in March.

- Inhabits marshes, lagoons and lakes surrounded by dense vegetation.
- *Flight:* slow with neck retracted and legs extending a long way behind tail.
- Diet: fish, small mammals, amphibians, nestling birds, snakes, lizards, crustaceans, water snails, insects and spiders.
- Breeds in colonies and builds a bulky nest of dead reeds, sticks.
- Eggs are bluish-green, 56 by 45 mm, clutch is four to five eggs.
- Both parents share incubation (24 and 28 days)
- Population is decreasing slowly.

Purple swamp hen [16]


Species

1. Western swamphen, Porphyrio porphyrio, southwest Europe and northwest Africa
2. African swamphen, Porphyrio madagascariensis, sub-Saharan continental Africa and Madagascar
3. Grey-headed swamphen, Porphyrio poliocephalus, Middle East, through the Indian subcontinent to southern China and northern Thailand
4. Black-backed swamphen, Porphyrio indicus, southeast Asia to Sulawesi
5. Philippine swamphen, Porphyrio pulverulentus, Philippine islands
6. Australasian swamphen, Porphyrio melanotus, Australia, New Zealand, and Oceania

- Scientific classification-

  Kingdom: Animalia

  Phylum: Chordata

  Class: Aves

  Order: Gruiformes

  Family: Rallidae

  Genus: Porphyrio

  Species: P. poliocephalus
- Occurs in Middle East, Indian subcontinent to southern China and northern Thailand.
- Male has an elaborate courtship display, holding water weeds in his bill and bowing to the female with loud chuckles.

Red muniya [17]

- Red avadavat or strawberry finch
- Scientific classification
  - ⇨ Kingdom: Animalia
  - ⇨ Phylum: Chordata
  - ⇨ Class: Aves
  - ⇨ Order: Passeriformes
  - ⇨ Family: Estrildidae
  - ⇨ Genus: Amandava
  - ⇨ Species: A. amandava
- Sparrow-sized bird
- Found in Bangladesh, India, Sri Lanka, Nepal, Pakistan, Spain, Brunei, Fiji, Egypt, Malaysia, Portugal, Puerto Rico, Singapore and Hawaii.

- Red munia diverged from the green munia about 9 million years ago.
- Habitat in the open fields mainly on flat plains, tall grasses or crops, near water
- Rounded black tail and bill is seasonally red.
- Rump is red and breeding male is red on most of the upper parts except for a black eye-stripe, lower belly and wings.
- White spots on the red body and wing feathers.
- Non-breeding male is duller, has the red-rump.
- Female -dull with less of the white spotting on the feathers
- Pairs stay together during the breeding season
- Call-low single note pseep given in flight.
- Feeds on grass seeds , insects -termites
- Build a globular nest made of grass blades.
- Clutch- five or six white eggs
- Beak -turn red in May, darkens in November-December, turns black in April and the cycle continues.

Wire-tailed swallow [18]

- Scientific classification-
  - ⇨ Kingdom: Animalia
  - ⇨ Phylum: Chordata
  - ⇨ Class: ves
  - ⇨ Order: Passeriformes
  - ⇨ Family: Hirundinidae
  - ⇨ Species: Hirundo smithii
- Small passerine bird in swallow family.
- Two subspecies:
  - ⇨ H. S. smithii (Africa)
  - ⇨ H. S. filifera (southern and southeastern Asia)
- Found in open country near water and human habitation.
- 18 cm in length, fast flyers.
- Bright blue upperparts, bright white underparts and a chestnut cap.
- Very long filamentous outermost tail feathers, which trail behind like two wires
- Immature birds lack tail wires.
- Both sexes are similar in appearance but female has shorter wires.
- Juveniles have a brown crown, back and tail. The Asian form, H.S. filifera, is larger and longer-tailed than the abundant African H.S. Smithii.
- Feed on insects and flies.
- Half-bowl nests are lined with mud collected in the swallows' beaks.
- Clutch-three to four eggs.

White-browed wagtail [19]

White-browed wagtail, large pied wagtail, dhobin, and washerwoman

- Scientific classification-
  - ⇨ Kingdom: Animalia
  - ⇨ Phylum: Chordata
  - ⇨ Class: Aves
  - ⇨ Order: Passeriformes
  - ⇨ Family: Motacillidae
  - ⇨ Genus: Motacilla
  - ⇨ Species: Motacilla maderaspatensis
- Medium-sized bird, 21 cm length.
- Largest member of wagtail family.
- Slender bird, long, constantly wagging tail
- Black upperparts, head and breast, with a white supercilium and large white wingbar

- Female has the black less intense than in the male
- Juveniles are like the females brown-grey where the adult is black
- Resident breeder in India , found in open freshwater wetland habitats.
- Seen in pairs or small groups near open water
- Call in the morning's wheezy "wheech".
- Breeding season -March to October.
- cup-shaped nest placed on the ground or rocks in a hole, ledge or mud bank
- Nest is made of grass, roots, algae and located close to water.
- Clutch-Three to five eggs.
- While taking this photographs and collecting information, I came to know very important question –Why the birds are in danger zone? The following causes I get from various sources, which everyone has to think in positive way.
  - ⇨ Changes in the physical environment
  - ⇨ Habitat fragmentation
  - ⇨ Chemical contamination
  - ⇨ Exploitation of birds for human use
  - ⇨ Trafficking
  - ⇨ Rivers getting flooded during breeding season nests in colonies on low sandbanks are destroyed.
  - ⇨ Non-native bird species have become invasive for many native bird species.

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Review Article

El-Nino and its Connection with Indian Monsoon

Ravi Kiran

Abstract

The unpredictable nature of monsoon affects directly the Indian economy. Earlier El Nino phenomena was thought to be affecting only the west coast of South America but now it is linked to Indian monsoon. El Nino refers to all the conditions associated with warmer than normal water in the Tropical Pacific region. The La-Nina is just opposite to El Nino. La Nina favors monsoon in India but El Nino does not. The accurate prediction of the El-Nino effect will certainly help the planners for making correct food policies in future.

Keywords: El-Nino; La-Nina.

Introduction

Sun is the only source of energy for driving all natural process on the earth. The uneven heating of earth causes general circulation of the ocean and atmosphere and determines broadly the annual climatic condition. Majority of the atmospheric circulations occurs in troposphere. The tropical atmospheric circulation is extended up to 50% or more of the earth's surface. Ocean currents also play important role in determining the weather conditions of atmosphere. The cool oceanic currents favour the stable weather and warm currents favors the instability in the weathers. The vagaries of southwest monsoon are well known for its influence on Indian economy. In recent years erratic dry and wet spells of weather in many parts of the world have drawn our attentions of the ocean on weather. Many experts have expressed their view that the weak monsoon activities over India in some years may be attributed to the famous El-Nino effect.

El Nino and Indian Monsoon

The Indian Meteorological Department, in its first long-range forecast for 2015 monsoon season predicted only 93 per cent of the "normal" rainfall during the season, due to El Nino phenomena. The IMD said parts of the northwest and central India are likely to be affected the most with less rainfall. This is a typical El Nino feature where northwest India and central India will receive less rainfall. There are 70 per cent chances that El Nino will continue during this monsoon, DS Pai, Head of the IMD's Long Range Forecast department, said. This is be the second consecutive year that India may witness reduced rainfall. From the times immemorial the fate of agriculture has been inseparably with the weather. The farmers have always been at the mercy of weather for raising the agriculture crops.

Of all weather elements, rainfall is the single most important factor affecting plant growth, agricultural production, irrigation schedule and generation. Summer monsoon which is also known as Southwest monsoon (June-September) rainfall constitutes about 75-80% of the total rainfall occurs in India and nearly four-fifth of the country receives rain during this season.

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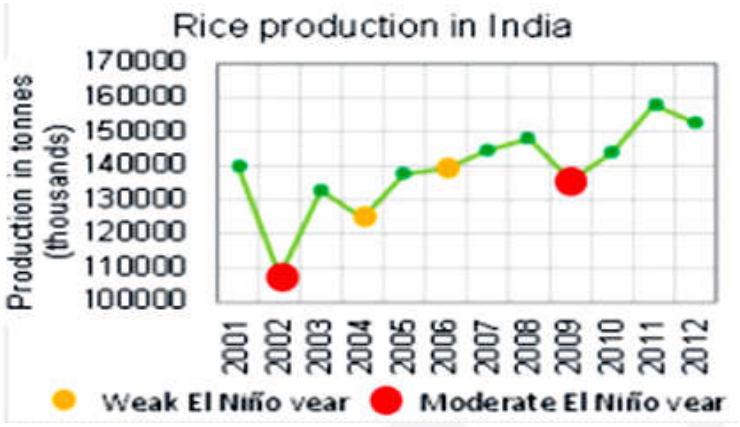

Graph 1:

Note: The impact of EI Nino on rice production is experienced the same year of the event
source: FAO Statistical database available from http://faostat.foa.org/

Table 1:

Year	Occurrence	Impact	Monsoon*
2004	EI Nino	Drought	88%
2005	neutral	normal	101%
2006	neutral	normal	103%
2007	La Nina	excess	110%
2008	La Nina	Above normal	105%
2009	EI Nino	severe drought	79%
2010	La Nina	normal	100%
2011	La Nina	normal	104%
2012	Mide EI Nino	Below normal	92%
2013	neutral	Above normal	106%

monsoon as percentoge of 50- year Avarage
source: skymet

The term El-Nino has come from the Spanish speaking people who live along the Pacific coast of Peru. Each year in late December, a southward moving current warms the water. The Peruvians started calling the warm current El Nino i.e. boy child, for the infant Jesus – because it comes around Christmas.

In an irregular interval, in every few years, the ocean warming is greater than normal and leads to disruption of the usually abundant fish and other marine life. El Nino was thought to be affecting only the west coast of South America. Now we know it has become a part of a global chain of ocean and atmospheric events.

El Nino is a condition in which the temperature of sea in the equatorial PacificOcean become unusually warm. It is known to have an effect on weather events worldwide, including the monsoon. La Niña means The Little Girl in Spanish.

El Nino is a condition in which the ocean temperatures in the equatorial Pacific Ocean become unusually warm. It is known to have an effect on weather events worldwide, including the monsoon.At the end of year, ocean surface temperatures warm along the coasts of Ecuador and northern Peru.referred to this annual warming as "El Niño," meaning "The Child," owing to to its appearance around the Christmas season. The appearance of El Niño signified the end of the fishing season. Every 2-7 years a much stronger warming appears along the west coast of South America, lasting for several months and is often accompanied by heavy rainfall in the arid coastal regions of Ecuador and northern Peru. Over time the term El Niño began to be used to referer to these warm episodes.

El Niño Southern Oscillation (ENSO), refers to the effects of a band of sea surface temperatures which are abnormally warm or cold for long periods of time that develops off the western coast of South America and causes climatic changes across the tropics and subtropics.

The "Southern Oscillation" is the variations in the temperature of the surface of the tropical eastern

Pacific Ocean, with warming known as El Niño. When air pressure is high around Australia, it is low in Tahiti. This is called southern oscillation. The pattern of low and high pressure over the Indian and Pacific Ocean gives rise to a vertical circulation along the equator (Walker circulation) with its reisiing limb over the low-pressure area and descending limb the high pressure area. The location of the low pressure and hence the rising limb over Indian Ocean is considered to be favorable for good monsoon rainfall over India.

It's shifting towards east from its normal position augers poor monsoon rainfall in India. By 1970's, scientists realized that El Nino and the southern oscillation are part of a huge ocean-atmosphere system that changes storm tracks. The effects are felt far away from its tropical home. The El Nino was linked to the World Meteorological Organization (WMO) began the Tropical Ocean and Global Atmosphere (TOGA) Programme. From 1985 to 1994, scientists tried to learn if some of the El Nino's effects could be predicted. As part of the experiment, approximately 70 weather buoys were moored across the tropical Pacific.

These radioed back air and ocean temperature via satellite to weather centers. By comparing the collected data to the weather events around the world, TOGA scientists developed computer models to predict El Nino's effects. The buoys situated in Tropical Pacific are still sending back the data and the computer models are constantly showing how El Nino and La Nino directly affecting the weather in Tropics. By 1996, Peru, Brazil and Australia were using forecast to predict El-Nino drought or rain three months ahead.

The forecast enabled the farmers to plant crops to make the best use of the most suitable conditions. Now El-Nino is being used to forecast the season in the tropical locations, but to estimate the effects more accurately some other factors have to be taken into account. The success of El-Nino predictions generated great interest among the scientists to use the ocean conditions in predicting the weather, especially to forecast what kind of average conditions in predicting the weather, especially to forecast what kind of average conditions to expect months or even a year in future. Now it has been well established that El Nino is the part of a cycle occurring in the Pacific Ocean.

Conclusion

The accurate forecasting of El Nino will go a long way to help the population over the whole globe. The southwest monsoon is highly variable in all time scale. The year-to-year variability is quite large. Over the last more than a decade the monsoon rainfall has been almost normal over the country, although there were variations from one region to other. The Indian Agriculture is dependent on timely receipt of rains, therefore correct forecast of the season incorporating the El-Nino effect will go a long way to help the planners for making correct food policies for the next season.

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Review Article

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Supramolecular Interactions of Cyclobisintercaland Molecules with the Single Stranded DNA

Nikita Dinger

Abstract

The interactions of DNA with synthetic molecules is being widely studied to empower scientists with better tools for human survival and for the betterment of the quality of life. Cyclobisintercaland molecules or macrocycles are cyclic compound which are designed to non-covalently interact with the single stranded DNA. These kind of interactions open up a wide arena of therapeutics which could involve drugs and treatments based on non-covalent binding. This paper intends to outline the study conducted so far on cyclobisintercaland molecules engineered and their biological applications.

Keywords: Cyclobisintercalands; Macrocycles; Noncovalent Interactions; Single Stranded DNA; Supramolecular Chemistry.

Introduction

DNA is regarded as a 'blueprint of life'. Ever since its discovery as the biomolecule carrying all hereditary information, extensive research is being carried out to understand its beautiful complexity and attempts are regularly being made to powerfully manipulate it to benefit mankind. Information stored in the DNA is used after amplification by subsequent RNA and proteins to phenotypically express it into observable traits. This phenomenon of gene expression had been beautifully articulated as the 'central dogma' of molecular biology by Francis Crick in 1958 [1].

Study of the genetic basis of diseases has focused the attention of researchers towards creation of molecular drugs that can bind to the complex DNA structure and bring about a beneficial change in the subsequent gene expression. Although, small synthetic molecules as well as large molecular structures may be used as drugs for gene binding or manipulation, small molecules have an added advantage of easy transport into the cell.

Supramolecular interactions of synthetic molecules with DNA primarily include non-covalent forces involved in the spatial organization of the two molecules favouring their mutual interaction. Since interactions *in vivo* between the nucleic acid and small molecules are non-covalent, examining these interactions is necessary to mimic them and use them with synthetic molecules. These recently discovered interactions of synthetic molecules with nucleic acids is of importance to mimic and influence the biological processes. Among the plethora of molecules synthesized, those that react selectively with single stranded DNA are rare and hence the recognition of these sites by synthetic molecules is important as they could act as potential anti-viral agents. This review aims to provide a recapitulation of the research conducted to date on the supramolecular interactions of chemically synthesized cyclobisintercaland molecules with the single stranded DNA.

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The DNA Single Strand

One of the greatest discoveries in the field of Molecular Biology was that of the structure of DNA double helix by James D. Watson and Francis H.C. Crick in 1953 [2]. Research since then has shown various other forms of DNA present in organisms.

Simplistically, a DNA is composed of 4 nucleotide bases- Adenine (A), Guanine (G), Thymine (T) and Cytosine (C).

Each nucleotide is attached to a deoxyribose sugar which is further attached to a phosphate molecule. The nucleotides are stacked on top of each other in the double helical structure with the sugar and phosphate molecules forming the backbone. The two polyanionic strands of DNA are intertwined around each other in the most energetically favorable manner and the bases pair via Hydrogen Bonds according to Watson-Crick base pairing rules (I.e. A=T and G ≡ C). Another base pairing, known as Hoogsteen Base Pairing is present but rarely observed. {Note: Figure 1 is basic and included only for reader's reference}

The single stranded DNA in most cases is referred to as a reminiscent of the double strand. Melting experiments can usually generate single stranded DNA synthetically. Generally, in solutions, single stranded DNA is prepared by completely melting both the strands of the ds helical DNA. The ssDNA retain some preferred conformations but their shape can be easily geometrically distorted [3]. In nature, the single stranded DNA is found mostly in viruses. Viruses containing these ssDNA release their DNA into the host which is then transported to the nucleus for transcription. ssDNA virus are largely icosahedral shaped.

However, the exact 3-dimensional structure of the DNA depends on its environment and sequence.

Most supramolecular DNA research conducted includes broadly 4 main DNA structures:

1. DNA Single Strand – Photocleavage Studies, Abasic site recognition and Hairpin Structure
2. Double Stranded DNA - Helical and ds Circular DNA; A,B and Z DNA interactions.

B DNA interactions are the most widely studied and include DNA binding majorly in these 5 sites:

a. Major Groove
b. Minor Groove
c. Sugar Phosphate Backbone
d. Intercalation between base pairs
e. Covalent binding/ Metal co-ordination to the bases [4]

3. DNA Triple Helix (Mainly DNA triple helix stabilization)
4. Tetraplex DNA or the G quadraplex

Binding of synthetic molecules to DNA/ polynucleotides includes 2 main approaches: synthesis of molecules that are sequence specific or of molecules that are structure specific. In accordance with the scope of this review, CBI interactions with single stranded DNA structures are discussed.

Cyclobisintercalands

The major synthetic molecules used to date to study single stranded DNA interactions include a broad range of macrocyclic compounds. For selective manipulation of the single stranded nucleotides by cleavage, the molecules capable of recognizing particular sequences/structures are attached with functional groups capable of cleavage.

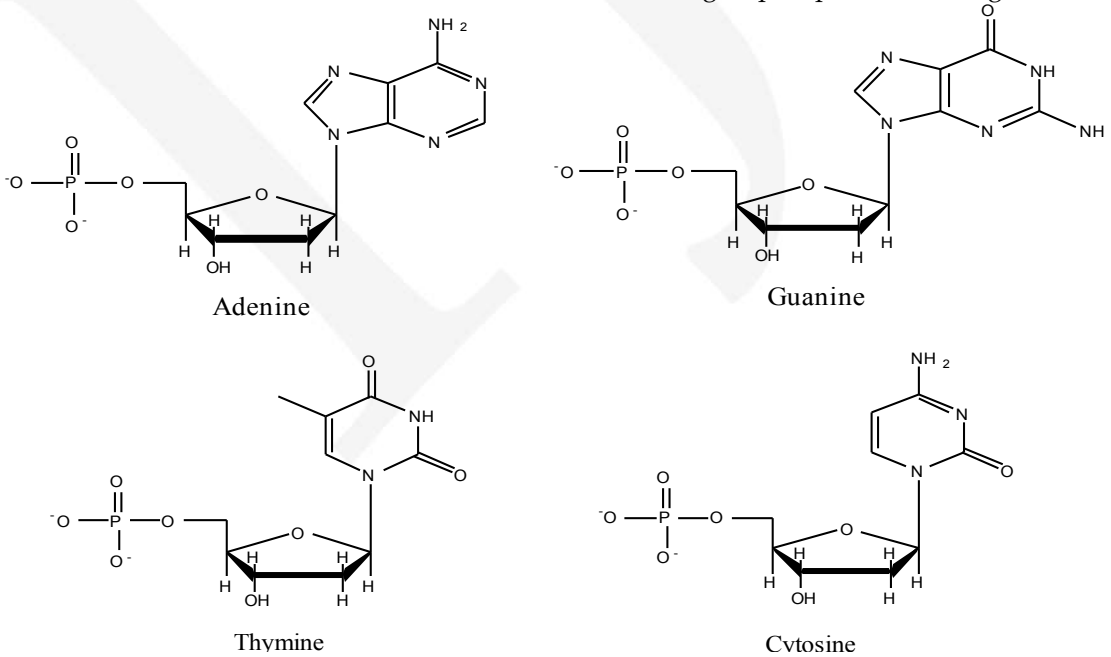


Fig. 1: Deoxyribonucleotides

A class of molecules known as the cyclobisintercalands, have been closely studied in the past few years. These molecules contain 2 planar subunits attached to each other by flexible or rigid linkers/bridges (designed according to need). This arrangement gives them an appropriate structure which when separated by appropriate distances can bind to nucleic acids and serve its intended purposes.

A type of macrocyclic compound includes the cyclo-bisintercaland (CBI) molecules. CBIs incorporate intercalating molecules into a particular macrocyclic structure with defined geometrical properties. The general structure of these cyclo-bisintercalating molecules is shown in Figure 2a. These molecules preferentially bind to ssDNA rather than dsDNA as will be discussed below. The ss DNA binding with cyclo-bisintercaland molecules shows the presence of the ssDNA between the 2 intercalands as depicted in Figure 2 b.

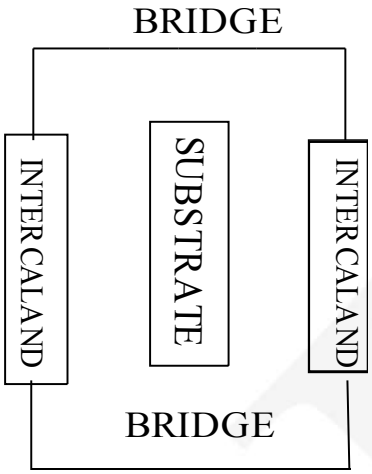


Fig. 2: Binding of the substrate (ssDNA) within the macrocyclic framework

Figure 3 further depicts the binding of CBI to single stranded DNA as compared to double stranded DNA. The preferential binding to single stranded DNA could be due to the fact that DNA binding to the ds DNA could be hindered by steric interactions of the ds framework and the chains acting as bridges between the two flat molecules [5].

The Macrocyclicbisintercalands containing porphyrin, diazapyrene, phenazine, naphthalene, acridine, anthracene and Phenanthridine have been synthesized for their applications in DNA intercalation. The synthesis has always been with the aim that it has a perfect geometry that can bind to polyanionic substrate (like nucleobases) strands and can be used in biological systems.

The positively charged CBI molecules, in aqueous solutions, strongly bind to anionic or neutral substrates.

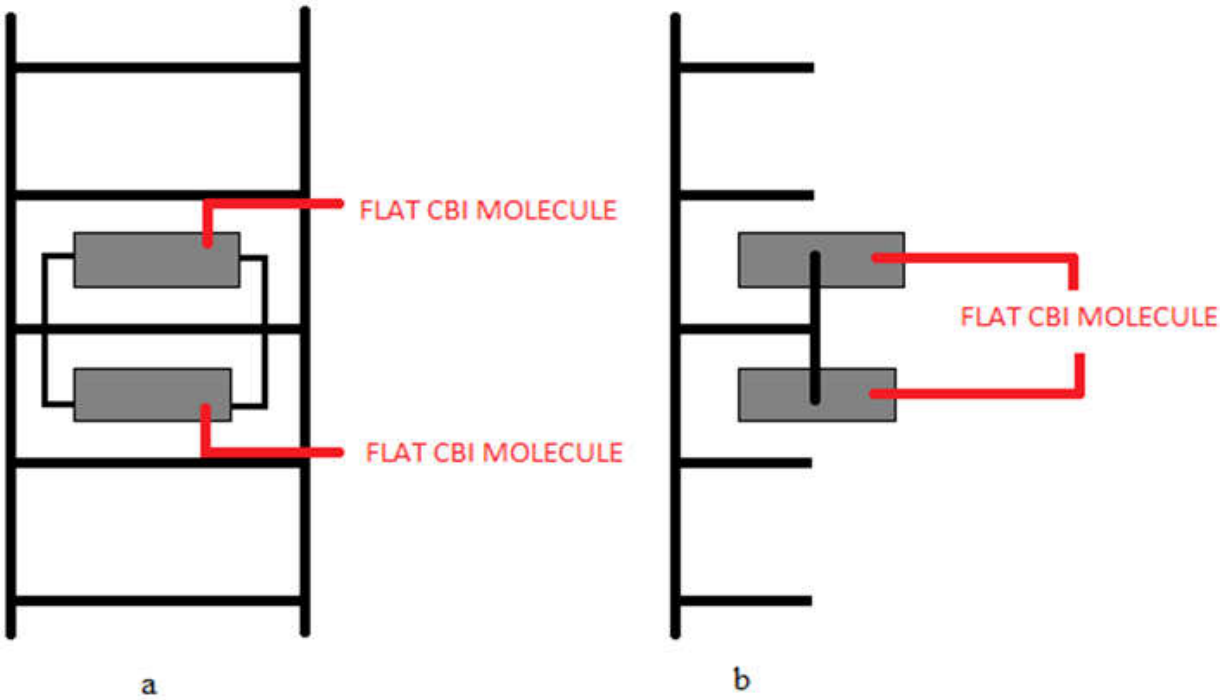


Fig. 3: Binding of a cyclobisintercaland molecule to
a. double stranded DNA and
b. Single stranded DNA
Please note: all mentions in red are the marking for naming the molecules

Synthetic Supramolecular Interactions of the Single Stranded DNA

The most interesting development in the study of the CBI binding to the single stranded nucleic acids, is the ability of these CBI molecules to differentiate between single and double stranded DNA.

DNA Hairpins

Several times, in nature, including in the human body, various non-double stranded structures play a major role in gene expression. These include the recognition of structural probes by enzymes or molecular machines for transcription, translation, replication or splicing. Hairpin motifs particularly regulate several human gene transcriptions [6,7]. Moreover, these Hairpin structures have been associated with various human diseases including the human fragile X-chromosome syndrome [8], human myotonic dystrophy [9] and promotion of 'kissing complex' of HIV dimerization [10,11]. All these factors make the study of Hairpin-binding compounds very useful to destabilize, form or manipulate this structure. In consideration of the scope of this paper, DNA Hairpin binding molecules have been discussed.

Majorly, the DNA Hairpin binding has been studied with respect to bisacridine molecules for their known preferential binding to single stranded structures. The experiments conducted have been recapitulated below:

1 (bisacridine) bound selectively to single stranded loops of the DNA hairpin structure while destabilizing double stranded polynucleotides. [5] The contribution of the above listed forces in the intercalation of the macrocyclic structure and the substrate was corroborated with experiments conducted on **2** [12].

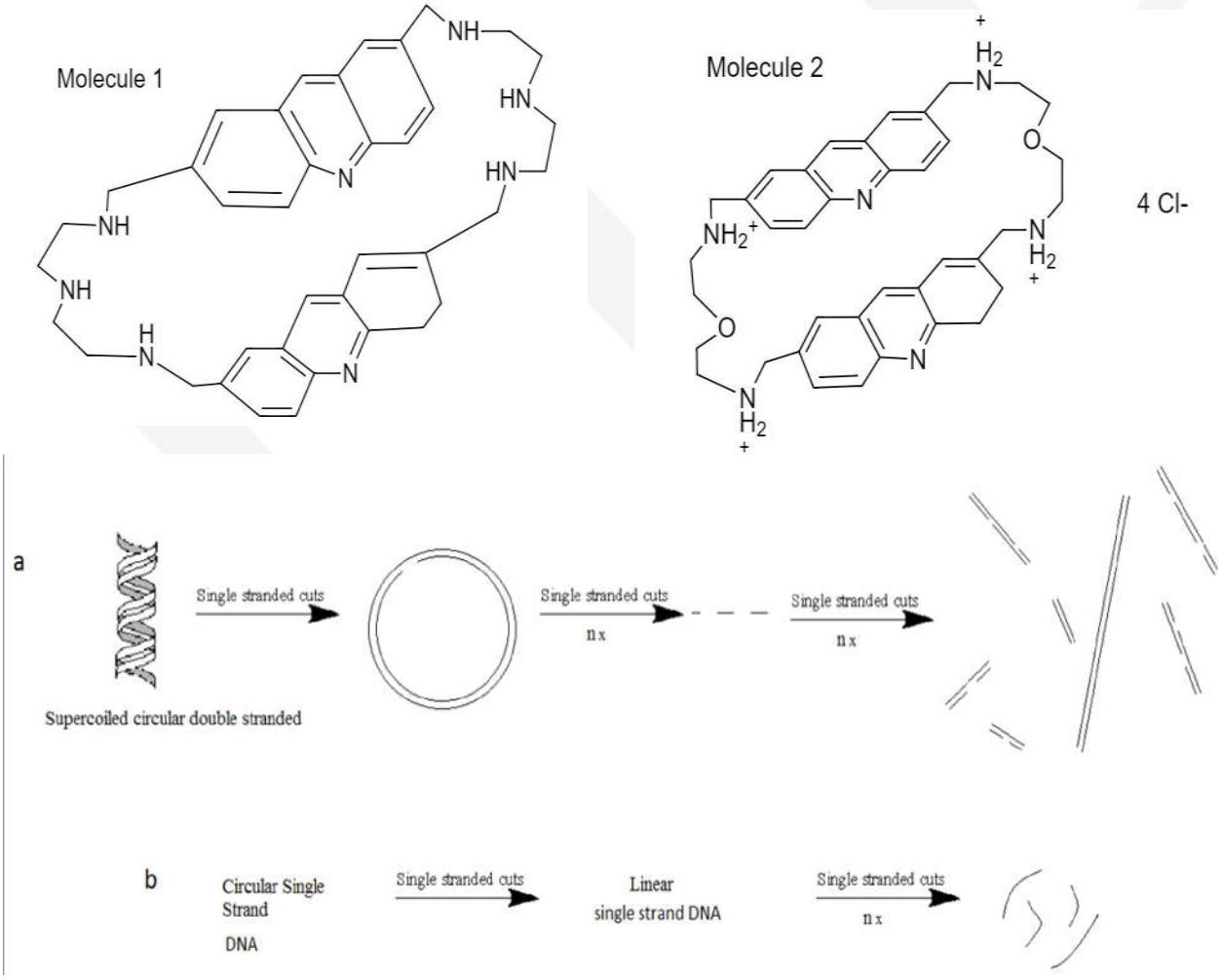


Fig. 4a: Single stranded cuts in the supercoiled double stranded DNA structure
b. Single stranded in circular single stranded DNA leading to formation of small DNA fragments

Another macrocyclic bisacridine molecule synthesized **(3)** containing positively charged flexible arms (that electrostatically bind with the phosphate groups of the oligonucleotide backbone) and uncharged flat acridine components (which stack themselves on the bases), was found to preferentially bind to and stabilize the DNA Hairpin structures. This macrocyclic molecule also was observed to stabilize the DNA Hairpin structure via a 25°C stabilization [13].

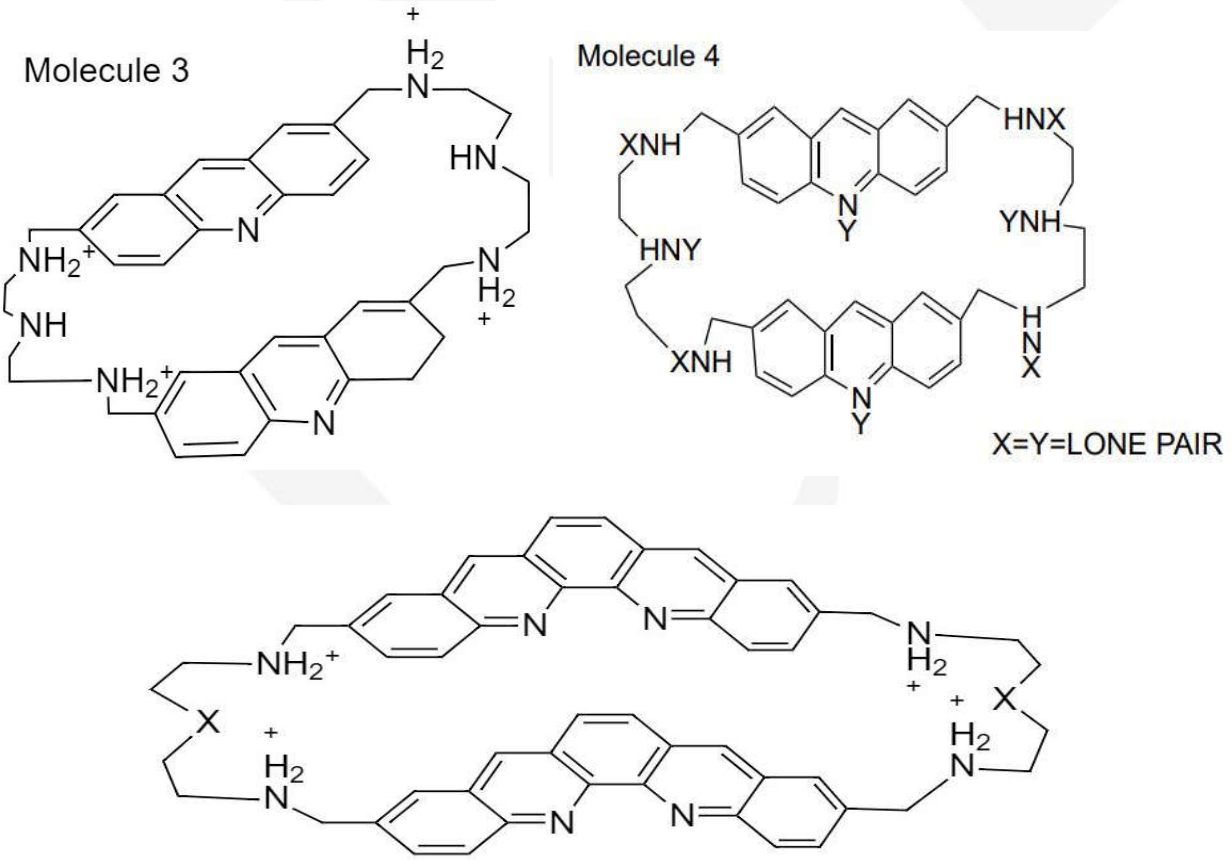
Successive competition experiments held verified the binding selectivity of the macrocyclic bisacridine **(3)** for DNA Hairpins compared to the double helical DNA. This selective affinity is the reason for a shift in the equilibrium observed from the duplex to the hairpin structures [14].

Molecular recognition of Nucleobases

In water, hydrophobic forces and electrostatic interactions play major roles.

Cyclo-bisintercaland receptor molecules based on acridine subunits synthesized were flat, positively charged and separated from each other by appropriate distances **(4)**. These molecules were soluble in water and were found to bind to nucleotides and nucleosides near biological pH. The results generated showed that the binding of these molecules could be used as a differentiator of purines and pyrimidine. On reaction with purine nucleotides, quenching of the emission was observed whereas on reaction with pyrimidine nucleotides, there was an enhancement of the fluorescence intensity. The results also established the contributions of electrostatic and hydrophobic interactions in the binding of the bisacridine molecules to the nucleobases [15]. The bisacridine macrocycles are however known to bind to flat substrates much more strongly than their parent bisnaphthalene compounds.

5 (a-c) containing crescent-shaped quinacridine structures joined by certain linkers were shown to bind to anionic aromatic nucleotides by electrostatic and ð stacking forces. These molecules were proven to bind to nucleoside monophosphates stoichiometrically in a 1:2 ratio and to nucleoside di and tri phosphates in a 1:1 stoichiometric ratio.



Higher affinities were recorded for guanosine derivatives and competition experiments even prove the binding selectivity for the guanosine derivatives in aqueous solution was preserved in the gas phase [16].

The bisporphyrin 6 was also shown to preferentially bind to single stranded DNA [5].

Naphthalene derivatives of these macrocyclic compounds, **7** (BisNP-O and BisNP-N) have been shown to bind to purines derivatives preferentially. **7 BisBP-N** was also shown to destabilize the double helical structure of the DNA by binding presumably to the single stranded regions of the DNA [17].

CBI (Cyclo-bisintercaland) molecules such as bis-naphthalene **(8)** and bis-acridine **(3)** were studied to destabilize the double helical structure of the DNA. This property of theirs was predicted due to their preferential binding to single stranded oligonucleotides [18].

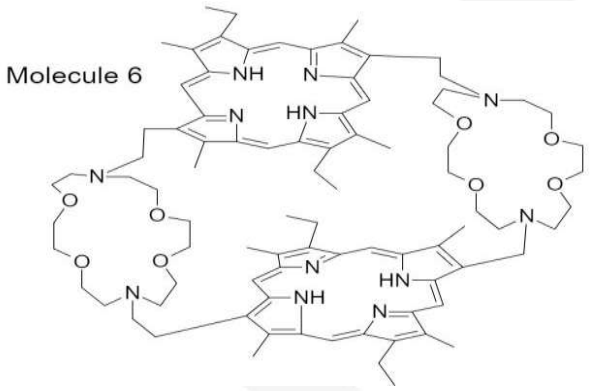
Photochemical Cleavage

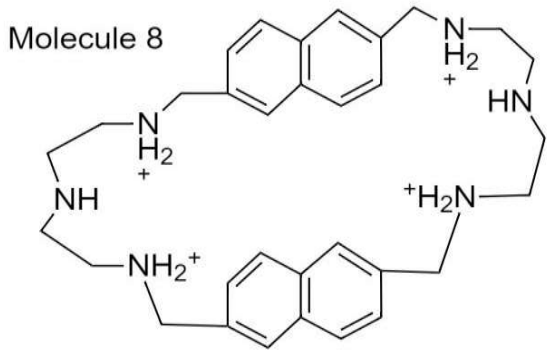
DNA is capable of *in vitro* and *in vivo* photocleavage as has been shown by various experiments in the past [19]. For functional cyclointercaland receptors, which are capable of photocleavage, the inclusion of photoactive intercalating groups into macrocycles is necessary.

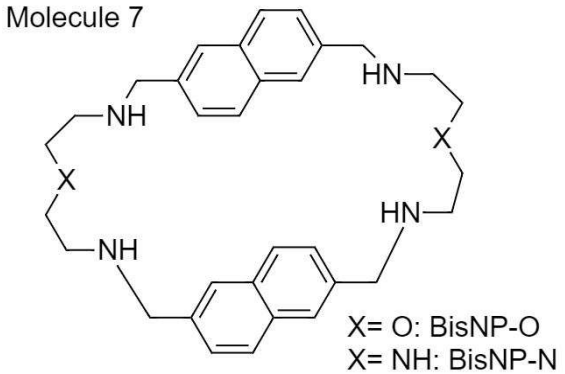
6 (bisporphyrin) has been shown to form more stable complexes with single stranded rather than double stranded polynucleotides. **6** was also tested for its Ss polynucleotide preferentiality by incubating and irradiating (for photocleavage) **6** with tRNAasp. The results obtained confirmed the binding sites of **6** with ssRNA and thus conclusively **6** prefers to bind to single stranded polynucleotides [5].

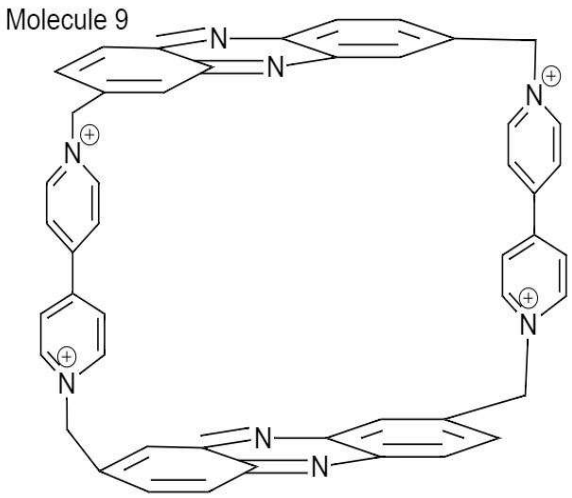
9 is a tetra-cation that essentially contains a rigid cavity for inclusion for aromatic substrates, photosensitizing intercalating phenazine subunits, electron acceptor viologen subunits. It was shown to cause significant nicks in the supercoiled DNA on irradiation. Kinetic studies conducted for this experiment also showed **9** to have a higher percent cleavage than its control counterpart **10** in the same defined time period [20].

2,7-Diazapyrenium di-cations ($MDAP^{2+}$) **(11)** had been shown to bind and photo-oxidize under irradiation with visible light molecular polyanions and thence, DNA. $MDAP^{2+}$ was shown to entirely cleave supercoiled ds DNA under visible light









irradiation resulting in nicked circular DNA upon cleavage of one strand and in linear DNA on cleavage of both strands on further reaction.

In comparison, the bis-DAP^{4+}**(12)** showed a greater degree of DNA cleavage generating multiple small fragments by cuts in both strands [21].

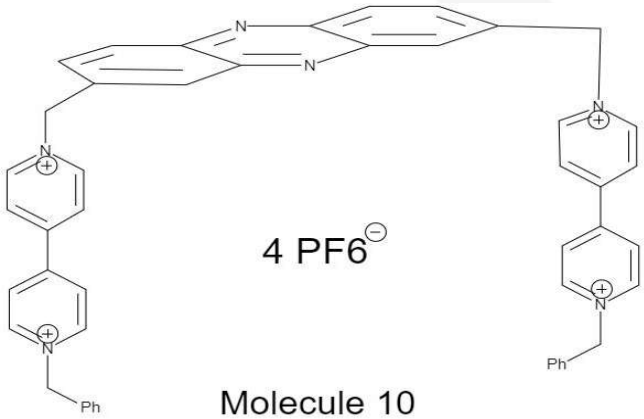
Photocleavage experiments have been mostly conducted on circular supercoiled dsDNA. The ds DNAs on being cut, changed to relaxed circular forms. This on further multiple cuts, gave linear ssDNA species.

Abasic Site

The formation of abasic sites results from the cleavage of a glycosidic bond from the DNA double helix structure. This act leaves behind a 2′-deoxyribose residue referred to as an AP site (apurinic/apyrimidinic). The formation of these sites has been shown to be accelerated greatly by the use of alkylating drugs. *In vivo* these AP sites are repaired by biological enzymes [22]. Being non-informative, the abasic sites are regarded as mutagenic or lethal entities. 9-aminoellipticine, 3- aminocarbazole, Lys-Trp-Lys peptides, hybrid molecules, bisnaphthalimide DMP 840, etc. have been engineered for their use as drugs related to abasic site [23].

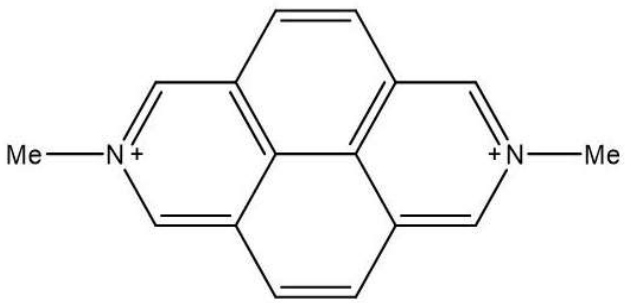
The abasic site is one of a strong thermodynamic destabilization of the ds DNA. Insertion of molecules such as the macrocyclic bisacridine leads to π-stacking interactions of acridine rings with base pairs and electrostatic interactions between the DNA groove floors and the linkers of the bisacridine macrocycle [23]. Mostly, the studies conducted on abasic site binding include bisacridine as they have already been shown to prefer single stranded structures such as the hairpin motifs. Hence, this can be extrapolated and they can be thought of as very useful binding probes for abasic DNA sites [22-25].

Adenine-triamino-acridine (ATAc), diaminopurine-triamino-acridine (DTAc) (**13**) synthesis has been reported. These structures recognize abasic sites in the DNA even at minute nanomolar concentrations and cleave the DNA at the AP-sites by β-elimination of the 3′-phosphate. They mimic the AP-endonuclease mechanism [22].



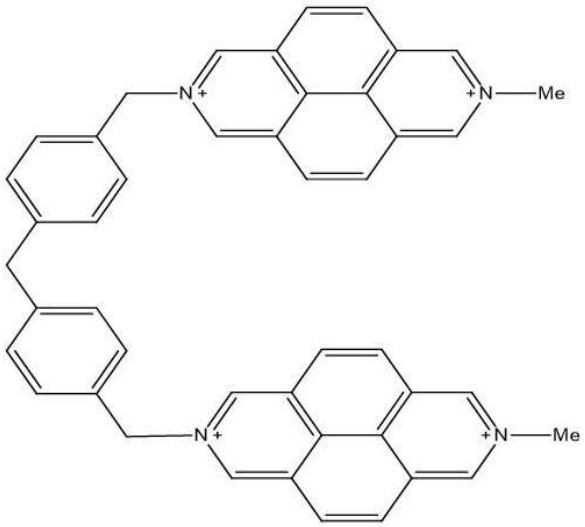
Molecule 10

Molecule 11



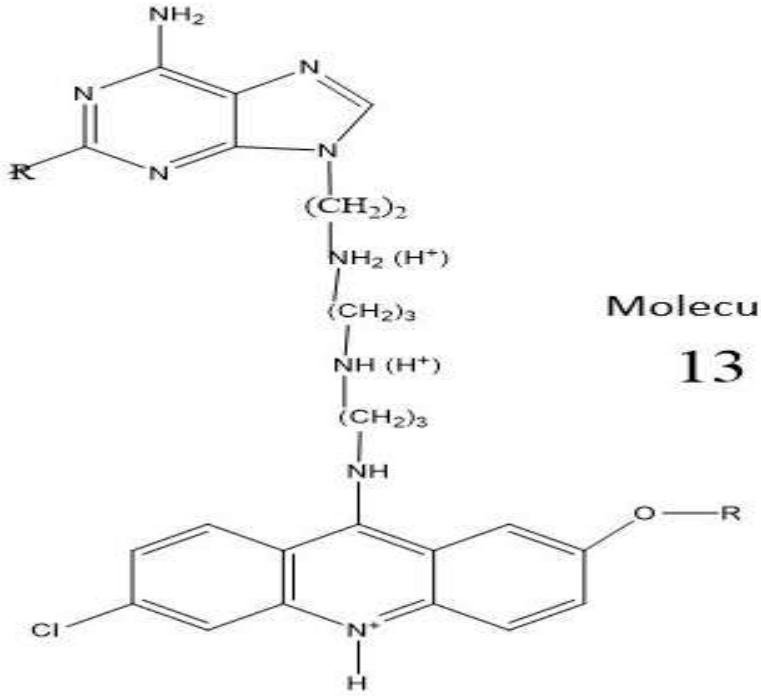
MDAP^{2+}

Molecule 12



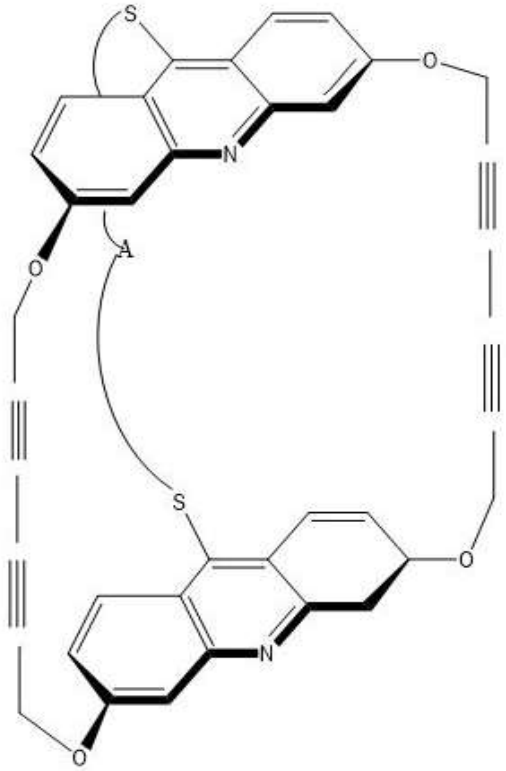
bis-DAP^{4+}

Finally, bisnaphthalene macrocycles synthesized **(7 BisNP-O)** were recorded to recognize T-T mismatch sites in ds DNA by one naphthalene ring occupying the region of the mismatched Thymine in the DNA while the other naphthalene ring binds at the A-T base pair. The polyammonium chains as usual, stabilize the complex [26].



Molecule 13

ATAc R=CH_3
DTAc R=CH_3



a A= -$(CH_2)_6$-
b A= -$CH_2CH_2OCH_2CH_2$-
c A= -$CH_2CH_2OCH_2CH_2OCH_2CH_2$-
a-Me_2^{2+} A= -$(CH_2)_6$-; bis-NCH_3^+

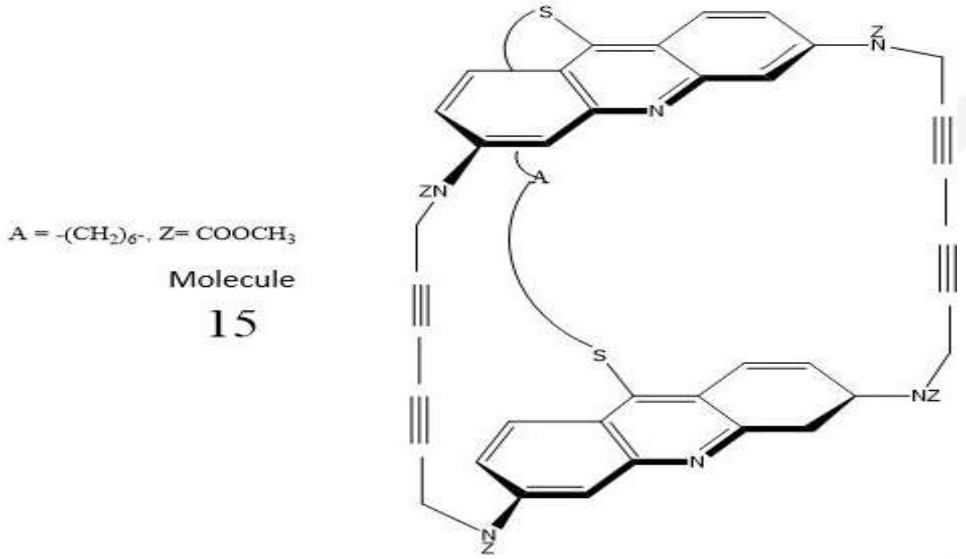
Molecule 14

CBI Binding Properties

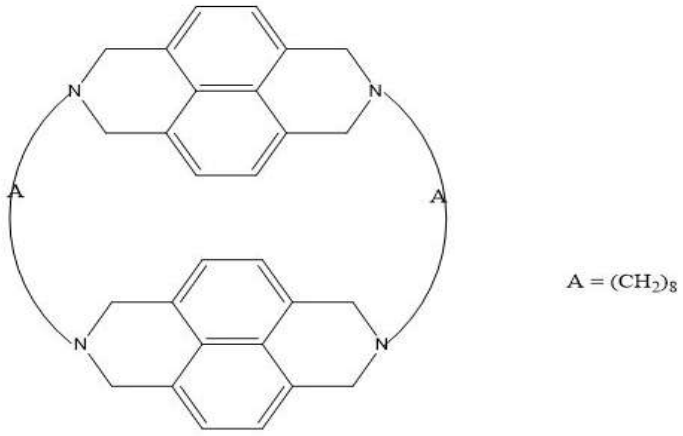
As mentioned earlier, synthesis of CBI molecules has been with the aim to create molecules either sequence specific or structure specific, which bind to the substrates in an environment similar to their *in vivo* characteristics. Amongst all species, the binding of flat **bisacridine** molecules **(14,15)** to the DNA has been widely studied. Some other acridine based bis-intercaland molecules synthesized have been shown to have a separation of about 4 Å similar to that of B-DNA. These molecules hence may also prove to be useful for binding and intercalation [27].

Another cyclo-bisintercaland **(16)** synthesized showed binding affinities to anionic substrates and hence may be able to bind to DNA [28].

Finally, cyclo-bisintercalands with *phenanthridine* subunits were synthesized containing two 8-amino-6-phenanthridinyl **(17-21)** and 8-amino-5-methylphenanthridinium-6-yl **(22)** respectively. These molecules, on complexation with aromatic substances/cyclization showed a marked increase in their electronic absorption coefficient and their fluorescence intensity. Aforementioned bisacridine subunit based cyclointercalands showed opposite results i.e. their cyclic structures showed a decrease. Ethidium Bromide, a phenanthridine derivative, is one of the most widely used fluorescent tag and a typical ds intercalator. The hydrophobic environment found between the base pairs is mainly regarded as the primary cause for its fluorescence [29]. Ethidium Bromide has also been shown to specifically intercalate between the G/C base pairs of DNA Hairpin [30] A diasterioisomeric CBI structure of two positively charged phenanthridinium subunits joined by aminobisacetylenic bridges **(23 and 24)** with a distance of 4.5 Å was also shown to selectively bind to single stranded polynucleotides primarily due to its structural geometry which is favourable for the same [31].

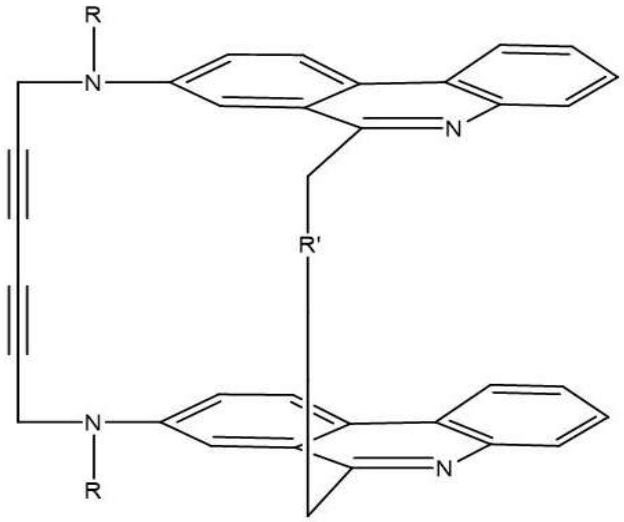


Molecule 15



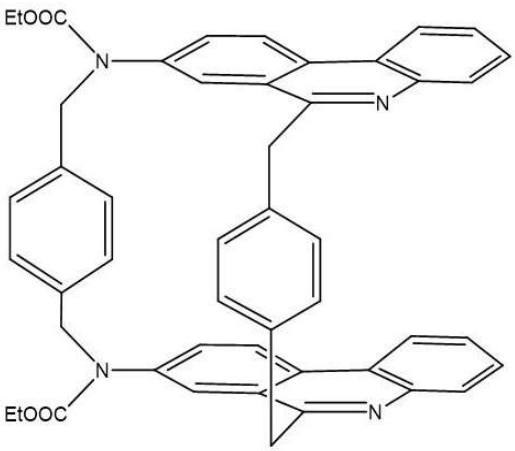
Molecule 16

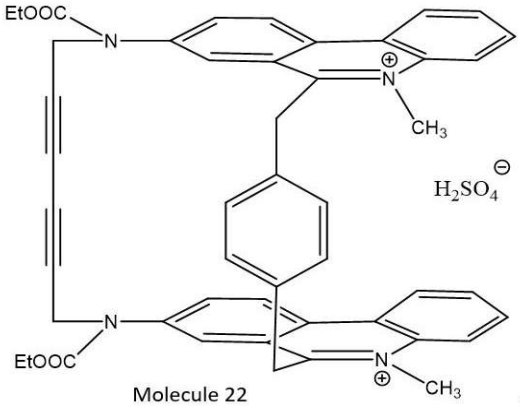
Molecules 17 to 20



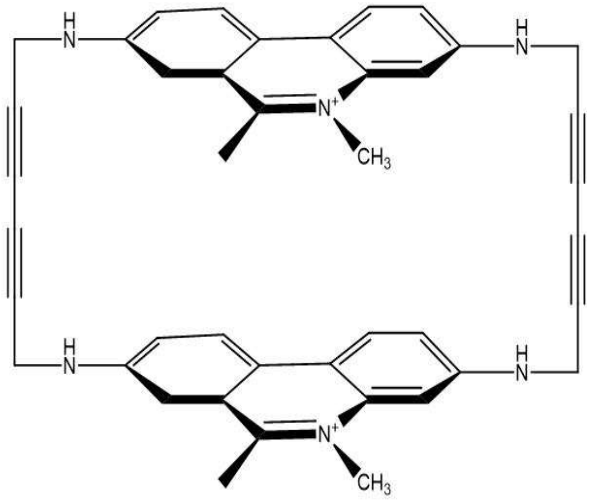
	R	R'
17	COOEt	p-C_6H_4
18	H	p-C_6H_4
19	COOEt	$(CH_2)_4$
20	H	$(CH_2)_4$

Molecule 21

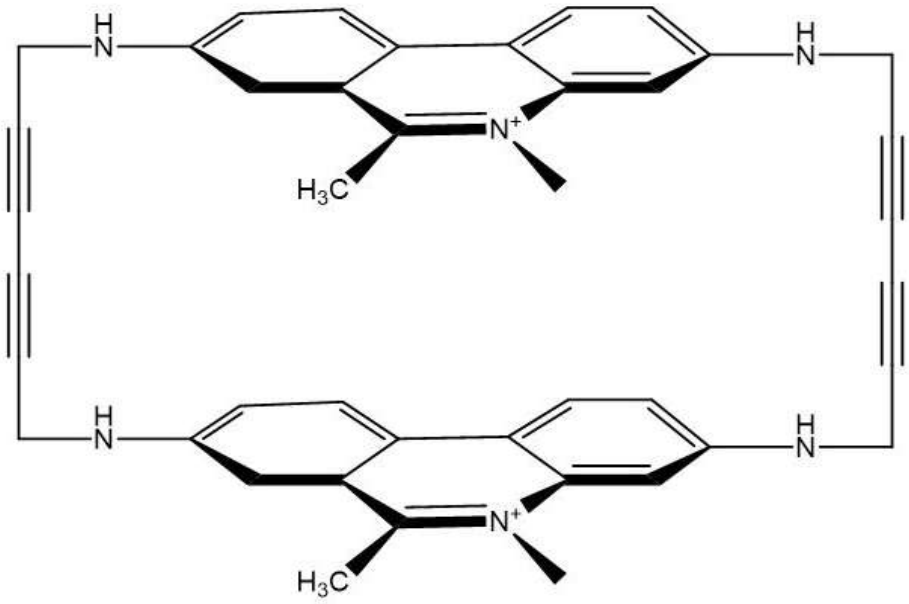




Molecule 22



Molecule 23



Molecule 24

Conclusion

The DNA intercalation phenomenon is reminiscent of DNA base-stacking. Bis-intercalands have been shown to have lower residence time on intercalation with the DNA. This phenomenon has been postulated to be the reason behind lower anti-tumor activity of many intercalands *in vivo [32].*

As for the nucleotides, very high affinity constants were measured (between 10^4 and 10^8 M^{-1}) [15,33]. The stoichiometry of binding for all substrates measured was found to be in a 1:1 ratio. Concluding results, the stoichiometry, hypochromism of $\pi-\pi$ systems stacking and characteristics of aromatic surface areas indicate towards sandwich type structural geometry of the substrates in between the flat receptor molecules. Certain crystal structures have also confirmed this [34].

Although, research on single stranded DNA intercalation is in its infancy, the probable future application we can predict include its use as drugs for the treatment of human diseases. The experiments of CBIs *in vitro* are now being conducted in conditions similar to those *in vivo*. This has increased its scope of application for human use. Nonetheless, various drugs mentioned in this paper have reached the chemical trials phase. Extraordinary opportunities remain, as delving deeper into biological applications will require sophisticated techniques.

Acknowledgements

I would sincerely like to thank Prof. J M Lehn (Nobel Prize in Chemistry 1987) from the Institut de Science et d'Ingénierie Supramoléculaires, University of Strasbourg, France for giving me an opportunity to work with him. This paper is largely inspired by my learning in his lab. I would also like to thank him for his support and suggested reading during the writing of this paper.

Conflict of Interest Statement

The author declares that there is no conflict of interest.

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Erratum

Original Research Article

Article Titled "**Biosynthesis and Characterization of Silver Nanoparticles by using Leaf Extract of Thalkudi (*Centella asiatica)* and its Antimicrobial Activity**"

Gitanjali Mishra*, Diptikanta Acharya, Sagarika Satapathy**, Manoja Das*****

Published in

Indian Journal of Biology

Volume 4 Number 2, July - December 2017

DOI: http://dx.doi.org/10.21088/ijb.2394.1391.4217.6

The original published version of this Article contained errors in the plant name **Thalkudi (*Centella asiatica)*** mentioned as **Tulsi (*Ocimum sanctum*)** in **abstract, Introduction, Results and Discussion and in Conclusion**. But actual name of plant in the study is "**Thalkudi (*Centella asiatica)*".**

Now Tulsi (*Ocimum sanctum*) read as, **Thalkudi (*Centella asiatica)*** in all the places in abstract, Introduction, Results and Discussion and in Conclusion of the article.

Mistake is regretted **Editor-in -chief**

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The text of observational and experimental articles should be divided into sections with the headings: Introduction, Methods, Results, Discussion, References, Tables, Figures, Figure legends, and Acknowledgment. Do not make subheadings in these sections.

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The title page should carry

1) Type of manuscript (e.g. Original article, Review article, Case Report)
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The second page should carry the full title of the manuscript and an abstract (of no more than 150 words for case reports, brief reports and 250 words for original articles). The abstract should be structured and state the Context (Background), Aims, Settings and Design, Methods and Materials, Statistical analysis used, Results and Conclusions. Below the abstract should provide 3 to 10 keywords.

Introduction

State the background of the study and purpose of the study and summarize the rationale for the study or observation.

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Results

Present your results in logical sequence in the text, tables, and illustrations, giving the main or most important findings first. Do not repeat in the text all the data in the tables or illustrations; emphasize or summarize only important observations. Extra or supplementary materials and technical details can be placed in an appendix where it will be accessible but will not interrupt the flow of the text; alternatively, it can be published only in the electronic version of the journal.

Discussion

Include summary of key findings (primary outcome measures, secondary outcome measures, results as they relate to a prior hypothesis); Strengths and limitations of the study (study question, study design, data collection, analysis and interpretation); Interpretation and implications in the context of the totality of evidence (is there a systematic review to refer to, if not, could one be reasonably done here and now?, What this study adds to the available evidence, effects on patient care and health policy, possible mechanisms)? Controversies raised by this study; and Future research directions (for this particular research collaboration, underlying mechanisms, clinical research). Do not repeat in detail data or other material given in the Introduction or the Results section.

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List references in alphabetical order. Each listed reference should be cited in text (not in alphabetic order), and each text citation should be listed in the References section. Identify references in text, tables, and legends by Arabic numerals in square bracket (e.g. [10]). Please refer to ICMJE Guidelines (http://www.nlm.nih.gov/bsd/uniform_requirements.html) for more examples.

Standard journal article

[1] Flink H, Tegelberg Å, Thörn M, Lagerlöf F. Effect of oral iron supplementation on unstimulated salivary flow rate: A randomized, double-blind, placebo-controlled trial. J Oral Pathol Med 2006; 35: 540-7.

[2] Twetman S, Axelsson S, Dahlgren H, Holm AK, Källestål C, Lagerlöf F, et al. Caries-preventive effect of fluoride toothpaste: A systematic review. Acta Odontol Scand 2003; 61: 347-55.

Article in supplement or special issue

[3] Fleischer W, Reimer K. Povidone iodine antisepsis. State of the art. Dermatology 1997; 195 Suppl 2: 3-9.

Corporate (collective) author

[4] American Academy of Periodontology. Sonic and ultrasonic scalers in periodontics. J Periodontol 2000; 71: 1792-801.

Unpublished article

[5] Garoushi S, Lassila LV, Tezvergil A, Vallittu PK. Static and fatigue compression test for particulate filler composite resin with fiber-reinforced composite substructure. Dent Mater 2006.

Personal author(s)

[6] Hosmer D, Lemeshow S. Applied logistic regression, 2nd edn. New York: Wiley-Interscience; 2000.

Chapter in book

[7] Nauntofte B, Tenovuo J, Lagerlöf F. Secretion and composition of saliva. In: Fejerskov O, Kidd EAM,

editors. Dental caries: The disease and its clinical management. Oxford: Blackwell Munksgaard; 2003. p. 7-27.

No author given

[8] World Health Organization. Oral health surveys - basic methods, 4th edn. Geneva: World Health Organization; 1997.

Reference from electronic media

[9] National Statistics Online—Trends in suicide by method in England and Wales, 1979-2001. www.statistics.gov.uk/downloads/theme_health/HSQ 20.pdf (accessed Jan 24, 2005): 7-18. Only verified references against the original documents should be cited. Authors are responsible for the accuracy and completeness of their references and for correct text citation. The number of reference should be kept limited to 20 in case of major communications and 10 for short communications.

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