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Original Article

Studies on the Socio-Economic Condition of Fishermen Community of Ornamental Fish Culture of West Bengal

Sarbani Dutta (Roy)*, A. K. Panigrahi, Anandamoy Mandal*****

Abstract

Ornamental fish can be defined as an attractive colourful fishes of peaceful by nature that is kept as a pet in confined space of an aquarium or a garden pool with the purpose of enjoying their beauty for fun and fancy. The fisher community is very much associated with this ornamental fish culture. So their socio economic condition, age factors, education, caste system, family size and type are affected due to the ups and downs of the culture. Khare and Punekar (2001) reported that socio economic status of fisher women were found to be positive and significantly correlated towards fish farming. So the paper enlightens the background information of the fishermen community of ornamental fish culture.

Keywords: Ornamental; Aquarium; Community; Culture; Economic Enlighten.

Introduction

Ornamental fisheries have developed into a multibillion dollar industry as an important sub-sector within the fisheries and aquaculture sector. A number of endemic and native ornamental fish species known as indigenous ornamental fishes are dominating the export market recent times. Thus the indigenous ornamental fishery is also showing the sunlight to the unemployed people by providing them a source of livelihood. According to Ghosh et al. (2003) availability of labour, favourable environment and mostly availability of a number of indigenous potential fishes made this state unique in India in case of ornamental fishery. For the culture of ornamental fishes the socio-economic condition of ornamental fish farmers plays a significant role. According to Gracy (1998) the women in the fishing communities play an important role in the fisheries sector in terms of their involvement in fish related activities such as drying, storing, fish packing, grading and net making. According to Sharma and Kumar (2001) the fishermen managed to income not less than five thousand per month through this ornamental fish culture. Valiakandathil (1978) reported that among the fishermen community 22.7% dominated by primary educated fishermen community. Caste system has also been observed in the fishermen community. According to Halder et al. (1988) majority of fishermen were belonged to Scheduled caste community in some villages of West Bengal. Generally the marketing and trade of ornamental fishery is increasing day by day.

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Materials and Methods

The studies were conducted in four districts of West Bengal i.e. North 24 Parganas, South 24 Parganas, Howrah and Hooghly. To conduct the study scientifically, a suitable research design was evolved in order to arrive at an authentic conclusion. This chapter deals with the details of the methodology adopted for the present study. Now for the sake of convenience the chapter is sub-divided in to the following sub-headings.

1. Locale of the study for selection of sampling areas
2. Selection of respondents.

3. Operationalization of variables and their measurement.
4. Methods of data collection.
5. Statistical analysis of the data.

1. Locale of the study for selection of sampling areas

Ornamental fishes are generally cultured in different districts of West Bengal namely Howrah, South 24 Parganas, Jalpaiguri, Darjeeling, Birbhum, Hooghly etc. But among those districts, South 24 Parganas, North 24 Parganas, Howrah and Hooghly districts were selected for this study because these districts are rich in ornamental fish and are adjacent to each other. The present study was carried out from the month of March' 2008 to February' 2011 among the selected districts.

2. Selection of Respondents

A list of all ornamental fish farmer of four selected areas was prepared. The number of fish farmers of four districts was seven hundred. Among them eighty ornamental fish farmers from each district and as a whole three hundred twenty fish farmers were selected by using simple random technique without replacement from the four districts. Thus three hundred twenty numbers of ornamental fish farmers i.e. respondents were taken as the sample size for this study.

Sum total nos. of respondents:

Howrah = 80 + Hooghly = 80+ North 24 Parganas = 80 + South 24 Parganas = 80

Total nos. of respondents: 320

3. Operationalization of the variables and their measurement

3.1. Personal Characteristics

I. Age

It refers to the chronological age of the ornamental fish farmers and consisted of the following three characteristics:

- Young ornamental fish farmers whose age upto 25 years.
- Middle aged ornamental fish farmers- It refers to between 26-50 years.
- Old aged ornamental fish farmers- It refers to those ornamental farmers whose age was above 50 years.

II. Education

It refers to ornamental fish farmers' academic qualifications acquired through formal schooling. The formal education levels, which were used in this study, are as follows-

- Illiterate- It refers to ornamental fish farmers whose education was nil.
- Middle- It refers to those ornamental fish farmers whose education upto middle class level (class V-VIII)
- Madhyamik- It refers to ornamental fish farmers who passed the school final examination.
- Higher Secondary- It refers to those ornamental fish farmers who passed the Higher Secondary examination.
- Graduate- It refers to those ornamental fish farmers whose education was upto graduation level.

III. Family Size

In a nuclear family it refers to total number of persons of the family of an ornamental fish farmer which includes the ornamental fish farmer's father, mother, sisters, brother and grandparents. The total number was taken as a family size.

IV. Gender

It refers to ornamental fish farmer's sex and considered of two categories i.e. male and female.

3.2. Socio Economic Characteristics

I. Economic Status

It refers to the ornamental fish farmer's earnings through fishing activities as well as through other agricultural activities. This study was done based on the categories like BPL (bellow poverty line) and APL (above poverty line) as directed by the Government.

II. Caste

It refers to caste of the ornamental fish farmers. Three categories of the caste were considered in this study namely scheduled caste, general caste and scheduled tribe.

III. Religion

It refers to religion of the ornamental fish farmers. Three categories have been identified i.e. Hindu, Muslim and others.

IV. Land Holding

It refers to the total land areas (cultivated and house hold) possessed by ornamental fish farmers which range from one kattha to five bighas.

3.3. Communicational Characteristics

I. Utilization of Information Sources

It refers to the various sources through which the respondent receives the information regarding fishery activities. According to the frequency of utilization sources had been allotted for individuals sources …. "most often"-4, "often"-3, "sometimes"-2 and "never"-1.

II. Personal characteristics

Age	: Chronological age in years
Education	: Schedule was developed for thestudy
Family Size	: Schedule was developed for the study
Gender	: Gender in male and female

III. Socio-economic Characteristics

Cast	: Schedule was developed for the study
Religion	: Schedule was developed for the study
Economic status	: Schedule was developed for the study
Land holding	: Schedule was developed for the study
Water body processing	: Schedule was developed for the study

4. Method of the data collection

The final data for this study were collected with the help of structured interview. The data were collected during March' 2008 to February' 2011. The respondents were contacted twice in order to establish rapport with them and obtaining factual information. The researcher herself individually interviewed the respondents selected in the sample in order to gather the required and relevant information for the study.

5. Statistical Analysis of Data

The collected data were checked and put in prepared format for bring out proper results. For making simple comparisons the frequency tables were constructed and the respective percentages were calculated. The acquired data were analyzed by following statistical techniques.

Mean

Mean or average, taken as representative of groups of item implies a measured degree of validity. The arithmetic mean is the simple average, which is calculated as the sum of the items divided by the number of items.

The formula for the mean of a series of numbers is as follows:

$$X = \frac{\sum X}{N}$$

Where $\sum X$ = Sum of the individual items.

N = Number of items.

Percentage

Percentages were used mainly for making simple comparison. For calculating percentages the frequency of a particular characteristic were divided by total number of respondents in that particular characters and multiplied by 100.

The formula for the percentage is as follows:

$$\text{Percentage} = \frac{\text{Frequency}}{N} \times 100,$$

where N= Number of items.

Results

The results of that study have been presented broadly in three parts here. The results occurring from descriptive analysis of the data regarding ornamental fish farmers have been presented. In the first part, the result regarding personal, socio-economical and communicational characteristics of ornamental fish farmers have been presented. The second part dealt with the cultural practices and the problems faced by the farmers. In the third part, the marketing system of ornamental fishes in the study area has been described.

1. Background information of the ornamental fish farmers

In order to understand the present status of ornamental fish farming clearly and comprehensive

for this study some of the personal, socio-economical, communicational characteristics were taken into account which include their age, education, family size, caste, land and water bodies holding and utilization of information sources of ornamental fish farmers.

1.1. Personal Characteristics

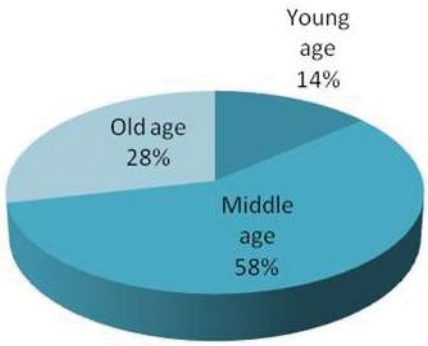
1.1.1. Age

The age of the ornamental fish farmers varied from 17 years to 60 years.

Table 1: Distribution of Respondents according to age (N=320)

Sl. No.	Category	Frequency	Percentage
1.	Young (upto 25 years)	44	13.75
2.	Middle (26-50 years)	185	57.81
3.	Old (above 50 years)	91	28.44

Fig. 1: Chart showing the Percentage of Age of respondents



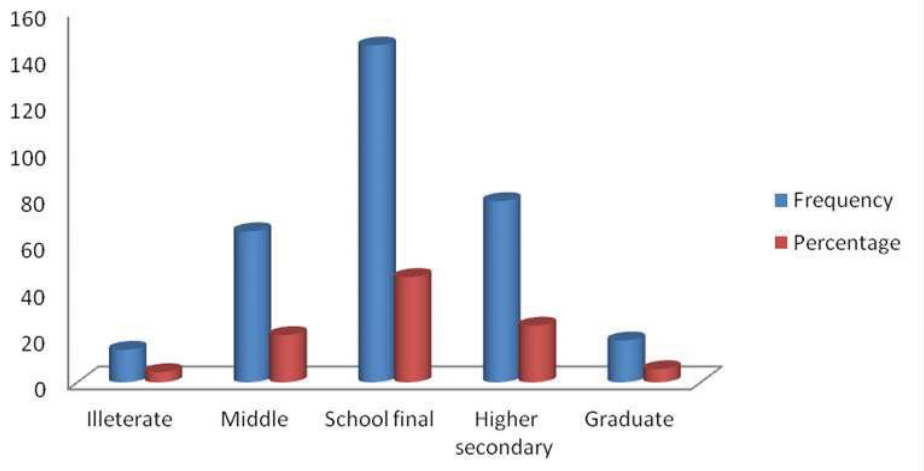
Out of 320 ornamental fish farmers, majority i.e. 185 numbers (57.81%) were in the age group of 26-50 years (middle), 91 (28.44%) were belonged to old category i.e. above 50 years and remaining 44 (13.75%) ornamental fish farmers were in age group of upto 25 years (young) (Table 1, Fig.1).

1.1.2. Education

Table 2: Distribution of respondents according to education (N=320)

Sl. No.	Category	Frequency	Percentage
1.	Illiterate	14	4.37
2.	Middle	65	20.31
3.	Madhyamik (School Final)	145	45.31
4.	Higher Secondary	78	24.37
5.	Graduate	18	5.62

Fig. 2: Chart showing the frequency and percentage of Education of respondents



The results showed that out of 320 ornamental fish farmers only 14 ornamental fish farmers (4.37%) were illiterate and rest of 306 (95.63%) were literate.

Out of 306 literate ornamental fish farmers 65 numbers (20.31%) were in middle level and majority i.e. 145 numbers (45.31%) were in Madhyamik level and 78 numbers (24.37%) were in higher secondary level where as only 18 ornamental fish farmers (5.62%) were graduate level (Table-2, Fig-2)

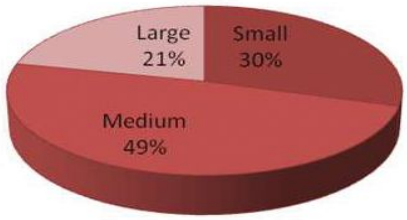
1.1.3. Family Size

The maximum and minimum numbers of family members of ornamental fish farmers in the study were 12 and 2 respectively.

Table 3: Distribution of respondents according to Family Size (N=320)

Sl. No.	Category	Frequency	Percentage
1.	Small (1-4 nos.)	95	29.68
2.	Medium (5-8 nos.)	156	48.75
3.	Large (9 and above nos.)	69	21.56

Fig. 3: Chart showing the percentage of Family size of respondents.



Out of 320 ornamental fish farmers, majority i.e. 156 (48.75%) were having medium size family (5-8 nos.) followed by 95 (29.68%) who were having small size (1-4 nos.) of family. Only 69 (21.56%) ornamental fish farmers were having large size family (9 and above) (Table-3, Fig.3).

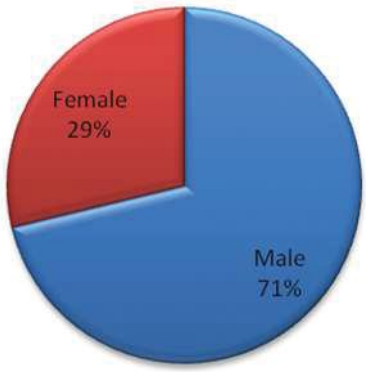
1.1.4. Gender

Both male and female ornamental fish farmers were found in the study area.

Table 4: Distribution of respondents according to their gender (N=320)

Sl. No.	Category	Frequency	Percentage
1.	Male	228	71.25
2.	Female	92	28.75

Fig. 4: Showing the percentage of Zender of ornamental fish farmers



The study indicated that out of 320 respondents, the majority were 228 (71.25%) male ornamental fish farmers and 72 (28.75%) female ornamental fish farmers out of 320 respondents (Table-4, Fig-4).

1.2. Socio-economic Characteristics

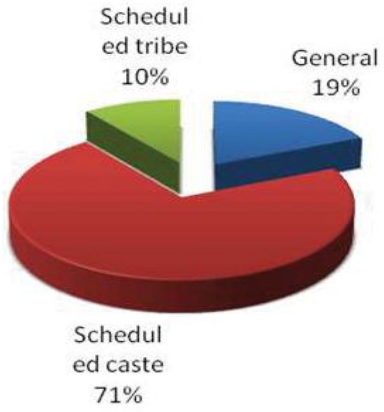
1.2.1. Caste

The findings of the study indicated that the majority of ornamental fish farmer belonging to scheduled caste community.

Table 5: Distribution of respondents according to their Caste (N=320)

Sl. No.	Category	Frequency	Percentage
1.	General	59	18.43
2.	Scheduled Caste	228	71.25
3.	Scheduled Tribe	33	10.31

Fig. 5: Showing the percentage of caste of ornamental fish farmers



So from the above data it can be concluded that majority of the respondents i.e., 228 (71.25%) belonging to scheduled caste and the rest respondents i.e. 59 (18.43%) belonging to general caste followed by 33 (10.31%) farmers belonging to scheduled tribe (Table 5, Fig. 5).

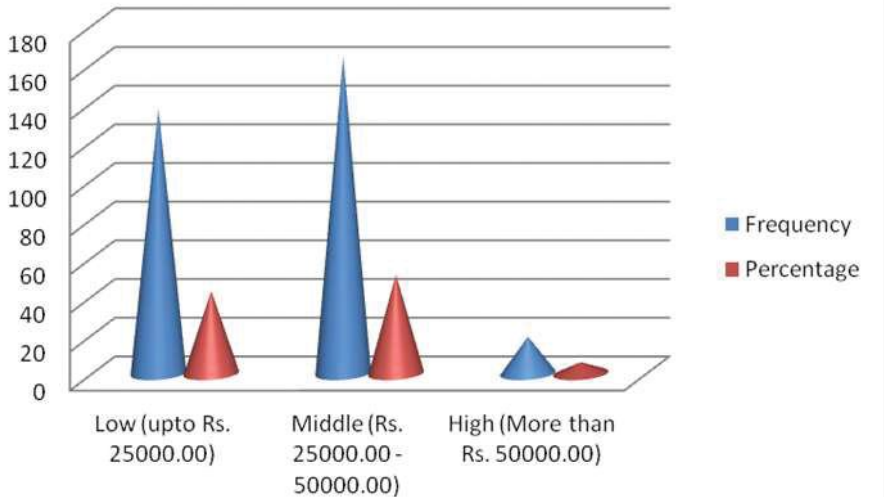
1.2.2. Annual Income

On the basis of annual income of the ornamental fish farmers they can be categorized as lower, middle and higher income groups.

Table 6: Distribution of respondents according to their Income (N=320)

Sl. No.	Category	Frequency	Percentage
1.	Lower (upto Rs. 25000.00)	137	42.81
2.	Middle (Rs. 25000.00 -50000.00)	164	51.25
3.	Higher (More than Rs. 50000.00)	19	5.93

Fig. 6: Chart showing the annual income of the ornamental fish farmers.



The present study revealed that out of 320 ornamental fish farmers, majority i.e. 164 (51.25%) farmers belonging to middle a income group (Rs. 25000.00-50000.00) followed by 137 (42.81%) ornamental fish farmers belonging to lower income group (upto Rs.25000.00) and the others i.e. 19 (5.93%) ornamental fish farmers belonging to higher income category (more than Rs.50000.00) (Table-6,Fig-6.)

1.2.3. Land Holding

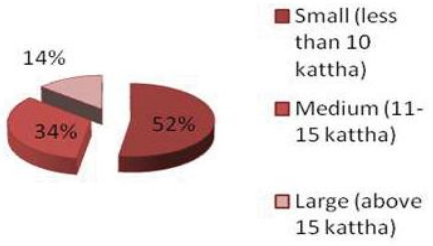
The land holding capacity of the respondents of the study area were reported from 4 cattah to 5 bigha

The majority of the respondents 168 (52.50%) had less than 10 katthas land, 108 (33.75%) respondents were reported of holding 11-15 katthas land and the rest i.e. 44 (13.75%) of respondents had large size land of above 15 katthas (Table-7, Fig- 7).

Table 7: Distribution of respondents according to their Land Holding (N=320)

Sl. No.	Category	Frequency	Percentage
1.	Small (less than 10 kattha)	168	52.50
2.	Medium (11-15 kattha)	108	33.75
3.	Large (above 15 kattha)	44	13.75

Fig. 7: Showing the percentage of land holding of the respondents



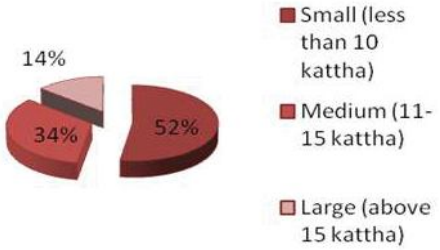
1.2.4. Total Water Bodies

The minimum and maximum water bodies owned by the respondents were 1.5 katthas and 15 katthas respectively.

Table 8: Distribution of respondents according to Total Water Bodies (N=320)

Sl. No.	Category	Frequency	Percentage
1.	Small (below 4 kattha)	158	49.37
2.	Medium (4-8 kattha)	84	26.25
3.	Large (above 8 kattha)	78	24.37

Fig. 8: showing the percentage of water bodies of the respondents



The present communication revealed that majority of the ornamental fish farmer i.e. 158 (49.37%) owned small area of water bodies followed by 84 (26.25%) owned medium areas of water bodies only 78 (24.37%) farmers had large water bodies (Table-8,Fig-8)

1.2.5. Communicational Characteristics

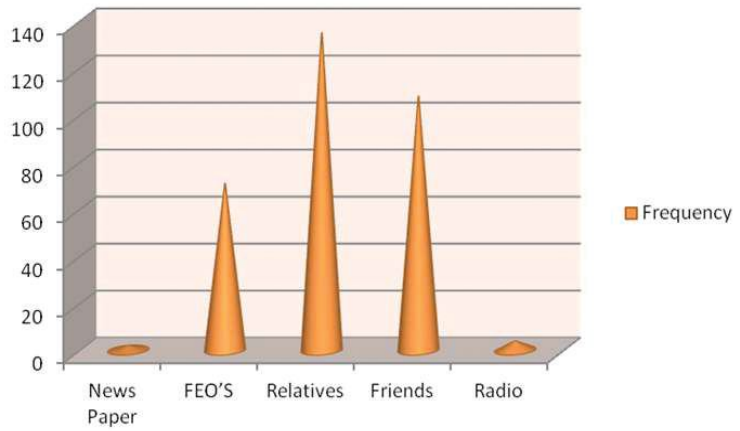
Information Sources

Ornamental fish farmers use different sources of information for the ornamental fish farming. They collected information related to culture practices and marketing. Sometimes they kept themselves in touch of various schemes and projects of the government.

Table 9: Distribution of respondents according to their use of Information Sources

Sl. No.	Category	Frequency	Rank
1	News Paper	2	5
2	FEO'S	71	3
3	Relatives	135	1
4	Friends	108	2
5	Radio	4	4

Fig. 9: Chart showing the frequency of source of information of the respondents



From the present study it was revealed that the majority of ornamental fish farmer i.e. 135 (42.18%) received necessary information from their relatives. Friends were the most credible sources of information to 108 (33.75%) ornamental fish farmers 71 (22.185) numbers of ornamental fish farmer used to get information from the Government officials. Only 2 (0.62%) received information from news papers and 4(1.30 %) received information through broad casting system (Table 9, Fig.9).

Discussion

The present study indicated that the majority of ornamental fish farmers (both male and female) belonged to middle age group i.e. age group of 26-50 years. In case of female farmers similar results have been reported by Sathiadas and Ashaletha et al., (2003) In case of family size of ornamental fish farmers the majority of the respondents belonged to medium size of family having 5-8 members. The present findings was corroborated with Halder et al., (1998) and Hug et al., (1985) with their findings which was reported that the average family size of the fish farmers. Males were dominated the ornamental fishery business but a large number of females are also engaged in this occupation. Females generally helps in water exchange, feeding , rearing and packaging of he fishes as the male went for other agricultural work. So, it is clear that other members of the family actively participated in ornamental fishery.

Regarding educational status, it is evident that majority of the ornamental fish farmers having madhyamik level of education. Out of 320 respondents only 14 respondents i.e. 4.37% were reported to be illiterate i.e. 95.63% respondents were literate. In this regard Maiti, A, (2003) reported that 90.67% respondents belonged to educational status of literacy level.

The present findings showed that the majority of respondents belonging to scheduled caste community. This work corroborated with the work of Halder et al., (1998), Bhaumik et at., (1991) and Das (2006) in this respect. The present communication also revealed that the majority of the ornamental fish farmers possessed small land area around less than ten katthas.

The present work also indicated that friend, relatives and Government officials were the most credible sources of information to the ornamental fish farmers.

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Original Article

Ecological Studies of Renuka Yellamma Lake, Peddapally, Karaimnagar District, Telangana

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Abstract

Yellamma Lake was constructed during the Kakatiya Regime and Dynasty. In this study we have estimated the total seventeen number of different Physico-Chemical parameters and four major groups of zooplanktons were studied. All the physico-chemical parameters are within the permissible limits. Four different major groups of zooplanktons are Rotifera, Copepoda, Cladocera and Ostracoda. The zooplankton of the selected lake mainly consists of Rotifera, Cladocera, Copepoda and Ostracoda, the total number of fifteen species were identified from the all the four orders during the present study. The total zooplankton population was dominated by Rotifera, Cladocera, Copepoda and Ostracoda respectively.

Keywords: Yellamma Lake; Physico-Chemical; Zooplankton.

Introduction

Water is a precious gift of Nature to this good earth and its bounty. Water is one of the few substances that can be found in all the three states (i.e. gas, liquid and solid) within the earth's climatic range. The solvent properties of water allow the uptake of vital nutrients from the soil and into plants; this then allows the transfer of the nutrients within a plant's structure. The ability of water to dissolve gases such as oxygen allows life to be sustained within bodies of water such as rivers, lakes and oceans.

In studying Hydrology the most common spatial unit of consideration is the catchment or river basin, this can be defined as the area of land from which water flows towards a river and then in that river to the sea. Towards the study of Hydrology, it is useful to consider the parameter of the Hydrological Cycle. This is a conceptual model of how water moves around between the earth and atmosphere in different states as a gas, liquid or solid. There are different scales that the hydrological cycle can be viewed at, but it is helpful to start at the large global scale and then move to the smaller hydrological unit of a river basin or catchment.

The term plankton is taken from the Greek verb meaning "to wander" and refers to organisms whose powers of movement are insufficient to prevent them from being moved by water currents. Commonly planktons are important food source to fishes in the aquatic field.

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Better Quality of water Described by its Physical, Chemical and Biological Characteristics. The physico-chemical and biological parameters are very important in estimating the constituents of water and concentration of pollutant or contaminant.

Methodology

YellammaLake was constructed during the Kakatiya Regime and Dynasty.It is situated in village and mandal of peddapalli, Karimnagar, Talanganaat 18º.36´.52.85½ (N) latitude and 79º.22´.17.47½ (E) longitude and it is distance about 120 kilometers from Kakatiya University campus.

Collection of Samples

Water samples were collected on monthly basis. Collections were made on specific dates of every month. Surface samples were collected using a clean plastic container for the study of various physico-chemical and biological parameters. All the sample

collection and observation were made in the morning time (i.e. 6:00am to 8:00am) and some of the parameters are calculated at the collection point and other parameters were estimated in the lab throughout the study period. Water samples collected for the purpose of estimation of various parameters were brought to the laboratory and subjected to analysis immediately as for as possible Standard Methods per Estimation of Water and Waste Water 20th Edition, 1998 [1] were referred for estimation of parameters.

Zooplankton

Zooplankton collections were made employing a modified Haron-Trantor net with a square metallic frame of area 0.0625 m^2 area. The filtering cone was made up of nylon bolting silk plankton net (No. 25 mesh size 50µ) was used for collection of zooplankton. Collected samples were transferred to labeled vial bottles containing 4% formalin.The qualitative estimation of zooplankton communities was carried out in the laboratory with using of Sedgwick-Rafter cell and planktonic organisms numerically counted and identified. The identification of zooplankton species was done by the Zoological Survey of India, Kolkata and the same was confirmed by [2-7].

Results and Discussion

During the period of study (i.e. October 2013 to September 2014) seventeen number of different physico- chemical parameters of RenukaYellamma Lake were studied.

In the present investigation atmospheric temperature varied between 19.2°C to 33.7°C. Water temperature varied between 17.1°C to 32.0°C. During the study period maximum temperature was recorded in the month of June 2014 and minimum was recorded in the month of December 2013. In the present investigation atmospheric and water temperatures followed more or less similar trend of oscillation [8, 9]. Also suggested, that meteorological conditions are responsible for seasonal changes of temperature.

During the study period transparency and turbidity values were negative correlated each of them. Transparency vales varied between 21.3 cms to 58.2 cms and turbidity values ranged from 9 NTU to 38 NTU. In this investigation maximum transparency values shows in the month of May and minimum values in the month of September. Whereas turbidity values are highest in the month of September and lowest in the month of May. Similar results were observed by [10].

In this study dissolved solids were ranged between 190 mg / lit to 350 mg / lit. Highest values of dissolved solids was recorded in the month of May and lowest values recorded in the month of September. Hydrogen-ion- concentration values varied from 6.9 to 8.6. Highest values of pH was recorded in the month of January and lowest values recorded in the month of May. Electrical Conductivity values varied between 401µmho / cm to 548 µmho / cm. In this study maximum values of Electrical Conductivity was recorded in the month of July and lowest values recorded in the month of January. Similar results were observed by [11].

The results are clearly stated that the more transparency values may be due to less dissolved solids. Similar reports were made by [12], while studying the lakes of Gulbarga district [13]. Observed that pH is positively associated with total dissolved solids, electrical conductivity.

During the study period Dissolved oxygen values varied between 3.8 mg / lit to 9.5 mg / lit. In this study maximum values was observed in the month of March and minimum values observed in the month of September. The values of free carbon dioxide varied between 0 mg / lit to 8.7 mg / lit. Free carbon dioxide values are absent in south west monsoon and north east monsoon seasons. The distribution of oxygen is a net result of consumption for oxidation of organic matter and replacement from the atmosphere.

Calcium, magnesium, carbonates, bicarbonates, sulphates, chlorides, nitrates and organic matter together associate and forms hardness and alkaline nature of water. In the total hardness calcium values are varied between 30 mg / lit to 64 mg / lit and magnesium values are between 68 mg / lit to 155 mg / lit. The hardness values are highest record in the month of July and lowest in the month of March. In the total alkalinity, carbonate values are varied between 1.6 mg / lit to 8.2 mg / lit and bicarbonate values are between 140 mg / lit to 347 mg / lit. The alkalinity values are highest recorded in the month of February and lowest in the month of July. The total alkalinity values provide guidance in applying proper doses of chemicals in water and waste water treatment processes, particularly in coagulation; softening and operational control of anaerobic digestion [14, 15] studied on physico-chemical parameters and observes the range of alkalinity values from 168ppm to 462ppm.

In the present investigation Chloride values are varied from 48 mg/lit to 92 mg/lit. Highest values recorded in the month of April and lowest values in the month of January. In the present investigation Sulphate values are varied from 6.4 mg/lit to 17.3 mg/lit. Highest values recorded in the month of September and lowest values in the month of October. In the present investigation Phosphate values are varied from 1.4 mg/lit to 2.2 mg/lit. Highest values recorded in the month of September and lowest values in the month of June. The concentration of chlorides in natural waters is generally bears a strong correlation with sodium content and specific conductance [16]. Also attributed high chloride value due to increase of organic matter. Phosphorous controls the algal growth and primary productivity. Similar results are also made by [17].

Biological oxygen demand is nothing but the amount of oxygen utilized by microorganisms to stabilize the organic matter. According to BIS specifications potable water should be zero. But Biological oxygen demand value of 3-4 mg/lit is permissible. In the present investigation Biological oxygen demand values are varied between 2.2 mg/lit to 4.6 mg/lit. Highest values were observed in the month of February and the lowest value in the month of October.

Zooplankton

Zooplanktons are usually considered to be good indicators of environmental changes and have a fundamental role in energy flow and nutrient cycling in aquatic ecosystems.

Table 1: Monthly variation of physico-chemical parameters of Renuka Yellamma Lake during the year 2013-2014.

S. No.	Parameter	13-Oct	13-Nov	13-Dec	14-Jan	14-Feb	14-Mar	14-Apr	14-May	14-Jun	14-Jul	14-Aug	14-Sep
1	Atmospheric Temperature	22.6	20.8	19.2	19.8	25.5	30	31.2	33.5	33.7	29.6	27.5	24.4
2	Water Temperature	21.1	19.3	17.1	17.6	24	28.5	28.7	32	31.5	28.1	26.5	23.1
3	Transparency	32	31.4	32.4	40.1	42.6	51.6	54.2	58.2	39.8	28.2	26.2	21.3
4	Turbidity	30	30	30	15	14	11	10	9	16	29	13	38
5	Total Dissolved Solids	270	290	220	266	250	260	320	350	320	230	200	190
6	pH	7.7	8.1	8.3	8.6	8.1	7.2	7.3	6.9	7.1	7.2	8.1	8.3
7	Electrical Conductivity	438	456	450	401	444	488	436	496	523	548	518	491
8	Phosphates	2.2	1.9	1.5	1.8	1.3	1.6	1.6	1.9	1.4	2.1	2.1	2.2
9	Chlorides	53	61	55	48	81	86	92	79	62	70	58	52
10	Sulphates	6.4	8.2	6.8	7.2	10.1	11.6	12.4	12.9	15.9	16.2	16.8	17.3
11	Total Hardness	35	39	44	50	39	31	41	30	43	58	62	64
12	Total Hardness-mg	135	136	126	132	78	68	80	138	155	144	136	134
13	Dissolved Oxygen	5	6.4	6.1	8.4	9.1	9.5	8.8	8.4	5.2	4.6	3.8	3.8
14	Free CO2	Ab	Ab	Ab	4.6	5	8.7	5.6	Ab	Ab	Ab	Ab	Ab
15	Alkalinity- CO3	1.6	2.4	2.6	2.2	2.9	4.2	4.8	8.2	8.1	6.2	5.3	2.4
16	Alkalinity-HCO3	288	262	258	266	347	326	320	292	196	140	192	187
17	Biological Oxygen Demand	2.2	3.6	3.1	4.2	4.6	4.5	3.9	3.4	2.9	2.9	2.4	2.6

In the present study a comparison account was studied in RenukaYellamma Lake. The annual and seasonal fluctuation and composition of zooplankton were depicted in Table 2.

The zooplankton of the selected lake mainly consists of Rotifera, Cladocera, Copepoda and Ostracoda, the total number of fifteen species were identified from the all the four orders during the present study. The total zooplankton population was dominated by Rotifera, Cladocera, Copepoda and Ostracoda respectively.

In the present investigation Rotifera group of zooplanktons showed high diversity followed by Copepoda, Cladocera and Ostracoda. Among the Rotiferans*Keratellatropica* was dominated species followed by *Brachionus falcatus, Keratellacochlearies, Brachionusangularies, Brachionuscaudatus Cephalodellagibba* and *Lecaneluna* respectively. In group the of Copepoda*Paracyclopsfimbriatus* was dominated followed by *Mesocyclopsleukarti* and *Mesocyclopshyalinus*. In the group of Cladocera*Ceriodaphniacornuta* species was dominated followed by *Moinabranchiata* and *Moinamacrocopa*. In Ostracoda group *Heterocypris* species was dominated followed by *Cypris* species [18]. Did studied on the diversity of zooplankton in Kandlapally lake, Telangana.

Table 2: Zooplankton diversity at station A of Yellamma Lake, Peddapally during the year 2013-2014

	13-Oct	13-Nov	13-Dec	14-Jan	14-Feb	14-Mar	14-Apr	14-May	14-Jun	14-ul	14-Aug	14-Sep	Total
Rotifera													
Brachionusangularies	44	58	72	70	89	98	61	52	36	22	16	31	**649**
B. caudatus	32	28	36	39	32	48	50	36	28	12	12	8	**361**
B.fulcatus	96	132	146	152	161	210	140	70	50	32	16	0	**1205**
Keratellatropica	38	160	172	180	210	221	152	110	62	30	0	0	**1335**
K. cochlearies	140	142	164	191	210	180	60	60	0	0	18	0	**1165**
Cephadellagibba	25	35	45	36	51	41	41	8	4	4	0	12	**302**
Lecunaluna	11	14	18	26	42	51	29	26	14	8	0	0	**239**
Total	**386**	**569**	**653**	**694**	**795**	**849**	**533**	**362**	**194**	**108**	**62**	**51**	**5256**
Copepopda													
Paracyclopsfimbratus	11	11	46	86	110	140	160	110	80	60	80	62	**956**
Mesocyclopshyalinus	14	18	26	48	112	124	82	62	28	10	8	8	**540**
Mesocyclopsleukarti	8	8	26	48	52	52	82	120	80	122	98	110	**806**
Total	**33**	**37**	**98**	**182**	**274**	**316**	**324**	**292**	**188**	**192**	**186**	**180**	**2302**
Clodocera													
Ceriodaphniacornuta	189	241	101	52	43	11	8	10	19	96	41	30	**841**
Moinamacrocopa	30	30	31	12	16	18	22	10	10	60	41	11	**291**
Moinabranchiata	42	36	18	22	26	10	8	8	36	82	62	42	**392**
Total	**261**	**307**	**150**	**86**	**85**	**39**	**38**	**28**	**65**	**238**	**144**	**83**	**1524**
Ostracoda													
CyprisSps.	12	18	16	42	70	110	122	136	82	34	42	28	**712**
Heterocypris	14	8	28	92	88	132	151	80	74	44	52	40	**803**
Total	**26**	**26**	**44**	**134**	**158**	**242**	**273**	**216**	**156**	**78**	**94**	**68**	**1515**

During the study period total highest number of zooplankton species were observed in the month of March (1446 No. of individuals / liter) and lowest number of zooplankton species were observed in the month of September (382 No. of individuals / liter)

Conclusion

In the present investigation all the selected parameters are within the permissible limits of Bureau of Indian Standards (BIS), American public health Association (APHA) and American Water Works Association (AWWA). The zooplankton diversity of this lake is also clearly stated that the selected lake was not much polluted.

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Original Article

Comparative Haemato-Biochemical Studies on *Cirrhinus mrigala* and *Cirrhinus reba*

Acharya Gayatri*, Prafulla Mohanty K.**

Abstract

The present investigation is an attempt to study the comparative haemato-biochemical parameters of *Cirrhinus mrigala* and *Cirrhinus reba.* Alive freshwater fishes were collected from a freshwater pond of Kausalyaganga, Bhubaneswar and Odisha. Blood samples were then taken from the caudal vein of fishes. Haematological parameters such as haemoglobin (Hb), total erythrocyte count (TEC), total leucocyte count (TLC), packedcell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) and the serum biochemical parameters like protein, albumin, globulin, glucose and cholesterol were thoroughly studied and each parameter was expressed as mean ±SE .All the hemato-biochemical parameters showed slight fluctuation between the species. The results showed that RBC count, haemoglobin, WBC count and MCHC showed higher value in *C. mrigala*. PCV, MCVand MCH were found to be an elevated level in *C. reba* than *C.mrigala*. Except MCV none of the haematological parameters differ significantly between the *Cirrhinus* species. All biochemical parameters showed higher value in *C.reba* than *C.mrigala* but are not significantly different except albumin and cholesterol. The findings of the present study used as a reference value as well as a valuable tool in monitoring fish health.

Keywords: Haemato-Biochemical Parameters; Freshwater; *Cirrhinusmrigala; Cirrhinusreba.*

Introduction

The haematological characteristics can be used as an effective tool to monitor physiological and pathological changes in animals. Normal ranges for various blood parameters in fish have been established by different investigators in fish physiology and pathology [4, 24]. Haematological and biochemical parameters are being used as indicators in the measurement of health conditions and toxicological symptoms of organisms [18].These parameters provide information about the health status of organisms, also indicates the abnormal environmental conditions[6]. Information about the existence, status and degree of possible sickness in organisms can be rapidly obtained by haematological and biochemical parameters. One of the difficulties in assessing the state of health of natural fish population has been the lack of reliable references of the normalcondition[10, 24]. Although fish haematology is an effective tool but normal range for blood parameters are inadequate and literature in this area is often incomplete [24]. Despite advances in fish medicine in recent years, interpretation of fish haematology is often troubled by a lack of meaningful reference values [2]. Only a few normal values for a small number of haematological parameters have been established for someteleosts, but these values vary widely due to the lack of standardized collecting and measuring techniques. Number of factors cause normal and abnormal variations in haematological data [2] such as species and strain [11], temperature [11, 14], age [26], season [7], stress[3], photoperiod [12], nutritional state [26,13], the cycle of sexual maturity, health condition [20] and water quality. The present paper deals with the comparison of important blood parameters of *Cirrhinus mrigala and Cirrhinus reba.*

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Materials and Methods

The samples were collected from a freshwater pond of Kausalyaganga, Bhubaneswar, Odisha

during August 2014 to December 2014. 30 alive fishes of each species (irrespective of sex and almost of medium size group) were taken and brought to the laboratory. Blood samples were drawn from caudal vein into two different vials, one containing EDTA (ethylenediaminetetra acetic acid) for haematological studies and the other without EDTA allowing to clot and serum to separate for studying some biochemical constituents. The blood samples were processed for haemoglobin (Hb), totalerythrocyte count (TEC / RBC), total leukocyte count (TLC / WBC) and packed cell volume (PCV) as follow.

Haemoglobin concentration (Hb) was measured by Sahli'sacid haematin method[22]. RBC and WBC were determined using a Neubauer haemocytometer. Haematocrit / PCV valuewas determined by the standard microhematocrit method [16] and expressed in percentage. The following erythrocyte indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) and were calculated as per following formula.

MCV (fl) = PCV / RBC x 10

MCH (pg) = Hb / RBC x 10

MCHC (%) = Hb / PCV x 100

Biochemical parameters of Blood were determined by using standard kits (Crest biosystems, Alto Santacruz Bambolim complex, Goa-403 202, India).

Statistical Analysis

The results are presented as mean± SE. Difference in parameters between *C.mrigala and C.reba* was analyzed using t-test.

Result and Discussion

The haematological parameters of *C.mrigala* and *C.reba* are discussed in detail (Table-1)

Haematological analyses of fish are important as these are linked to fish health. Fish are exposed to

Table 1: The haematological parameters of *Cirrhinus mrigala* and *Cirrhinus reba*

Haematological parameters	*Cirrhinusmrigala* (30)	*Cirrhinusreba* (30)
RBC(mllions/mm³)	1.83±0.04	1.75 ±0.05
Hb(gm/dl)	6.26 ±0.26	6.21 ±0.23
WBC(thousands/mm³)	5 ±0.26	4.56 ± 0.24
PCV(%)	20.4 ± 0.79	21.8 ±0.76
MCV (fl)	112.41± 4.65*	126.61± 4.92*
MCH (pg)	34.33 ± 1.42	36.32±1.72
MCHC(%)	30.8 ±0.63	28.79± 0.89

(Figures in parentheses indicate number of observations, *significant at p<0.05).

different factors in natural habitat such as pollution, water quality, microorganisms for which they adapt some what to these adverse conditions by changing their physiological activities. Findings of reference value for fish species will help to establish and identify the causes of disease in fish which presents challenge for the ichthyologists. The RBC of fish determines the dissolved oxygen carrying capacity. The result of the RBC count of this work for *C.mrigala* and *C.reba* are 1.83±0.04 to 1.75 ±0.05 millions / mm³ respectively. This finding is similar with the corroboration [5] for control group of *C.mrigala.* The result of Hb obtained of this work for *C.mrigala* and *C.reba* are 6.26±0.26 to 6.21±0.23 g / dl respectively. There is a slight fluctuation in haemoglobin level which may be due to different species but the level of haemoglobin is higher when compared to other carps such as *Catlacatla and Labeorohita* (4.0 ± 0.08 and 4.0 ± 0.05) respectively [9]. Further the result of PCV of this study is20.4 ± 0.79 to 21.8 ±0.76 % for *C.mrigala* and *C.reba* respectively. The value of WBC is slightly higher in *C.mrigala* than *C.reba* but does not vary significantly. In fishes, the significance of WBC and their biological function are not clearly understood. The number of WBC in some species of fish varies greatly with age, season and maturation[17]. The MCV value reflects the size of red blood cells by expressing the volume occupied by a single red blood cell. The present study shows significantly higher value of MCV in *C.reba*than *C.mrigala.* The value of MCH is found higher in *C.reba* than *C.mrigala* but does not vary significantly. The MCHC is observed to be higher in *C.mrigala* than *C.reba*. The high level of MCHC indicates more Hb in a unit of RBC [21].

Serum biochemistry can be influenced by many biotic and abiotic factors such as temperature of water, seasonal pattern, food, age and sex of the fish [8]. In this study, the results of biochemical parameters (Table 2) showed higher value in *C.reba* than *C.mrigala* but are not significantly different except albumin and cholesterol.

Table 2: The biochemical parameters of *Cirrhinus mrigala* and *Cirrhinus reba*

Biochemical parameters	*Cirrhinusmrigala* (30)	*Cirrhinusreba* (30)
Protein(g/dl)	5.68 ± 0.42	6.61±0.42
Albumin(g/dl)	2.78 ± 0.18*	3.31± 0.2*
Globulin(g/dl)	2.9 ±0.41	3.29 ± 0.42
Glucose(mg/dl)	106.3 ± 3.43	106.46 ±3.34
Cholesterol(mg/dl)	186.19 ± 7.91*	210.07±7.61*

(Figures in parentheses indicate number of observations, *significant at p<0.05).

Among all the organisms, the highest level of protein is found in fishes. The level of protein found in our study for *C.mrigala* and *C.reba* are 5.68 ± 0.42 and 6.61±0.42 g/dl respectively. This value is higher in comparison to *Catla catla and Labeo rohita* which is 4.1 ± 0.23 and 3.9 ± 0.3 respectively. The Serum albumins accomplish important functions in the vertebrates, participating in the filtration of tissue fluid, in the transport of biomolecules and in the plastic metabolism [1]**.** In the present study, significantly higher level of albumin is recorded in *C.reba* than *C.mrigala.* Globulin found in *C.mrigala* and *C.reba*is2.9 ±0.41 and3.29 ± 0.42 g/dl respectively. Blood glucose has been shown to be a sensitive biochemical indicator of environmental stress [25, 27]. The blood sugar level represents a dynamic balance between the rate at which the sugar is entering the blood from the liver and the rate at which it is being removed by the body tissue from the blood [23].In this study, the level of glucose is found insignificantly higher in *C.reba.* The elevated blood glucose level reflects an increase in the rate of transportation of glucose probably from the liver to muscle where high energy demand was met due to brisk and erratic movements[19].The cholesterol concentration in this study was 186.19 ± 7.91 and 210.07±7.61mg/dl for *C.mrigala* and *C.reba* respectively. Cholesterol concentration varies both among and within fish species because of variation in diet activity and sexual development [27].

Conclusion

The results of this study provide the knowledge of the characteristics of haematological and biochemical parameters of *C.mrigala* and *C.reba.* This investigation may be helpful as a tool to monitor the health status of this and other related fish species. The evaluation of hematological parameters will grant early detection of clinical pathology as well as the presence of disturbance in the environment.

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Original Article

Role of Thyroxine (T_4) in Calcium, Phosphorus Metabolism in Tissues of Amphibia (Bufo melanostictus)

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Abstract

Current studies, find the role of thyroid hormone, thyroxine (T_4) in regulating the levels of calcium and phosphorous metabolism in bone, blood and muscle of Indian toad, *Bufo melanostictus*. Following seven days of thyroxine treatment, the body weight of toad increased significantly at both dose levels as compared to the control values. Thyroxine at lower doses showed a more pronounced effect with respect to the calcium and phosphorous parameters, corroborating the earlier view that the hormone is anabolic at lower doses, at least in poikilotherms. Both calcium and phosphorous in three tissues (blood, bone, muscle) showed higher levels following thyroxine treatment at higher doses. Such an observation possibly points to speculations that the hormone influences their levels by regulating their rate of absorption in the digestive tract or rate of excretion in kidney tubules. Alternatively one might suggest inhibitor of thyrocalcitonin by exogenous thyroxine directly through feed-back control or indirectly via the actions of parathyroid hormone.

Keywords: Thyroxine (T_4); Calcium; Phosphorus and Bufo Melanostictus.

Introduction

Ionic calcium is an integral requirement for many biological metabolic processes such as muscular contraction, nerve stimulation, change in permeability of cell membrane, the mediation of hormonal and cell receptor activities, fertilisation of the ovum, separation of chromosome in dividing cells, the beating of flagella, and the enzymatic cascade concerned with blood coagulation and so on. Its determination in biological system is extremely complex. Ionic calcium acts as a bridge between stimulus on one hand and biological and chemical responses on the other. It is useful for formation of bone and teeth. Deficiency develops rickets and muscle spasms.

Phosphorus or phosphate is important for formation of bone, teeth and bio-membranes. It keeps muscle and nerve activity normal, synthesis of nucleic acid (DNA, RNA) and ATP. Deficiency of phosphate causes loss of bone minerals, many metabolic disorders including cardiac muscle nerve disorder. It helps in energy transfer, cell division, and phosphorylation reactions etc.

Small quantity of calcium and inorganic phosphates present in the extracellular fluid are in equilibrium with the immense reservoir of calcium and phosphate in bones. The ions of calcium and phosphate are widely distributed in the extracellular fluid is under control by the parathyroid hormone (PTH). PTH influences both intestinal absorption of calcium, renal re-absorption of calcium and phosphates which are mediated through cholecalciferol (Vit-D_3). It mobilises calcium from the skeleton and calcitonin inhibits the release of calcium from the bones. Together they may be responsible for the remodelling and growth of bones. Cholecalciferol not only a vitamin to prevent rickets but also modulates the effects of PTH on osteoclasts in bone. Calcitonin is secreted when calcium level is high in the blood, then it lowers the calcium level by suppressing release of calcium ions from the bones. It influences tubular re-absorption of many ions like Ca, P, Na, K, Mg. Role of calcitonin in man is not clear. Thus calcitonin has an action opposite to that of PTH hormone on calcium metabolism.

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Bone formation involves the formation of matrix and deposition of calcium salts. These processes are influenced by a number of endocrine factors. The role of parathormone and of thyrocalcitonin in the control of calcium and phosphorous metabolism in tissues of vertebrates are not withstanding. There are few reports on the involvement of thyroid hormones in the metabolism of two parameters like estimation of calcium and phosphorous particularly in bone tissues. Adequate thyroid hormone is necessary for normal bone development. In hypothyroidism, skeletal growth and maturation are in paired while in hyperthyrodism children, linear growth is accelerated (Mundy and Raisz, 1979; Schlesinger et al., 1973). In adult hyperthyrodism, decreased bone mass is associated with increased osteoclastic activity and a high rate of bone turnover (Melsen and Mosekilde; 1977).In cartilage, thyroid hormone can enhance growth by increasing somatomedin production or sensitivity (Phillips and Vassilopoulou-Sellin, 1980 a; b; Thorngren and Hansson, 1977). The molecular mechanism by which thyroid hormone affects skeletal development is unclear (Raisz and Kream, 1981). In spite of increased osteoclastic bone re-absorption by thyroid hormones invitro, it could be responsible for enhanced bone turnover and remodelling (Mundy et al., 1976).In the view of above findings, in the present study, the calcium and phosphorus turnover in some tissues like bone, muscle and blood of Indian toad, *Bufo melanostictus* by thyroxine (T_4) treatment is accessed.

Materials and Methods

The common Indian toad, *Bufo melanostictus* of mixed sexes were collected from nature during evening time and were transferred to the laboratory within 12 hours. They were maintained in lab condition in wire netted wooden cages (75 x 40 x 35 cm in size) containing a moist sand bed for about 5 days. They were forced feed with about 1 gm of goat liver (Composition mg/gm wet-weight: 110 $\pm$41 protein, 84 $\pm$ 16 lipid, 2.3 $\pm$1.1 glycogen) each on every day and water was provided *ad libitum*.

Treatment

After laboratory acclimation, animals were divided into control and experimental groups. The experimental groups of toad were injected intramuscularly with thyroxine ($T_{„}$) Na-salts (fluke AG) at a dose of 0.5 µg / gm (Treated – I) and 2.0 µg / gm (Treated – II) in separate batches, dissolved in 0.65 % of NaCl solution pH = 8.3. The control animals received an equal volume of 0.65 % of NaCl solution pH = 8.3. This injection schedule was continued daily for 7 days so that each animal receives 7 doses. On the eighth day of treatment, the animals were sacrificed for estimation of biochemical parameter after taking their final body weight.

Collection of Tissues extract

At the end of the treatment, the animals were sacrificed by pitching on the head; blood, muscles & bone were quickly dissected out. Blood was collected from heart of the animal with the help of a hypodermic syringe containing 2ml of 2% sodium citrate solution. The muscles from hind limb were transferred to cold Amphibian Ringer solution and adherent connective tissues, blood vessels, nerve fibres were removed. Then blotted off with whatman filter paper No – 1. Long bones (Humerus) were taken out and cleaned off adherent materials in distilled water. All these 3 tissue extracts were collected by centrifuging at 2000 rpm for 10 minutes; those were used for estimation of biochemical parameters.

Estimation of Calcium

The calcium contents of 3 extracts of Bone, Muscle &Blood were estimated independently by the method of Kramer and Tisdall (1921) as modified by Clark and Collip (1925).

Estimation of Phosphorus

The phosphorus contents of 3 extracts of Bone, Muscles & Blood were estimated by the method of Fiske and Subbarow (1925).

These data were statistically analyzed by the student ***t – test*** (Abramoff and Thomson, 1966; Bishop, 1966.

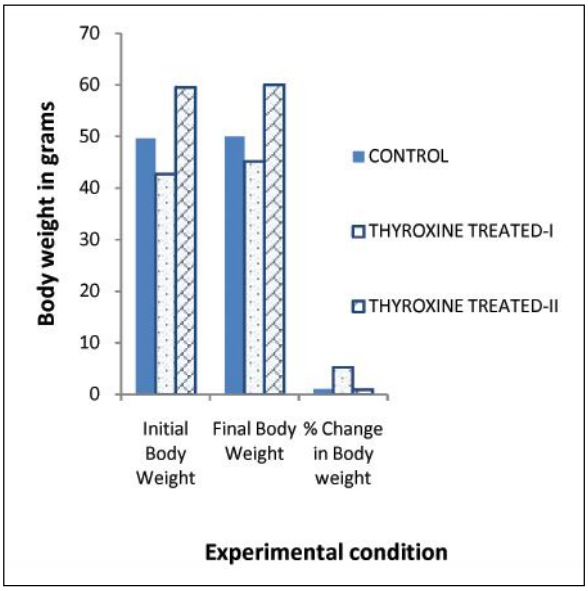
Results

Body weight: During the course of experiment the body weight of all animals have been found to increase. But the rate of increase (% change) in body weight based on individual values were found to be significantly high in T_4 treated animals as compared to that of the control animals (table-1, Fig-1).

Table-1: *In vivo* affects of thyroxine (2 doses) on body weights of Indian toad, *Bufo melanostictus. Values for initial and final body weights in grams (mean values). The % changes in body (mean value) are based on the individual values of initial and final body weights. Numbers in parentheses indicate the number of animals used.*

Experimental Condition	Initial Body Weight	Final Body Weight	% Change in Body weight
Control	49.7 (24)	50 (24)	1.058
Thyroxine treated-i	42.77 (22)	45.18 (22)	5.29
Thyroxine treated-ii	59.55 (20)	60.1 (20)	0.91

Fig. 1: Effects of thyroxine (0.5µg/gm; 2 µg/gm) on body weight and % change in body weight after 7 days of treatment, of *Bufo melanostictus*. Values for body weight are in gram. Columns represent the mean values



Calcium content

Thyroxine treatment for 7 days did not change the calcium content in any of the tissues studied at lower doses (0.5 mg / g) when compared to the control values. Thyroxine treatment at higher doses (2.0mg/g) increased significantly (P< 0.001) the levels of calcium in blood, muscle & bone after 7 days of treatment.

A comparison of data from two treated doses showed significantly high levels of calcium in all the 3 tissues of higher dose treated animals. (Figs. 2-4, table 2-4).

Phosphorus content

Thyroxine treatment for 7 days did not change the phosphorous content in bone & muscle tissues studied at lower doses (0.5mg/g) when compared to the control values, but in blood the content decreased significantly when compared to the control values.

Thyroxine treatment at higher doses (2.0mg/g) increased significantly (P<0.001) the levels of phosphorus in blood, bone, and muscle after 7 days of treatment.

A comparison of data from two treated doses showed significantly high level of phosphorus in bone, muscle (P0.001) and blood (P<0.01) of higher dose treated toads (Fig. 2-4; table 2-4).

Table 2: *In vivo* effects of thyroxine (2 doses) on calcium and phosphorous levels in blood of Indian toad, *Bufo melanostictus. Values are mg/100 ml of blood (Mean±SEM). Numbers in parentheses indicate the number of animals used, NS, not significant at 0.05 confidence level.*

Experimental Condition	Calcium	Phosphorous
Control	40.79 ± 2.18 (12)	3.1 ± 0.204 (9)
P	NS	< 0.05
Thyroxine treated-i	43.67 ± 1.02 (6)	2.47 ± 0.241 (8)
P	< 0.001	< 0.01
Thyroxine treated-ii	80.74 ± 4.26 (9)	4.24 ± 0.36 (8)
P	< 0.001	< 0.001

Fig. 2: Effects of thyroxine (0.5µg/gm;2µg/gm)on the calcium and phosphorous content of blood after 7 days of treatment.Values for calcium and phosphorous are mg/100 ml of blood; columns represent the mean values and vertical bars SEM.

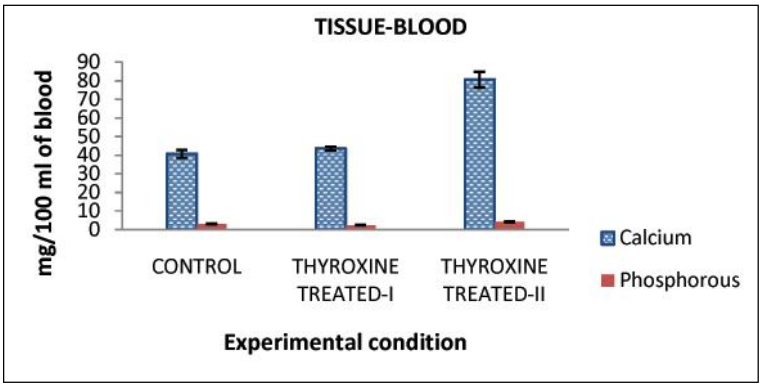


Table 3: *In vivo* effects of thyroxine (2 doses) on calcium and phosphorous levels in muscle of Indian toad, *Bufo melanostictus. Values are mg/gm t*issue wet-weight *(Mean±SEM). Numbers in parentheses indicate the number of animals used, NS, not significant at 0.05 confidence level*

Experimental Condition	Calcium	Phosphorous
Control	0.409 ± 0.018	0.177 ± 0.0089
P	(10)	(14)
	NS	NS
Thyroxine treated-i	0.442 ± 0.026	0.179 ± 0.015
P	(9)	(8)
	< 0.001	< 0.001
Thyroxine treated-ii	0.94 ±0.030	0.38 ± 0.014
P (between control and treated-"ii")	(9)	(10)
	< 0.001	< 0.001

Fig. 3: Effects of thyroxine (0.5µg/gm;2µg/gm)on the calcium and phosphorous content of muscle after 7 days of treatment. Values for calcium and phosphorous are mg/gm tissues wet weight; columns represent the mean values and vertical bars SEM.

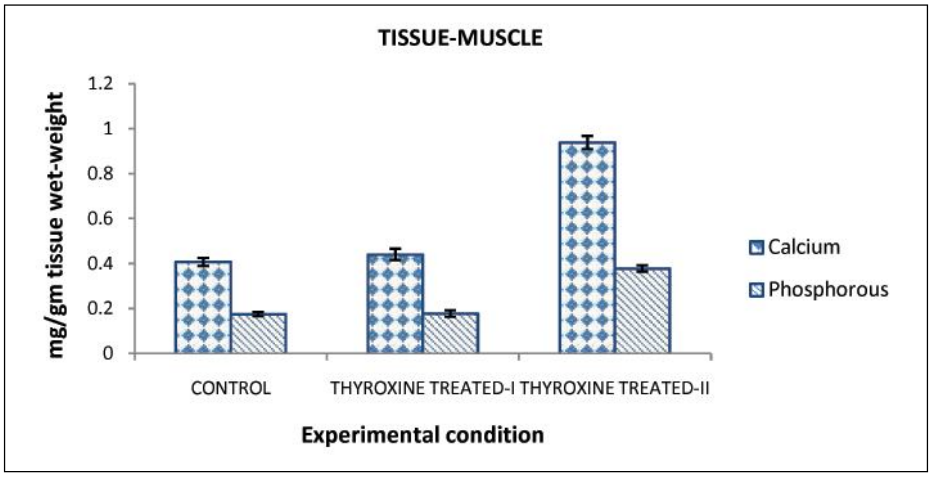
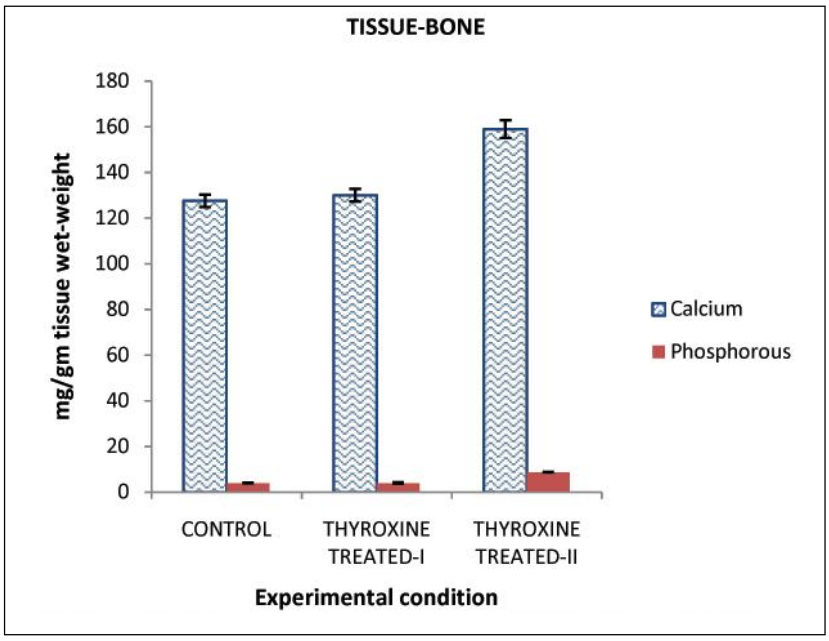


Table 4: *In vivo* effects of thyroxine (2 doses) on calcium and phosphorous levels in bone of Indian toad, *Bufo melanostictus. Values are mg/gm t*issue wet-weight *(Mean±SEM). Numbers in parentheses indicate the number of animals used, NS, not significant at 0.05 confidence level.*

Experimental Condition	Calcium	Phosphorous
	127.63 ± 2.72	3.99 ± 0.275
Control	(14)	(15)
P	NS	NS
	130.12 ± 2.74	3.95 ± 0.37
Thyroxine treated-i	(6)	(9)
P	< 0.001	< 0.001
	159.06 ± 3.95	8.73 ± 0.16
Thyroxine treated-ii	(10)	(10)
P	< 0.001	< 0.001

Fig. 4: Effects of thyroxine (0.5µg/gm;2µg/gm)on the calcium and phosphorous content of bone after 7 days of treatment. Values for calcium and phosphorous are mg/gm tissues wet weight; columns represent the mean values and vertical bars SEM.



Discussion

The thyroid gland synthesises and releaseT_3 and T_4. The biological active hormones T_3 (K.Boelaert, JA Franklyn, 2005) and T_4 play a significant role in growth, development and function of all major tissues. The hormone thyroxine appearing during the course of evolution in vertebrates only, has attracted the attention of biologists as a substances involved in the control of cell multiplication (Rudland and Jumnez, 1976; Gibson et al., 1978). The thyroid hormones increase cellular respiration and thereby increase the basal metabolic rate (BMR) (Jack DeRuiter, 2001).This hormone appears to have regulatory influence on almost all the metabolic events, although the mechanism of action at subcellular level has become a subject of intensive investigative effort and furious debate in recent years. Like steroid hormones, the thyroid hormones are relatively hydrophobic and lipophilic, solubilised and transported in the plasma with aid of carrier proteins and exert their action intra-cellularly after penetrating the plasma membrane (Kochupillai and Ramalingaswami, 1990).

Thyroid hormone synthesis and secretion is regulated by the negative feedback system that involves the hypothalamus, pituitary and the thyroid gland (Shupnik et al, 1989). Thyroid hormones are particularly important as regulators of differentiation during development. A closely related function is that of a stimulator of oxidative reactions and general regulators of metabolic rates in the body. Increased thyroid hormone is associated with increased oxygen consumption, body temperature, pulse systolic blood pressure, mental and physical vigour, irritability, the lipolysis and decreased cholesterol level in blood. Although a number of effects of thyroid hormones on specific metabolic reactions have been demonstrated, a unifying concept of mechanism is not yet apparent. This is, in part, due to the different effects noted when the hormone is studied at physiologic levels or at unphysiologic high doses.

Thyroid hormone involvement in ionic regulation is general and metabolic pathway like calcium & phosphorus in particular. Small amounts of thyroxine given to young growing animals enhance the retention of calcium. This is probably secondary effect resulting from the protein anabolic action of the hormone which facilitates the deposition of new bone matrix. In hyperthyroid stages, there is increased mobilisation of calcium from the skeleton and increased loss through urine and faeces without affecting the Ca concentration of blood (Turner, 1966). Thyroid hormone not only controlling the Ca & P metabolism, but also influences the metamorphosis of tadpole larvae in general and ossification process in particular (Duellman and Trueb, 1986) promoted us to carry out this study. The dose and route of administration of thyroxine to these animals was determined following the reports of several workers (Medda & Ray; Ghosh, 1982, Begum *et al*, 1984, Achary, 1986). These authors have clearly shown that thyroid hormones are anabolic at lower

doses and catabolic at higher doses. However species specific differences in the responses to doses of thyroxine have been observed. A dose considered anabolic for a species may have catabolic effects on the other.

Administration of thyroxine to toads, every day for 7 days caused an overall increase in the body weight at both dose levels. However the percentage increase in the body weights in animal treated with lower (0.5 µg / g) dose of thyroxine was higher than those treated with higher (2µg / g) dose. However these results are consistent with the observations in certain groups fishes (Barrington *et al.* , 1961 ; Higgs *et al.* , 1961; Higgs *et al.,1976*) , reptiles (Achary, 1986) and birds (Thapliyal *et al*.,1983). Administration of thyroid hormones to premetabolic tadpoles induced myofibrillar protein synthesis and muscle fibre formation in undifferentiated tadpole hind limb tissues (Dhanarajan, 1980).

One might, therefore speculate similar anabolic changes with respect to nitrogen metabolism in adult amphibians of the present study. Adequate thyroid hormone is necessary for normal bone development. Data on Ca & P content of bone, blood & muscle of adult toads showed increased levels following thyroxine treatment at higher doses.

These observation clearly points to increased retention of these substances in the tissues of toads by thyroxine, making them sufficiently available for incorporation into bones and / or to be utilised by other tissues for different metabolic processes. Such retentions of Ca & P could possibly be mediated through an increase in somatomedin production or sensitivity (Phillips and Vassilopoulou-Sellin, 1980 a, b; Thorngren and Hansson, 1977). Another possibility is that thyroxine might be causing Ca & P retention by way of influencing the rates of their absorption in the digestive tract or the rates of their excretion by the kidney tubules.

Exogenous thyroxine as is administered in the present study, might be inhibiting thyroid activity in toads, thus reducing the release of such a factor, consequently leading to increase in the concentrations of both calcium & phosphorus. PTH involved in Ca & P metabolism, however there has been lot of debates as to which of these two (parathormone or thyrocalcitonin) is the actual agent for the control of Ca & P (Turner, 1966).

It has been suggested that parathyroid itself is not the source of hypocalcemic principle but that the parathyroid produce a humoral substance (releasing factor) which stimulate the thyroid to release thyrocalcitonin. In view implies that parathyroid and thyroid tissues are needed for the regulation of Ca & P in animals (Gittes and Irvin, 1965)

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Original Article

Hematological Analysis of Three Breeds of Cows

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Abstract

Three breeds of cows namely, non descriptive (ND), Red Sindhi (RS) and cross breed Jersey (CBJ) cow, and each having three different age groups (2 years, 6 years and 10 years) were used in this study. Since the reports on the hematological profile with respect to these breeds and age are inadequate, the present study is undertaken. Among the three breeds, the highest (14.36±0.02g/dl) and the lowest (8.51±0.04g/dl) mean hemoglobin concentration were recorded in 2 years CBJ and 10 years ND cow respectively. The highest average total erythrocytes count (11.01±0.06 x10^6) and PCV (51.16±0.60%) were recorded in 6 years ND and 2 years CBJ cow respectively while the lowest value of total erythrocyte count (4.89±0.13 x 10^6) and PCV (26.56±0.21%) were recorded in 2 years and 10 years ND cow respectively. The highest average MCH (22.61±0.62pg) and MCV (71.94±2.11fl) were observed in 2 years ND whereas the lowest average MCH (10.77±0.04pg) and MCV (34.08±0.04fl) were noted in 6 years ND cow. The highest MCHC (32.88±0.22%) and the lowest (28.02±0.33%) were recorded in 2 years RS and 2 years CBJ cow respectively. The highest and the lowest total leukocyte count were recorded in 2 years ND and 6 years RS cow respectively. The significant difference at $p<0.05$ and $p<0.01$ was found among the different age groups of breeds for all the hematological parameters and no significant difference was found for eosinophil and basophil count. The difference may be due to differences in age, breed and physiological status.

Keywords: ND Cow; RS Cow; CBJ Cow.

Introduction

The hematological value during different physiological situations is essential for the diagnosis of various pathological and metabolic disorders, which can adversely affect the productive and reproductive performance of cows, leading to heavy economic loss [1]. Physiological equilibrium is maintained mainly by the blood in the body [2] but this equilibrium is altered in various physiological conditions changing the homeostasis of animals. In veterinary medicine, hematological examinations present an effective tool in monitoring the health and nutritional status of animal [3]. Age [4-7], sex [8], breed [4, 9], exercise [4, 10], pregnancy and lactation [11-13] and emotional states] [4] are variables to be considered when establishing reference values in domestic animals. Physiological variables such as recent activity and stress have an impact on hematological value in cattle. Despite the range, sensitivity and technology used, cattle hematology reference intervals are uniformly broad [14]. A complete blood count is a good indicator of general health, as stress and seasonal illnesses can modify hematological parameters, especially with regard to erythrocyte and lymphocyte count [15]. Seasonal parasite burdens may also alter the complete blood count (CBC) [14]. A complete blood count is undisrupted, the most important diagnostic method available to veterinarians, along with proper anamnesis and a physical examination of the animal [15]. Seasonal and environmental changes may influence the value of hematology hematology [16]. Numerous hematological and biochemical changes are associated with liver damage caused by liver flukes [17]. Aging results in WBC changes. As bovine adults age, the concentration of neutrophils and lymphocytes decreases but lymphocytes continue to be the predominant cell type [14]. Ruminants hemoglobin are of particular interest because of the large amount of polymorphism that occurs between

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species, breeds and even within the individual as it develops from embryo to adult [14]. The packed cell volume (PCV) is one of the most valuable techniques for determination of the percentage of cellular component of blood in the clinical laboratory [16]. Mean corpuscular volume is more valuable than blood film examination in assessing the true size of erythrocytes [18]. Basically an experienced pathologist performs bright-field microscopy in order to characterize the cells – red blood cells, white blood cells and platelets. However, differential counting of WBC is one of the major pathological issues in diagnosing of many health hazards. [19]. Reports on the hematological profile with respect to breed (non descriptive (ND), Red Sindhi (RS) and cross breed Jersey (CBJ)) and age, (2 years, 6 years and 10 years) are inadequate, for which the present study is designed.

Materials and Methods

Hematological method

After disinfecting of the sampling area, blood samples were taken from the jugular vein [4, 20] of each individual cow and blood smears were immediately performed. After drying the smears by waving the air [4, 18] they were treated with methanol (Qualigens scientific India Pvt. Ltd., Mumbai, Maharashtra, India) for 1-5 minutes [20]. Since collection of blood directly into a vacuum tube is preferred to collection of blood by syringe and transfer to vacuum tube which reduces platelet clumping and clot formation in samples for CBC determination [21] dry and sterilized needle (Dispo Van Single Use Needle, Hindustan Syringes & Medical Devices Ltd., Faridabad, India) and dry syringe (Dispo Van Single Use Syringe, Hindustan Syringes & Medical Devices Ltd., Faridabad, India) was used for collection of blood [4]. Since EDTA is an excellent anticoagulant [4, 21], the needle of syringe was inserted through the purple cap of EDTA vial (K_3 EDTA, 2ml * 13 75mm, Mfg By: HXS Tech Co., Ltd.PRC. For: Peerless Biotech Pvt. Ltd., Chennai, Tamilnadu, India). Estimation of hemoglobin was done by Sahli's acid hematin method [22] with Sahli's haemometer (HiMedia GW 191-1NO, Plane haemometer (Square Type), HiMedia Laboratories Pvt. Ltd., Mumbai, Maharashtra, India). PCV was done by centrifugation (REMI CENTRIFUGE, Catalogue No.C852 7/94 and Serial NO. GCLC-1632, REMI MOTORS, Bombay, Maharashtra, India) of blood at 3,000 rpm for 15 minutes [23].

Total erythrocyte count (TEC) and total leukocyte count (TLC) were done by using the conventional method [20] by using Neubauer's counting chamber. Erythrocyte indices like, MCH, MCV and MCHC were studied according to the methods described by earlier worker [4]. Differential leukocyte (DLC) [4] was assessed by staining the smeared slide (BLUE STAR), PIC 2, Polar Industrial Corporation, Mumbai, India) with Giemsa stain prepared from Giemsa powder Qualigens CAS NO.51811-82-6 Product NO. 39382, scientific India Pvt. Ltd., Mumbai, Maharashtra, India] following the standard hematological procedure [24] under 40X objective of light microscope (LABOSCOPE MICROSCOPES Research microscope M.No. BD-08 B, S. No. 21320 Mfd. by B.D. INSTRUMENTATION, Ambala Cantt, India).

Statistical analyses

Mean ±SE were calculated for each parameter by using Microsoft Office Excel 2007. For comparison of means statistical analyses were done by Paleontological statistics (PAST) version 2.17 [Natural History Museum, University of Oslo] for One-Way Analysis of variance (ANOVA) followed by Turkey's pair wise comparison tests. Differences were classified as significant at $P<0.05$ and highly significant at $P<0.01$.

Results

The value of erythrocyte parameters with respect to age and different breeds of cows are illustrated (Table 1) and the values of leukocyte parameters with respect to age and different breeds of cows are illustrated (Table 2). Among the three breeds the highest and the lowest mean hemoglobin concentrations were recorded in 2 years CB Jersey and 10 years ND cow respectively. The highest average total erythrocytes count and PCV were recorded in 6 years ND and 2 years CBJ cow respectively while the lowest values were recorded in 2 years and 10 years ND cow respectively. The highest average MCH and MCV were observed in 2 years ND whereas the lowest were noted in 6 years ND cow. The highest and the lowest MCHC were recorded in 2 years Red Sindhi and 2 years CBJ cow respectively. Different hematological parameters of ND cow, RS cow and CBJ cow are recorded (Fig.1, Fig.2 and Fig.3). Average percentages of leukocytes in ND cow (Fig.4), RS cow (Fig.5) and CBJ cow (Fig.6) is shown.

As shown (Table 1) for concentration of hemoglobin (g/dl), 2 years CBJ cow reflect highly

Table 1: Erythrocyte parameters with respect to age and different breeds of cows

Erythrocyte parameters/Age-groups and breeds		Hb (g/dl)	TEC (x10^6 per mm^3)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (%)
2 years	ND (3)	11.04±0.07^{a}	4.89±0.13^{a}	35.13±0.47^{a}	71.94±2.11^{a}	22.61±0.62^{a}	31.43±0.21^{a}
	RS (3)	10.35±0.02^{a,b,c,d}	7.96±0.05^{a,d}	31.50±0.28^{a,b,c,d}	39.57±0.09^{a,b,c,d}	13.00±0.06^{a,b,c,d}	32.88±0.22^{d,f}
	CBJ (3)	14.36±0.02^{a,b,c,d,e,g}	7.74±0.029^{a,c,e,f,g}	51.16±0.60^{a,b,c,e,f,g}	66.23±2.17^{a,b,c,e,g}	18.59±0.70^{a,b,c,d,e,f}	28.02±0.33^{a,b,c,d,f,g}
6 years	ND (3)	11.86±0.01^{a,b}	11.01±0.06^{a,b}	37.53±0.24^{a,b}	34.08±0.04^{a,b}	10.77±0.04^{a,b}	31.60±0.15^{b}
	RS (3)	13.31±0.06^{a,b,c,d,e}	6.31±0.01^{a,b,d}	45.34±0.08^{a,b,c,d,e}	71.81±0.02^{b,c,d,e}	21.08±0.05^{b,c,d,e}	29.36±0.08^{a,b,c,d,}
	CBJ (3)	13.31±0.05^{a,b,c,d,g}	7.40±0.03^{a,b,c,e,f}	44.50±0.76^{a,b,c,d,g}	60.33±0.98^{a,b,c,d,e,f,g}	17.96±0.16^{a,b,c,d,e,f}	29.80±0.74^{a,b,c,d,g}
10 years	ND (3)	8.51±0.04^{a,b,c}	5.79±0.03^{a,b,c}	26.56±0.21^{a,b,c}	45.85±0.11^{a,b,c}	14.69±0.01^{a,b,c}	32.04±0.95^{e}
	RS (3)	13.86±0.06^{a,b,c,d,e}	6.78±0.04^{a,b,c,d,e,f}	46.16±0.60^{a,b,c,d,f}	68.05±0.55^{b,c,d,f}	20.44±0.11^{a,b,c,d,f}	30.04±0.28^{c,d,f}
	CBJ (3)	13.38±0.03^{a,b,c,d,g}	7.13±0.04^{a,b,c,d,g}	44.36±0.31^{a,b,c,d,g}	62.22±0.23^{a,b,c,d,e,f}	18.76±0.08^{a,b,c,d,e,f}	30.15±0.19^{c,d,g}
F value		1662**	221.9**	316**	174.1**	148.1**	22.14**

[1]Mean±SE with similar superscripts in the same column differ significantly at p<0.05 and p< 0.01

[2]Significant at **p<0.01

[3]Figures in parentheses represent the number of observations in each case

F value: Fischer's value

Table 2: Leukocyte parameters with respect to age and different breeds of cows

Leukocyte parameters/Age groups and breeds		TLC (x10^3 per mm^3)	Lymphocytes (%)	Monocytes (%)	Neutrophils (%)	Eosinophils (%)	Basophils (%)
2 years	ND(3)	26.00±0.26^{a}	72.33±3.17	5.00±1.52^{a}	14.00±2.501^{a}	4.66±0.33	1.33±0.33
	RS(3)	11.73±0.27^{a,b,d}	80.00±6.00^{d}	3.66±0.33^{c}	8.00±4.16^{d}	1.66±1.20	3.00±1.15
	CBJ(3)	14.33±0.14^{a,b,c,d,e,f,g}	56.50±1.50	3.50±0.50^{c}	22.00±1.00^{e}	7.00±0.00	1.00±1.00
6 years	ND(3)	16.68±0.23^{a,b}	74.50±14.50^{b}	3.00±1.00^{b}	16.00±11.00^{b}	4.00±1.00	2.50±1.50
	RS(3)	8.93±0.10^{a,b,c,d,e}	40.66±3.28^{b,d,e}	2.33±0.33^{c}	46.33±1.45^{a,b,c,d,e}	2.00±0.00	2.00±1.00
	CBJ(3)	10.78±0.11^{a,b,c,d,e,f,g,h}	65.00±8.62	2.66±1.20^{c}	17.66±3.17^{e}	10.33±4.80	1.00±1.00
10 years	ND(3)	11.08±0.07^{a,b,c}	51.00±7.00	24.00±3.05^{a,b,c}	17.66±6.88^{c}	2.33±2.33	1.33±0.33
	RS(3)	15.51±0.21^{a,b,c,,d,e,f}	73.00±1.00^{e}	4.33±1.20^{c}	16.66±2.33^{e}	1.00±0.00	1.66±0.88
	CBJ(3)	15.71±0.04^{a,b,c,d,e,g,h}	72.00±3.00	1.00±1.00^{c}	11.00±0.00	10.50±1.50	0.50±0.50
F value		764.50**	4.67*	23.21**	7.02**	2.14^{NS}	0.71^{NS}

[1] Mean±SE with similar superscripts in the same column differ significantly at p<0.05 and p< 0.01

[2]Significant at *p<0.05 and **p<0.01, NS means not significant

[3]Figures in parentheses represent the number of observations in each case

F value: Fischer's value

Fig. 1: Different hematological parameters of Sindhi non descriptive cow

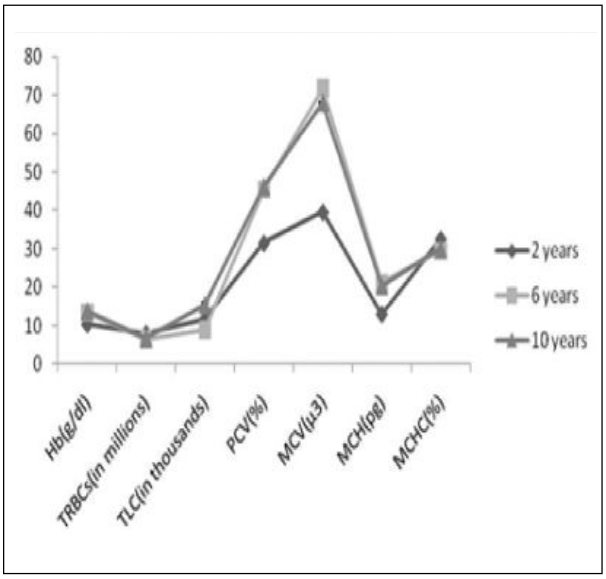


Fig. 2: Different hematological parameters of cow.

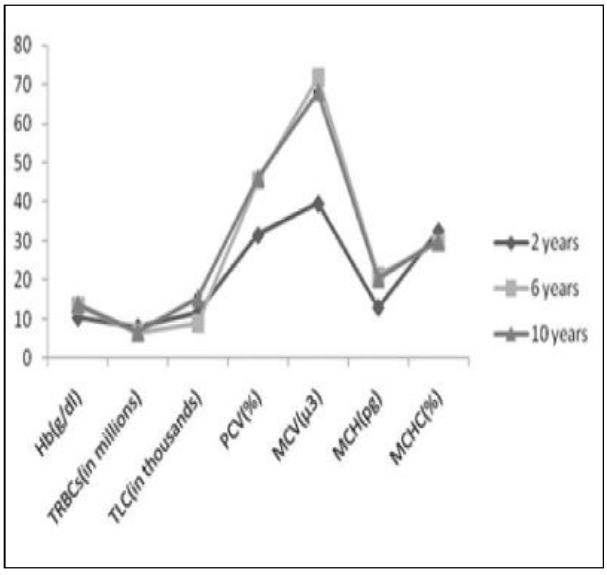


Fig. 3: Different hematological parameters of cross breed Jersey cow

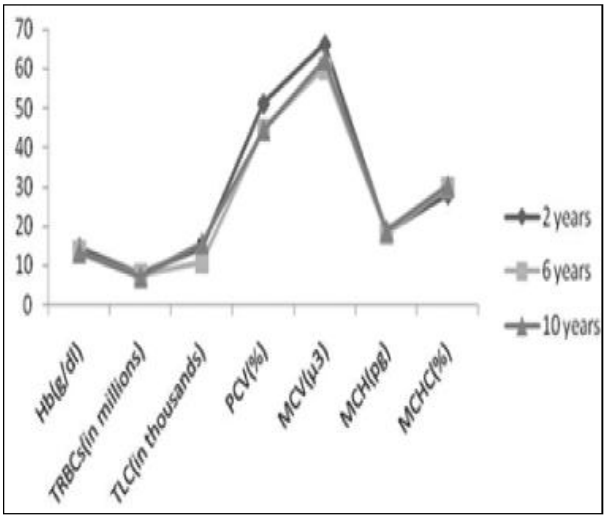


Fig. 4: Average percentages of leukocytes in non descriptive cow

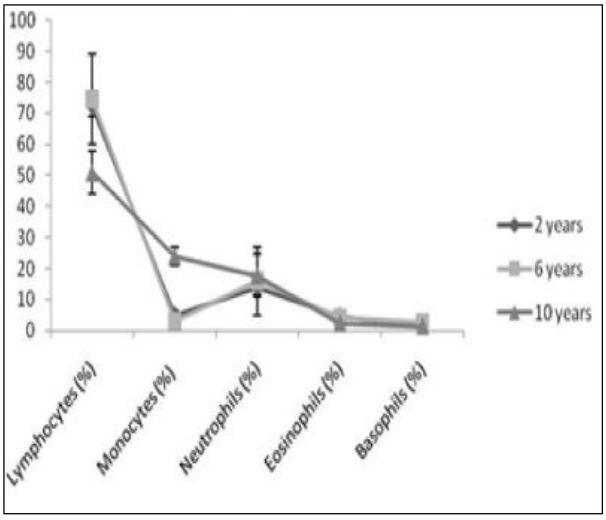


Fig. 5: Average percentages of leukocytes Red Sindhi cow

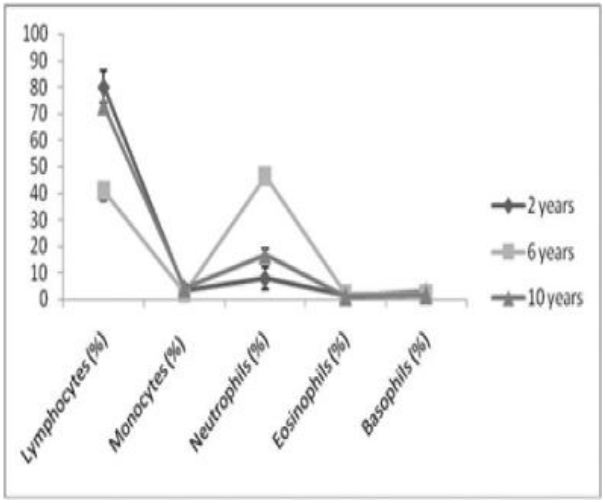
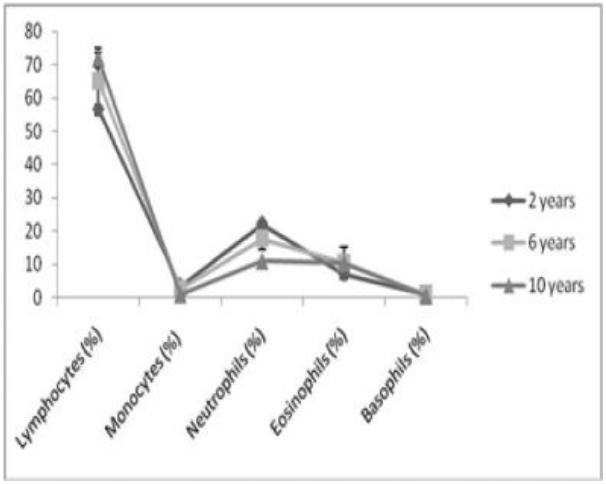


Fig. 6: Average percentages of leukocytes in cross breed in Jersey cow



significant difference (p=0.0001) with each age group of ND and RS cow. Ten years has highly significant difference (p = 0.0002) with each age group of ND, 2 and 10 years RS and 2 years CBJ cows.

For total RBCs count, there exists highly significant difference (p=0.0002) between 2 years and 6 years ND cow. Ten years ND cow has highly significant difference (p<0.01) with 2 years and 6 years ND cow. Two years RS has highly significant difference with 2 years and 6 years ND cow and 10 years RS cow (p=0.0002). Both 6 years and 10 years RS have highly significant difference (p=0.0002) with 2 years and 6 years ND cow. Both 2years and 10 years RS cows have highly significant difference (p<0.0005) with 10 years ND cow. Both CB Jersey 2 years and 6 years have highly significant difference (p=0.0002) with each age group of ND cow and RS cow of 6 years and 10 years. Ten years CB Jersey has highly significant difference (P=0.0002) with each age group of ND cow and RS cow with the exception to 10 years RS cow. Significant difference (p=0.03) exist between CBJ of 2 years and 10 years.

For PCV, significant differences (p=0.029) are there between 2 years and 6 years ND cow. Ten years ND cow has highly significant difference (p=0.0002) with 2 years and 6 years ND cow. There exist highly significant differences (p=0.0002) between 6 years Red Sindhi cow with all the age groups of ND cows and 2 years RS cow. Two years, 10 years RS and all age groups of CBJ cows have highly significant differences (p=0.0002) with all the age groups of ND cow. Besides that, 2 years CBJ has also significant differences (p=0.0002) with 6 years and 10 years Red Sindhi cow. Both 6 years and 10 years CBJ cows have highly significant differences with 2 years CBJ cow (p=0.0002).

For MCV, 6 years ND cow has highly significant difference with 2 years ND cow. Ten years ND has highly significant difference (p=0.0002) with both 2 years and 6 years ND cow. Two years RS has highly significant difference (P=0.0002) with 2 years ND cow. Significant difference (p=0.043) is there between 2 years RS and 6 years ND, and between 2 years RS and 10 years ND cow (p=0.015). Six years RS has highly significant differences (p=0.0002) with 6 years and 10 years ND cow and with 2 years RS cow. Ten years RS has highly significant difference (p=0.0002) with 6 years and 10 years ND cow and with 2 years RS cow. Two years CBJ has significant difference (p<0.05) with 2 years ND and 6 years RS cow. Six years CBJ shows highly significant (p=0.0002) with each age group of ND and RS cow. Two years and 6 years CBJ reflect significant difference (P=0.025). Each age group of ND and RS cow besides 10 years RS have highly significant difference (P<0.0004) with 10 years CBJ cow. Two years CB Jersey and 10 years CBJ reflect significant difference (p<0.05) between them.

For MCH, 6 years ND has highly significance difference (p=0.0002) with 2 years ND cow. Ten years ND cow has highly significant difference (p<0.0002) with both 2 years and 6 years ND cow. Six years RS reflect highly significant difference (p<0.0002) with 6 years and 10 years ND and 2 years RS cow. Ten years RS cow has highly significant difference with each age group of ND and 2 years RS cow. All the age groups of CBJ have highly significant difference with each age group of ND and 2 years and 6 years RS cow. Two years CBJ has significant difference (p<0.05) with 10 years RS cow. Six years and 10 years CBJ has highly significant difference (p<0.05) with 10 years RS cow.

For MCHC, highly significant difference (p<0.01) occur for 6 years RS cow with each age group of ND and 2 years Red Sindhi cow. For 10 years Red Sindhi cow highly significant difference (p<0.009) are there with 10 years ND and 2 years Red Sindhi cow. Ten years Red Sindhi has highly significant difference (p<0.01) with 10 years ND and 2 years Red Sindhi cow. Highly significant difference (p=0.002) exists for 2 years CB Jersey with each age group of ND and 2 years Red Sindhi cow. Two years CBJ and 10 years Red Sindhi reflect highly significant (p=0.007) between them. Highly significant difference (p<0.003) exist between 6 years CBJ and 10 years ND and between 2 years Red Sindhi and 6 years CBJ cow. Ten years CBJ shows significance difference (p<0.05) with 10 years ND cow. Highly significant differences (p<0.005) exist between 2 years and 10 years CBJ cow and between 2 years RS and 10 years CBJ cow (p<0.0005).

The highest and lowest total leukocyte counts were recorded in 2 years ND and 6 years RS cow respectively. As shown in Table II for TLC, 2 years ND has highly significant difference with 6 years ND cow. Both 10 years ND and 2 years RS cow have highly significant differences with 2 years and 6 years ND cow. Six years RS cow has highly significant difference with each age group of ND cow and 2 years RS cow. Ten years RS shows highly significant differences with each age group of ND cow and with 2 years and 6 years RS cow. Two years CBJ has highly significant difference (p<0.008) with each age group of ND and RS cow. Six years CBJ has significant difference (p<0.05) with 2 years and 6 years ND cow and highly significant difference (p<0.003) with 10 years ND and 2 years RS cow. Highly significant difference (p=0.0004) exists between 10 years CBJ and 2 years RS cow and significant difference (p=0.022) exits between 2 years and 6 years CBJ cow. Ten years CBJ cow reflect significant difference (p=0.013) with 10 years ND cow and highly significant difference (p<0.005) with 2 years RS cow and 2 years CBJ cow.

For percentage of lymphocytes, significant differences (p< 0.05) are there between 6 years ND and 6 years RS cow, between 2 years RS cow and 6 years RS cow and between 6 years RS and 10 years RS cow. For percentage of monocytes, highly significant differences (p<0.01) are there between 2 years and 10 years ND cow and between 6 years and 10 years ND cow. Ten years ND has highly significant differences (p<0.01) with all the age groups of RS and CBJ cow. Six years RS has highly significant difference (p<0.01) with all the age groups of ND and 2 years RS cow. Six years RS has highly significant difference (p<0.01) with 2 years RS and 6 years CBJ cow and significant differences are there between 6 years RS and 2 years CBJ cow. No significant difference is found for percentages of eosinophils and basophils among the breeds with respect to age groups.

Discussion

Previous author [4] opined normal ranges for hematological value of cattle, i.e., Hb (g/dl) range from 8-15, RBCs ($x10^6/mm^3$) range from 5-10, PCV (%) range from 24-26, MCV(μ^3) range from 40-60, MCH (μμg) ranges from 11-17, MCHC (%) range from 30-36, WBC ($x10^3/mm^3$) range from 4-12, neutrophils range from 15-45%, lymphocytes range from 45-75%, monocytes range from 2-7%, eosinophils range from 2-20% and basophils range from 0-2%. Our findings

regarding RBCs ($x10^6/mm^3$) are in close agreement with author [4] for 2 years and 10 years ND cow and all the age groups of RS and CBJ cows. Regarding PCV (%) our findings are in close agreement with [4] for all the age groups of ND cow, 2 years and 6 years RS cow, 6 years and 10 years RS cow. Regarding Hb (g/dl) are in close agreement with each age group of three breeds of cows. In this present study, MCH of 10 years ND cow is in close accordance with [5]. The present results for MCHC are in close accordance with [4] for all the age groups of ND cow and 2 years RS cow. The present studies for WBC are in close agreement with [4] for 10 years ND, 2 years and 6 years RS and 6 years CBJ cow. Regarding percentage of monocytes, our present findings are in close agreement with 2 years and 10 years ND cow, all the age groups of RS cow and with 2 years CBJ cow. The results of present findings with regard to percentage of eosinophils are in close agreement with [4] for 2 years and 6 years ND cow, 2 years RS cow, 2 years and 10 years CBJ cow.

The present study regarding hemoglobin content of CBJ cow (Table 1) is accordance with earlier worker [25] who reported a decrease in hemoglobin content when calves grew older. The average amount of hemoglobin in the blood of normal beef and dairy cattle of various ages has been reported by various workers to vary from 10.9 to 13.2g per 100ml of blood [26-32]. The study is in close agreement with [26-32] for 2 years, 6 years and 2 years RS cow; differences are found for other breeds may be due to their body weight, breed differences, age differences, and seasonal variations. Late pregnancy and onset of lactation is a period when slight anemia exits [33]. This statement can be interpreted with 6 years ND cow which is deviated from the statement as described by earlier author [26-32]. A range of 13.2 to 13.95g/dl and a range of 13.2 to 14.35g/dl hemoglobin are found in two age groups (6 years and 10 years) RS cow and three different age groups of CBJ cow respectively in our study which may lead to hemoconcentration.

Our study for all the breeds except 6 yrs ND cow is agreement with those [25, 30, 34-37] who observed a range of 4.9 to 9.98 million red blood cells per cu mm of blood. In this study the mean for red blood counts is slightly lower than 6.55 millions, i.e., 6.31 millions in 6 years RS and slightly higher than 6.55 millions, i.e., 6.79 millions as explained by earlier author [38] for Jerseys and mean red blood counts of CBJ is closely related with Guernseys having 7.49millions /cu mm as explained by [38]. Mean TRBC observed in this study for ND cow is deviated from the other breeds of dairy cows such as Jerseys, Guernseys and Holsteins [38] indicating the differences in breed among the dairy cows.

The breed difference for PCV (%) found in this study has also been documented in different breeds of dairy cattle namely Holsteins (39.5%), Jerseys (42.3%) and Guernseys (46%) [37]. Haematocrit values or PCV (%) observed in our study except in 10 years ND cow whose average PCV (%) is slightly lower and 2 years CBJ whose average PCV (%) is slightly higher are in close agreement with those [39-40, 25] who reported that a range in average haematocrit values in dairy cattle varies from 28 to 50%. The average haematocrit values obtained in this study has also been documented in beef cattle from 31% to 48% [32] with the exception for 10 years ND and 2 years CBJ cow. The differences are found due to breed difference.

For MCV, [25] calculated that mean corpuscular volume on 233 dairy calves from birth to one year of age whose extreme values were 28 to 112 cubic microns. Our study can be interpreted with [25] that after one year of age the values were also within the range.

Previous worker [39] has reported a range of 14.2 to 18.5 micro micrograms with mean of 15.7 micro micrograms for mean corpuscular hemoglobin content of the blood of the adult cow. The present results are in accordance with the author [38] for MCH of 10 years ND and 6 years CBJ cow. Our findings regarding MCHC are in close agreement with previous worker [36] for each age group of ND cow and 2 years RS cow who observed average of 32.0 and 37.2% for mean corpuscular hemoglobin in dairy and beef cattle. The average for MCHC obtained in present study except 10 years ND and 2 years RS cow differed from the result obtained by [36] who found average of 32.0% and 37.2% for MCH for dairy and beef cattle respectively.

The range of TLC with respect to age and breed observed in this study has also been documented for different age groups of dairy calves as stated by [25] who found that number of leukocytes per cu mm of blood, in dairy calves from birth to a year of age, ranged between 4,500 and 15,000 with the majority ranging from 6,500 to 11,500 per cu mm. Earlier worker [25] has reported a decrease in the number of lymphocytes as calves grew from birth to one year of age and observed the number of monocytes, neutrophils and eosinophils to be variable from age to age while basophils were absent. Our study can be interpreted with [25] for variable number of leukocytes in different age groups of cows.

One author [36] opined normal ranges for differential leukocyte counts of cattle, i.e., neutrophils range from 1-15 which has been observed for 2 years

ND and 10 years CBJ cow in this present study, eosinophils range from 1-15, which is seen in all the breeds of cows except in 6 years CBJ cow and 10 years ND cow, basophils range from 0-1, lymphocytes range from 40-70 which has been seen in 10 years ND, 6 years RS and 2 years CBJ cow in our present study and monocytes range from 3-15, which is observed in 2 years RS and 2 years CBJ cow in our study. According to [4] more exercise causes leukocytosis which can be interpreted with increase in TLC in 2 years ND cow. MCHC is mostly normal but in certain anemia there is reduction of hemoglobin while the cell volume is normal; for example in iron deficiency MCHC is decreased [4].

Age [4-7], breed [4, 9], exercise [4, 10] and emotional states [4] influence the differential leukocyte count which can be interpreted with our study. Our study is in close agreement with [4] who stated that in cattle lymphocytes are always more in number than neutrophils. In 6 years RS cow there is more neutrophils than lymphocytes which is in accordance with [4] who opined that emotional states also influence the differential leukocyte count, i.e., besides the high leukocyte counts there is neutrophilia in fear and there is neutrophilia during exercise. This increase in the neutrophil count may be due to increased level of cortisol because of stress [41]. However, neutrophilia has also been reported during excitement, exercise, adrenaline and ACTH release [42]. Lymphocytes decrease around parturition mainly due to reduced lymphocyte proliferation [43].

According to [4] at birth TLC is high in calf, exercise causes leukocytosis and in fear high leukocyte counts are there which can be interpreted with 2 years ND cow having more TLC having one of the above causes.

The differences found for each hematological parameter were may be due to differences in age, breed and physiological status. Numerous reference values for domestic cattle have been reported and reveal few breed differences. Breed differences have been reported for beef cattle, which have higher RBC values, compared to dairy cattle breeds [14]. Care must be taken to use reference intervals that include similar environmental conditions and seasons as well as physiologic variables [14]. Reference values are influenced by age, sex, physical activity, etc. [5-8, 44-46] and in veterinary medicine the additional species, breed and management factors greatly magnify the effort required to generate reference values for each subpopulation of interest [47]. The accuracy and precision of the laboratory techniques as modified by reagents, temperature and instrumentation etc can effect reference values from a par ticular population [44, 46, 48-50] .

Discrepancies in values for various hematological parameters between our findings and previous studies may be explained by differences in sampling interval, methods used, number of cows sampled, and / or degree of metabolic disturbances as described by some authors [51]. Moreover, genetic differences between cows [52] and environmental conditions [51] of the present study might have played a role for the differences with other studies. Differences may also be due to their body weight, breed difference, age differences, and seasonal variations.

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Original Article

A Health GIS Based Approach to Portray the Influence of Ambient Temperature on Goat Health in Two Different Agro-Climatic Zones in West Bengal, India

Mihir Bhatta*, Debasish Das, Probal Ranjan Ghosh*****

Abstract

The spatial and temporal distribution patterns of the livestock health status in the developing countries like India are complex. In this regards, the application of Geographical Information System (GIS) is valuable as it has many features that make it an ideal tool for use in animal health surveillance, monitoring, prediction and its management strategy. The goal of the present study is to find out the effect of ambient temperature on goat health in two different agro-climatic zones in West Bengal, India with the additional help of GIS technology. The highest mean value of temperature (42.6 ± 1.5 ºC) has been reported during the month of April or May in the season of pre-monsoon in Purulia. Survey of India (SOI) topographical sheets (73 I/3 and 79 B/5) are used to map the study areas. Top sheets are scanned, geo-referenced and then digitized with the help of GIS software. The biochemical and meteorological data are entered to the newly prepared digitized map as the non-spatial data or attributes. Moreover, the present work aims to confer an indication of the potential applications and usages of a GIS in the field of animal health for advancing the knowledge about this innovative approach of goat heath surveillance and monitoring.

Keywords: Goats; GIS; Pre-Monsoon; Post-Monsoon; Purulia; Nadia.

Introduction

Our earth is now undergoing through a rapid demographic and ecologic changes, including tremendous pressure of population growth with successive increasing problems of food insufficiency[1], Inadequate development in public health sector, climate changes and simultaneous loosing of biodiversity, and the impacts on ecosystems which in common affects human and animal health [2]. The most important reason for using a GIS in an animal health information system is to facilitate the spatial component of animal health to be included in the reporting and analysis of animal health data. The applications of GIS can be divided into three main areas, which can be explained differently as inventory, analysis and management applications [3] or data visualisation, data exploration, and data modelling in an alternate way [4]. The problem is to consider whether a GIS for sustainably developing countries like India is able to provide the full range of functions required by such a system. An animal health information system has the necessary purposes of gathering, storing, analysing as well as reporting information on animal health [5]. Insertion of GIS into an animal health information system allows the spatial component of animal health to be incorporated into this process. The efficiency of data collection system has no impact here, or how equipped the data storage method, excluding data analysis, and ultimately reporting of the animal health information, the system provides no useful function [6]. Now in the developing country like India, GIS is becoming very popular in the observation as well as to track and to monitor the vector-borne and water-borne diseases [7].

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Methodology

Survey of India (SOI) topographical sheets of 1: 50,000 scales (No. 73 I/3 and 79 B/5) are used to map the study areas. Topographical sheets are scanned, geo-referenced and then digitized with the

Fig. 1: Non-spatial attributes including biochemical parameters of the pre-monsoon in the study area of Purulia

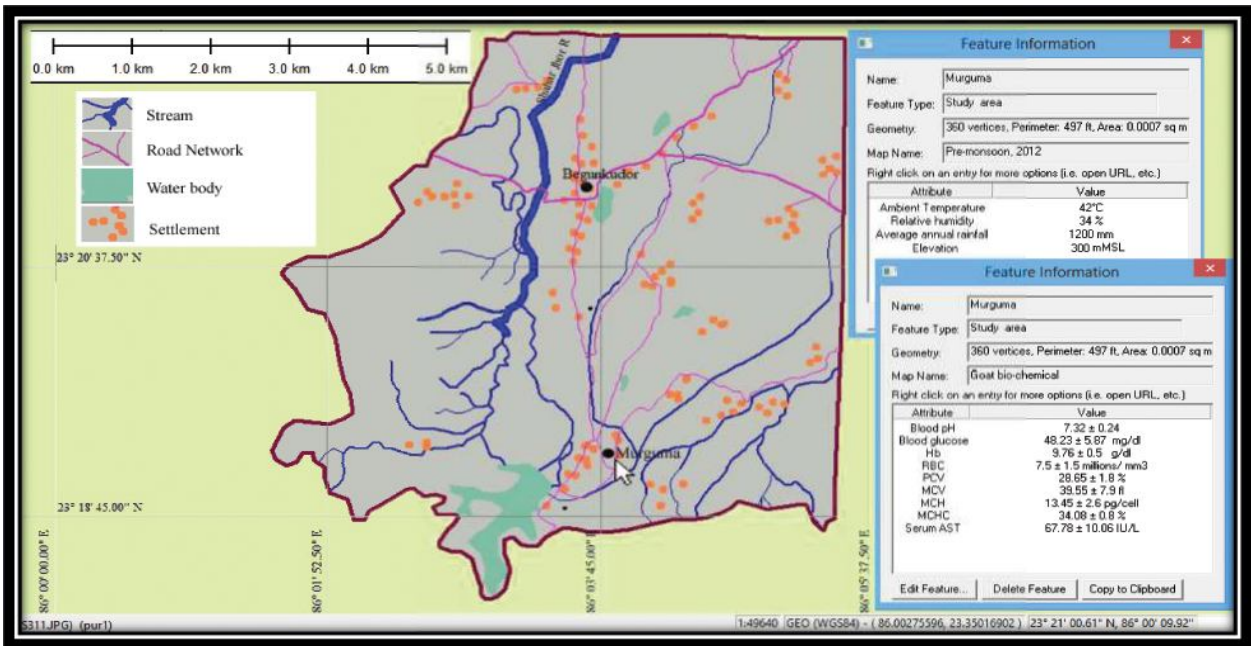
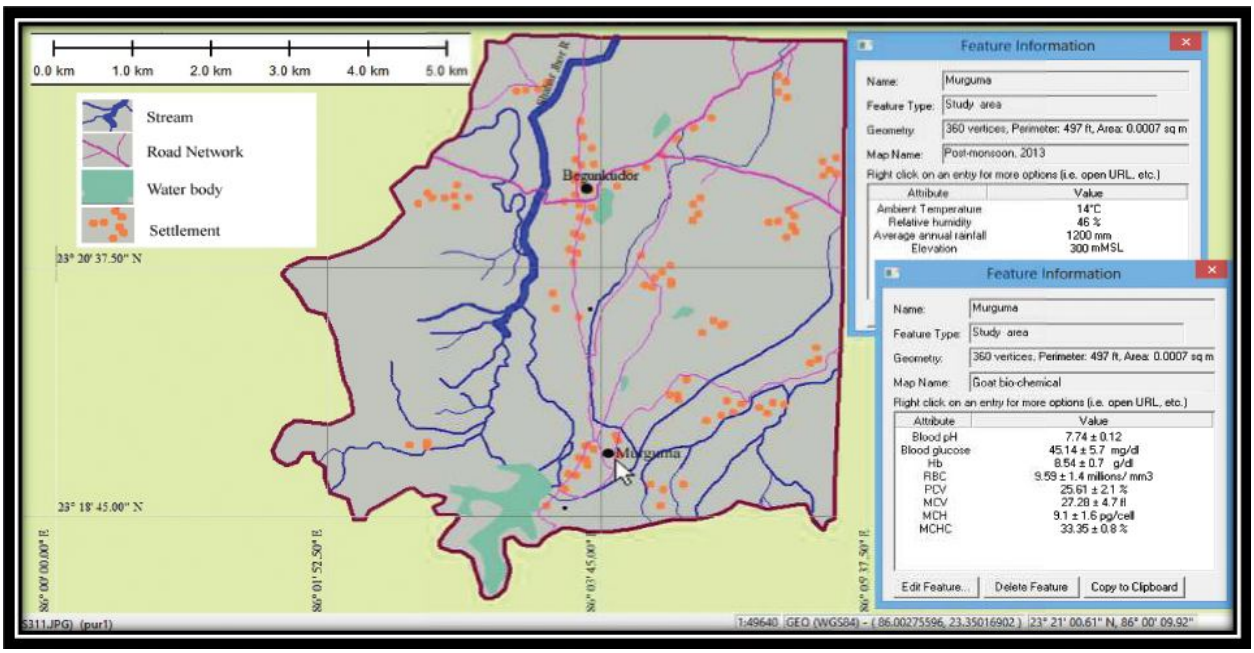


Fig. 2: Non-spatial attributes including goat's biochemical parameters of the post-monsoon in the study area of Purulia



help of GIS software [8] such as TNTmips® 7.2packages (©2015 Micro Images Inc.), Global Mapper® etc. The biochemical parameters such as blood glucose (mg / dl), total RBC (millions / mm3), total haemoglobin or Hb (g / dl), packed cell volume or PCV (%), mean corpuscular volume or MCV (femtoliter per cell or fl), mean corpuscular haemoglobin or MCH (in picogram per cell or pg) and mean corpuscular haemoglobin concentration or MCHC (%) and meteorological data such as ambient temperature (ÚC), relative humidity (%), average annual rain fall (mm) and elevation (mMSL) from previously published article[9] and from some recent work of the same authors, has been entered to the newly prepared digitized map as the non-spatial data or attributes. The data have been entered in such a manner so that, when the mouse (computing) has been ported and clicked on any selected point subsequently opening one or more windows containing the information about different non-spatial attributes (Figure 1, 2, 3 and 4). Latitude and longitude of the different study sites have been measured using GPS (eTrex, Garmin International, Inc.).

Fig. 3: Non-spatial attributes including goat's biochemical parameters of the pre-monsoon in the study area of Nadia

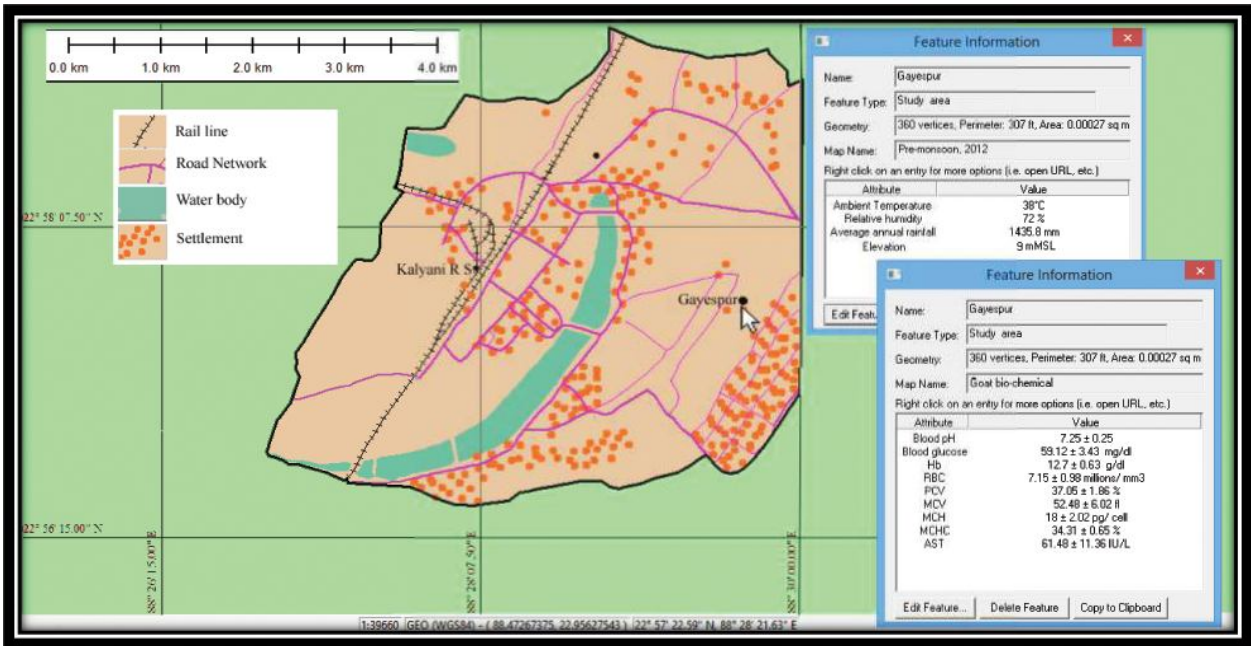
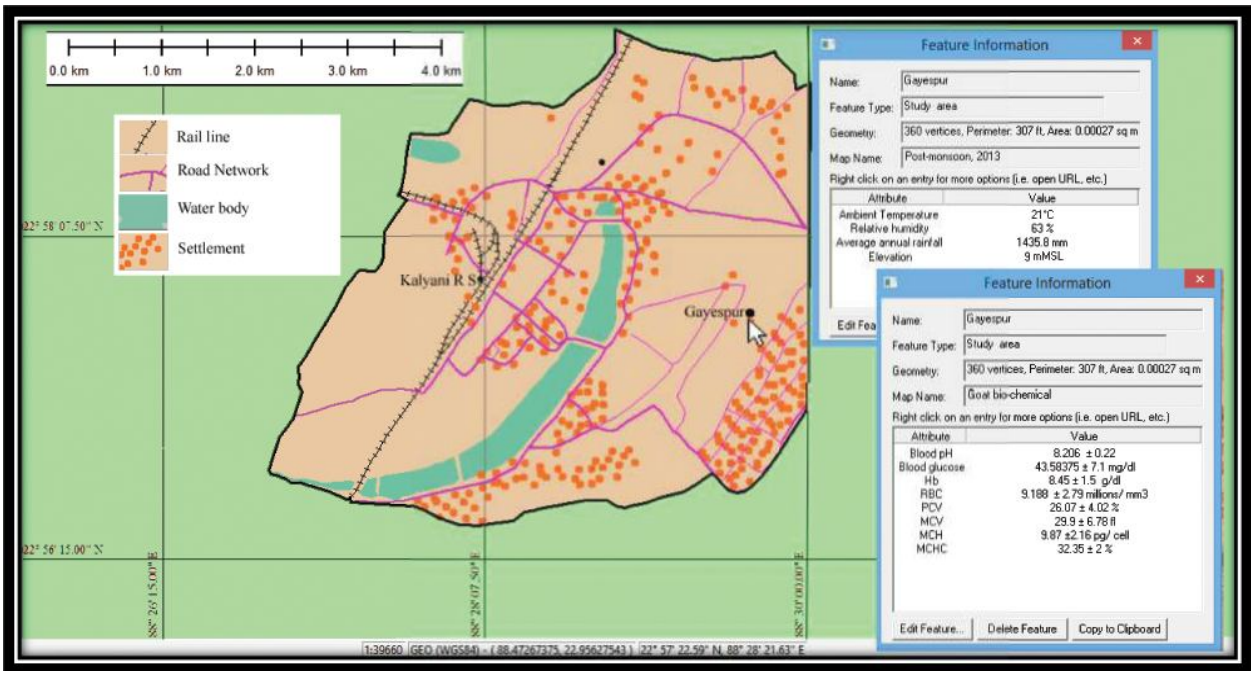


Fig. 4: Non-spatial attributes including goat's biochemical parameters of the post-monsoon in the study area of Nadia



Results and Discussion

The physiological, biochemical and meteorological data, from previously published article by the same authors [9] has been entered to the newly prepared digitized map as the non-spatial data or attributes. An effective linking of geographical and attribute information is an essential part of the function of a GIS. As a result, most of the GIS have very potent, and simple to use data linking capacities. This makes them well suited to the task of integrating the many different data sources that are necessary in an animal health information system. Keeping this view in mind the present work has been done. The maps are prepared from the topographical sheet 73 I/3, showing Murguma, Purulia and the maps are prepared from the topographical sheet 79 B/5, showing Gayespur, Nadia. The non-spatial attributes i.e. biochemical parameters of the goat blood during

the pre-monsoon season in the study area Murguma, Purulia have been entered in the map (Figure 1). Figure 2 showing the non-spatial attributes i.e. biochemical parameters of the goat blood during the post-monsoon season in the same study area of Purulia. Figure 3 and 4 showing non-spatial attributes i.e. biochemical parameters of the goat blood in the study area of Gayespur, Nadia, for the season of pre-monsoon and post-monsoon respectively.

The one of the present study area Murguma, situated in Purulia district, which fall under Eastern Plateau and Hills (agro-climatic) region of India [10]. It receives about 1200 mm of rainfall annually. During pre-monsoon season the average ambient temperature has been found 42^{0}C and relative humidity has been found 34 % (Figure 1). Whereas during post-monsoon season the average ambient temperature and relative humidity has been found 14^{0}C and 46 % respectively (Figure 2). The other study area of the present work is Gayespur from Nadiadistrict, which fall under Lower Gangetic Plains (agro-climatic) region of India [11]. Here annual rainfall ranging between1200 mm to 1700 mm. During pre-monsoon season the average ambient temperature has been found 38^{0}C and relative humidity has been found 72 % (Figure 3). Whereas during post-monsoon season the average ambient temperature and relative humidity has been found 21^{0}C and 63 % respectively (Figure 4).

The maps of Figure1 and Figure 2 are clearly showing marked differences in biophysical and biochemical parameters between two seasons in Murguma, Purulia [12]. Similarly Figure 3 and Figure 4 show some differences biochemical parameters between two seasons in Gayespur, Nadia. Here we can compare the Figure 1 with Figure 3, i.e. comparing bio-chemical parameters of goats under study of pre-monsoon season between the two present study areas of Purulia and Nadia. Which show significant difference in many aspects [9]. However, there are less significant difference in between the two study areas Purulia and Nadia during the post-monsoon seasons (comparing Figure 2 and Figure 4). Some other important findings from the prepared map are: blood pH values in pre-monsoon are lower than post-monsoon in both the region i.e. Purulia and Nadia. The blood glucose level always has been higher in post-monsoon. MCHC values remain unchanged throughout the year as well as in two different agro-climatic regions. As many workers [13] reported the steady level of MCHC during different seasons.

We know that the purpose of an animal health information system is to give information that provides a better understanding of the epidemiology of disease and the continuous study of goat's bio-physical parameters such as rectal temperature, heart rate, pulse rate and bio-chemical parameters such as blood pH, blood glucose, RBC, PCV, MCV, MCH, MCHC values can give an idea of the goat heath in real time [14 &15]. Examination of the spatial component of animal health data yields another important advantage of GIS – the capability to quickly identify the data errors. Lost and out-of-range data can be simply identified when the data going to incorporated into the map. Previously GIS have been effectively applied to a number of definite problems in veterinary epidemiology, such as calculating the risk of East Coast fever to the livestock in Africa [16]. An effective linking of geographical and attribute information is an essential part of the function of a GIS. As a result, most of the GIS have very potent, and simple to use data linking capacities. This makes them well suited to the task of integrating the many different data sources that are required in an animal health information system [14].

Conclusion

GIS has been efficient in collection and presentation of spatial and non-spatial data as well as disease occurrences, which assist to prepare immediate remedial and preventive approaches for disease prevention and management [17]. It is obvious that the systems in most developing countries fall far short of the optimal, due to a range of constraints strange to the developing parts of the world [18]. In the present context, the ability of GIS to link graphic and non-graphic data facilitates prevailing analysis of different non-spatial distribution and related issues. It will be feasible and quite easy to draw the maps and visualize possible temporal and spatial risk factors [19]. Particular problems exist in the collection of unbiased, reliable, timely information on the health status of livestock populations, and in the management of animal health data [20]. GIS provides strong spatial analytical potentialities [21], but the limited availability of consistent animal health data, such as that collected by dynamic surveillance, the analysis is of limited value. These systems are being increasingly applied to animal disease control as an integral component of supporting system concerning decisions in the field of veterinary science. Thus, GIS can be sighted as a possible tool for a novel move towards of science [22], to endorse the animal health in terms of monitoring, observation as well as disease management policies.

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Original Article

Inter-Relationship Between Physicochemical Parameters of River Churni, Nadia, Westbengal, India

Ashis Kumar Panigrahi*, Avijit Bakshi, Sarbani Dutta (Roy)***, Somsuvra Dasgupta****, Anandamoy Mondal*****

Abstract

Water samples were collected monthly from nine sampling sites of River Churni of Nadia district, West Bengal for two annual years (2012-2013). A total of 216 samples were analyzed first to determine the values of each water quality parameters. Parameters *viz.*, temperature, pH, dissolved oxygen, biochemical oxygen demand, chemical oxygen demand, total hardness, total alkalinity, total nitrogen, total phosphorus, total solid and total coliform were determined to understand the current status of water quality of the river. Correlations between physicochemical parameters were established by the use of Pearson correlation matrix (n) to determine the interrelationship between the parameters. Correlation coefficient values between these pollution indicator parameters were found to indicate good interrelationship between them.

Keywords: River Churni; Water Quality Parameters; Correlation.

Introduction

River Churni is an important river of Nadia district of West Bengal. It originates from a distributary of River Padma named River Mathabhanga near Krisnagang, Nadia. After flowing about 54 km it pours its content to the River Bhagirathi-Hooghly. The entire stretch of the river is situated within the district Nadia. According to Panigrahi and Bakshi (2014), the river is the one of the main sources of surface water of Nadia district. The river was described to face a loss of fish species along with degrading ecological condition by Ghosh and Konar (1991). Bakshi and Panigrahi (2012) had identified various reasons behind the ecological degradation. They had cited that the river was affected by various point and non-point pollution sources at various places. 63.6% of fish species had been recorded to be eliminated from river Churni by Das and Chakrabarty (2007). Loss of benthic macro-invertebrates had also been reported for this river mainly due to increasing anthropogenic pollution load (Das et al., 2007).

Water quality parameters were determined during the total study period as these are said to be good pollution indicators. Several studies on water quality were done focusing on the physicochemical characteristics of waters (Wazir*i et al*., 2009; Izonfuo and Bareweni, 2001). It was very essential to identify the interrelations between the various physico-chemical parameters for this river to understand the accumulated impact of the parameters.

The aim of the total study for two annual years (January, 2012- December, 2013) was to determine the level of some pollution indicators and establish relation between different parameters.

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Study area and sampling sites

The investigation was carried throughout the complete stretch of River Churni (about 54km). Nine sampling sites were selected as follows: Krishnaganj (S1), Hanskhali (S2), Mamjoani (S3), Aranghata (S4), Kalinarayanpur (S5), Aishtala (S6), Ranaghat Bridge

Point (S7), Anulia (S8) and Shibpur (S9). The geographical position of the sampling stations was listed in Table 1.

Table 1: Sampling sites of the study area and their geographical positions

Sites	Name of the areas (present sampling sites)
S-1	Krishnaganj (23.403965°N, 88.709667°E)
S-2	Hanskhali (23.358589°N, 88.60.8795°E)
S-3	Mamjoani (23.304460°N, 88.581861°E)
S-4	Arranghata (23.263231°N, 88.6008545°E)
S-5	Kalinarayanpur (23.205668°N, 88.559752°E)
S-6	Aishtala, Ranaghat (23.192428°N, 88.566960°E)
S-7	Ranaghat Bridge Point (23.177679°N, 88.558168°E)
S-8	Anulia (23.158119°N, 88.544505°E)
S-9	Shibpur, Payradanga (23.133389°N, 88.502662°E)

Materials and Methods

Water samples were collected monthly from each sampling site. Samples were collected monthly from lotic zones of two banks and mid-stream of each sampling site. Average value was taken as the monthly data for each site. Water samples were collected at a depth of 6-10 cm from the surface water during 9 a.m. to 12 a.m. of the sampling days. Sample water was collected in sterilized plastic bottles with stoppers. Dissolved oxygen and temperature of the water were measured on the spot by using portable DO meter (IC 70, UK) and simple mercury thermometer (range 0°C to 1000°C) respectively. A portable pen pH meter (pHep Tester, Hanna instrument, Romania) was used to determine the pH of water. All other parameters were analyzed using the guideline of APHA (1995, 19th edition). Average values of total 216 observations were taken into consideration for the correlation analysis. A statistical software, XLSTAT-2015, was used to calculate the range of the parameters and the correlation between them.

Result and Discussion

In the Table 2, measuring units for each parameter are listed.

Ranges of the parameters are listed in the Table 3. The interpretations of the tabulated values are described in the following paragraphs.

Hydrogen ion concentration of water is determined by pH value. It is also an important pollution indicator as low pH value is an indication of presence of dissolved carbon di oxide in water. Jhingran (1991) has stated that pH more than 9.0 is responsible for stress in carp culture; moreover, pH 11.0 is lethal to the fishes. The pH value of the river water has been found to vary between 6.4 to 8.4 with an average value of 7.758 and standard deviation value of 0.428.

Water temperature of any aquatic ecosystem is a very important parameter to study as temperature below 16.7°C and above 39.5°C have been reported to be fatal for the fishes especially IMCs (Indian major carps) (Jhingran, 1991).The temperature of the river water has been found to vary between 14.1°C to 33.3°C with an average value of 25.74°C and standard deviation value of 5.139.

DO level of any water body indicates the pollution load on the system. Low DO is fatal for aquatic life. According to Jhingran (1991), both high and low value of DO is responsible for adverse effect over fish production. The river water shows a trend of decreasing DO value by the year. Minimum value of DO is observed in May, 2012 at S-1 (0.4 mg/L) whereas; Maximum value (i.e., 7.2 mg/L) is recorded in December, 2012 at S-9.

BOD is also an important water quality parameter as high value of BOD is detrimental for aquatic lives.BOD value is found to be the maximum in January, 2012 at sampling site-1 (37.5 mg/L).

Total alkalinity and total hardness values of river water are observed to remain closer to slight alkaline state.

Total nitrogen and total phosphorus value of water is the indicator of nutrient levelof any aquatic system. Total nitrogen value ranges from 2.96 mg/L to 5.64 mg/L and total phosphorus value ranges from 0.17 mg/L to 2.01 mg/L during the total study period.

Pearson correlation matrix is constructed using all the values of the parameters. The matrix is cited in Table 4.

Interpretation of table 4 is put down in the following paragraphs.

Coefficient of Pearson correlations are listed in the aforesaid table and put down in the brackets in the following part of the paragraph. Significant positive correlations are observed between pH & total hardness (0.320), pH & total solid (0.394),pH & COD (0.289), pH & total nitrogen (0.211), pH & total alkalinity(0.458), temperature & total phosphorus (0.444), temperature & total nitrogen (0.287), temperature & total coliform (0.486), BOD & total hardness (0.154), BOD & COD (0.230), BOD & total solid (0.156), BOD & total alkalinity (0.284),COD &

total hardness (0.460), COD & total solid (0.568), COD & total alkalinity (0.389),total hardness &total alkalinity (0.779), total phosphorus & total coliform (0.590),total alkalinity & total solid (0.598), and total hardness & total solid (0.643). Significant negative correlations are found between pH & total coliform (-0.701), pH & total phosphorus (-0.473), temperature & COD (-0.398), temperature & total solid (-0.343), temperature & BOD (-0.264), temperature & total alkalinity (-0.445),temperature & total hardness (-0.278), DO & total harness (-0.206), DO & BOD (-0.633), DO & COD (-0.182), DO & total alkalinity (-0.237), DO & total solid (-0.363), BOD & total coliform (-0.180), COD & total coliform (-0.295), COD & total phosphorus (-0.258), total hardness & total coliform (-0.307), total hardness & total phosphorus (-0.343), total alkalinity & total coliform (-0.428), total alkalinity & total phosphorus (-0.403), total phosphorus & total solid (-0.515) total nitrogen & total coliform (-0.300) and total solid & total coliform (-0.440). The correlation study shows non-significant relations between rests of the parameters.

Table 2: Units of different water quality parameters

Parameters	Units
pH	-
Temperature	°C
Dissolved Oxygen	mg L^{-1}
Biochemical Oxygen Demand	mg L^{-1}
Chemical Oxygen Demand	mg L^{-1}
Total Alkalinity	mg L^{-1}
Total Hardness	mg L^{-1}
Total Nitrogen	mg L^{-1}
Total Phosphorus	mg L^{-1}
Total Solid	mg L^{-1}
Total Coliform	MPN/dL

Table 3: Table showing total observations, Observation with missing data, Observation without missing data, Minimum and Maximum value of the parameters, Mean and Standard deviation value of each parameter

Variable	Observations	Obs. with missing data	Obs. without missing data	Minimum	Maximum	Mean	Std. deviation
pH	216	0	216	6.400	8.400	7.758	0.428
Temp	216	0	216	14.100	33.300	25.740	5.139
DO	216	0	216	0.400	7.200	3.227	1.434
BOD	216	0	216	0.500	37.500	6.144	6.699
COD	216	0	216	34.500	235.390	118.668	44.702
Total Hardness	216	0	216	100.600	406.120	214.932	58.012
Total Alkalinity	216	0	216	96.400	380.600	210.275	51.016
Total Nitrogen	216	0	216	2.960	5.640	4.185	0.540
Total Phosphorus	216	0	216	0.170	2.010	1.026	0.526
TS	216	0	216	253.700	610.500	430.983	98.557
Total Coliform	216	0	216	0.090	22.450	5.041	4.958

Table 4: Pearson correlation matrix (n) (Values in bold are different from 0 with a significance level alpha=0.05)

Variables	pH	Temp	DO	BOD	COD	Total Hardness	Total Alkalinity	Total Nitrogen	Total Phosphorus	TS	Total Coliform
pH	1	-0.51	-0.09	0.120	0.289	0.320	0.458	0.211	-0.473	0.394	-0.701
Temp	-0.51	1	-0.01	-0.26	-0.39	-0.278	-0.445	0.287	0.444	-0.34	0.486
DO	-0.09	-0.01	1	-0.63	-0.18	-0.206	-0.237	-0.086	0.097	-0.36	0.108
BOD	0.120	-0.26	-0.63	1	0.230	0.154	0.284	0.045	-0.041	0.156	-0.180
COD	0.289	-0.39	-0.18	0.230	1	0.460	0.389	0.197	-0.258	0.568	-0.295
Total Hardness	0.320	-0.27	-0.20	0.154	0.460	1	0.779	0.022	-0.343	0.643	-0.307
Total Alkalinity	0.458	-0.44	-0.23	0.284	0.389	0.779	1	-0.050	-0.403	0.598	-0.428
Total Nitrogen	0.211	0.287	-0.08	0.045	0.197	0.022	-0.050	1	0.052	0.020	-0.300
Total Phosphorus	-0.47	0.444	0.097	-0.04	-0.25	-0.343	-0.403	0.052	1	-0.51	0.590
TS	0.394	-0.34	-0.36	0.156	0.568	0.643	0.598	0.020	-0.515	1	-0.440
Total Coliform	-0.70	0.486	0.108	-0.18	-0.29	-0.307	-0.428	-0.300	0.590	-0.44	1

Conclusion

The study represents the variation in different water quality parameters which help to assess the present ecological condition of the river. The result shows that water quality of the river is not very good for the aquatic bio life. Correlation coefficient may predict the average value of one parameter with the help of other.

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Original Article

Some Important Physico-Chemical Parameters and Sodium, Potassium Ion Concentrations in Common, Available and Widely Consumed Soft Drinks in India

Monojit Ray*, Chandrima Nag**

Abstract

In the present study we tried to find out the pH, Conductance, Total Dissolved Solids (TDS), Salinity, Sodium ion concentrations and Potassium ion concentrations within some common, available and widely sold soft drinks in India. For this purpose we had studied total 21 soft drinks among which seven are widespread selling fruit drinks. Most of them show high acidity, high conductance, high TDS and high salinity values. The sodium ion concentrations and potassium ion concentrations are significant and vary widely among these drinks.

Keywords: Physico-Chemical Parameters; Sodium; Potassium; Soft Drinks; India**.**

Introduction

Sodium ions and potassium ions play vital roles within our body, so, they are very significant. Sodium ion regulates, blood volume, blood pressure, pH and osmotic pressure of blood. Sodium ion is the most important extra-cellular ion, whereas, potassium ion is the most significant intracellular ion. The refreshing soft drinks or fruit drinks provide those ions to our body after consumption of them. In India during every summer large number of people consumes these packed soft drinks and fruit drinks. Conductance value indicates the amount of ions presents within these soft drinks. Salinity denotes the amount of salt present and TDS reflect the amount of soluble substances. The pH value denotes the order of acidity within soft drinks. Sodium ion concentrations and potassium ion concentrations within human body fluid and blood are almost constant. The exact concentrations of the ions are different for different type of cells. The extracellular sodium ion concentration is 3.45 g per liter (approx) whereas; the intracellular sodium ion concentration is 0.23 g per liter (approx). The extracellular potassium ion concentration is 0.2 g per liter (approx), at the same time; the intracellular potassium ion concentration is 6 g per liter (approx). The salinity of human blood is 9g / liter. The pH of human blood lies between 7.15-7.45 (approx).

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Materials and Methods

The entire study was carried out at the Research Laboratory, Department of Chemistry, Nabadwip Vidyasagar College, Nabadwip, Nadia. All the soft drinks samples used were sealed metal cans of 300 ml or pet bottlesof 500 ml/ 200 ml, and manufactured within last one month of study date. Temperature, pH, conductance, TDS and salinity were measured using EUTECH Mult-parameter PCSTester 35. Sodium ion concentrations and potassium ion concentrations were measured using Systronics Flame photometer 130 of Department of Chemistry, Nabadwip Vidyasagar College. Redistilled and ion free water, prepared at laboratory, were used for all the analysis. All the measurements were carried out between 18° - 20°C.

Results

Table 1: Energy value and Maker of few common, available and widely consumed soft drinks in India

No.	Brand Name	Company	Energy value Kcal/100ml
1	RC Cola	Iceberg Foods Limited	44.41
2	RC Q Orange	Iceberg Foods Limited	53.7
3	Sprite	The Coca Cola Company	48
4	7UP	PepsiCo Inc.	40
5	Fanta	The Coca Cola Company	52
6	Mirinda	PepsiCo Inc.	55
7	Pepsi	PepsiCo Inc.	43
8	Coca Cola	The Coca Cola Company	44
9	Coca Cola Zero	The Coca Cola Company	0.3
10	Diet Coke	The Coca Cola Company	0.2
11	Diet Pepsi	PepsiCo Inc.	0.3
12	Thumbs Up	The Coca Cola Company	40
13	Limca	The Coca Cola Company	44
14	Appy Fizz	Parle Agro	54
15	Appy	Parle Agro	63
16	Maaza	The Coca Cola Company	54
17	Frooti	Parle Agro	65
18	Slice	Varun Beverages Limited	63
19	RedBull	Rauch Trading AG	45
20	Peach Coolada fruit Drink	FieldFreshFroots Private Limited	52.7
21	Litchi Flavored Drink	Pran Exports Ltd.	59.2

Table 2: Physico-chemical Parameters and Na, K ion concentrations of few common, available and widely consumed soft drinks in India (Temp = 18° - 20°C)

No.	Brand Name	pH	TDS mg/lit	Salinity mg/lit	Conductance µS/cm	Na^+ mg/lit	K^+ mg/lit
1	RC Cola	2.50	730	508	1032	24	40
2	RC Q Orange	2.60	490	334	694	55	04
3	Sprite	3.34	335	224	483	117	05
4	7UP	3.38	452	308	602	152	00
5	Fanta	2.80	408	278	514	66	09
6	Mirinda	2.65	388	268	550	51	00
7	Pepsi	2.71	472	530	1010	35	38
8	Coca Cola	2.45	624	430	893	40	49
9	Coca Cola Zero	3.18	645	473	860	114	80
10	Diet Coke	2.77	795	555	1120	83	79
11	Diet Pepsi	3.18	613	422	865	108	49
12	Thums Up	2.45	826	572	1163	47	49
13	Limca	2.76	356	242	500	47	03
14	Appy Fizz	3.30	631	439	887	141	114
15	Appy	3.00	603	416	850	140	107
16	Maza	3.44	712	495	1004	135	155
17	Frooti	3.34	708	493	1001	146	154
18	Slice	3.10	582	402	820	45	161
19	RedBull	3.30	1140	757	1586	>200	03
20	Peach Coolada fruit Drink	3.00	552	380	777	30	140
21	Litchi Flavored Drink	3.18	455	312	640	110	38

Discussion

The energy value /100 ml soft drinks are listed in Table 1. Appy, Frooti and Slice provide maximum energy, while, Diet Coke, Diet Pepsi and Coca Cola Zero provide minimum energy to human body. Study of physico-chemical parameters shows that all the soft drinks and fruit drinks have pH value below 3.5, i.e., all are acidic in nature. Table 2 clearly shows that Coca Cola, Thums Up and R C Cola are most acidic. Maza is the least acidic. Total dissolved solid (TDS) is maximum for Red Bull and least for Sprite. Conductance values suggests that R C Cola, Pepsi, Diet Coke, Thums Up, Maza, Frooti contain relatively high ion concentrations. Red Bull contains highest ion concentrations. Relatively low ion concentrations are found within Limca, Fanta and Sprite.

Sprite, 7UP, Fanta, Mirinda, Slice, Frooti, Maza, Appy, Appy Fizz, Peach Coolada fruit Drink, Limca, RC Q Orange and Litchi flavored drink does not

contain any caffeine. Some drinks contain caffeine, the stimulating agent used for refreshing body and specially mind. These drinks are Pepsi, Diet Pepsi, Coca Cola, Diet Coke, Coca Cola Zero, Thums Up, Red Bull and RC Cola. Some drinks contain original fruit parts, viz., slice contains 15% alfanso mango pulp, Frooti contain 19% mango pulp, Maza contain 19.5% mango pulp and Appy contain 14% apple juice. Appy Fizz contains 12.7% apple juice. Peach Coolada fruit drink contain mango pulp and peach bits**.**Sprite,7UP, Coca Cola, Diet Coke, Coca Cola Zero, Thums Up, Fanta, Mirinda, Diet Pepsi, Pepsi, Red Bull, Limca, RC Q Orange, RC Cola and Litchi flavored drink contain no fruit**.**

Though Litchi flavored drink contain vitamin C 15 mg / 100 ml. still Red Bull is the only drinks which contain added vitamins. Every 100 ml Red Bull contain niacin 8 mg, pantothenic acid 2 mg, Vitamin B_6 2 mg, Vitamin B_{12} 2 mg, Vitamin B_2 0.06 mg. Among these drinks Appy and Frooti, are the only drinks which contain no added preservatives.

According to the World Health Organization (WHO) drinks having TDS value less than 300 mg / liter may be considered as "excellent" where as drinks having TDS value between 300 mg / liter and 600 mg / liter may be considered as "good" for the health. Among these twenty one drinks only ten have TDS vales between 300 and 600 mg / lit. TDS value of Appy is 603 mg / lit.

Healthy 19 to 50 years old adult should consume 1.5 g sodium ion and 2.3 g potassium ion per day. Any human body having 70 kg weight, contain 15 liters extracellular fluid, which contain approximately 50 g sodium ion and this is the 90% of the total body sodium ion. Sweating during summer release huge sodium ions from the body fluid. Among the selected studied soft drinks, Red Bull contains maximum sodium ion concentrations. Appy Fizz, Appy, Maza, Frooti, 7UP, Sprite, Coca Cola Zero, Diet Pepsi and Litchi Flavored Drink contain relatively high sodium ion concentrations. The sodium ion concentration is less (i.e., below 50 mg / lit) within RC Cola, Peach Coolada Fruit drink, Pepsi Coca Cola, Slice, Thums Up and Limca.

Potassium ions prevent stroke, osteoporosis, kidney stone and hypertension. Relatively high potassium ion concentrations (i.e., above 100 mg / lit) are found within Maza, Slice, Frooti, Appy Fizz, Appy and Peach Coolada fruit drink. RC Q Orange, Sprite, Fanta, Limca and Red Bull contain very low potassium concentration. 7UP and Mirinda do not contain any potassium ions. Patients suffering from kidney diseases, should not consume drinks containing high potassium concentrations, so, 7UP and Mirinda should be preferred.

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Original Article

In Vitro Screening of Salt Tolerant *Bacillus* from Rhizosphere of Tomato (*Lycopersicon esculentum*) Showing Plant Growth Promoting Traits

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Abstract

PGPR enhance plant growth either by direct or indirect mechanisms. The direct growth promoting mechanisms involve nitrogen fixation, solubilization of minerals, production of phytohormones and the indirect approach occurs when PGPR lessen or prevent the deleterious effects of plant pathogens. In the present study, rhizospheric soil samples were collected from different tomato (*Lycopersicon esculentum*) cultivated fields from Raebareli district, Uttar Pradesh, India. A total of twenty three (23) bacterial strains were isolated from soil samples and identified on the basis of their morphological and biochemical characteristics and classified as genus *Bacillus.* Out of these twenty three isolates, seven bacterial isolates showed salt tolerance and three isolates (RBL-1, RBL-5 and RBL-6) showed tolerance against 10% (w/V) NaCl. All salt tolerant rhizobacterial isolates showed potential plant growth promoting (PGP)traits (production of IAA, HCN, NH_3, siderophores and phosphate solubilisation) and the isolate RBL-1 showed significantly high IAA production (78.20 µg/ml).

Keywords: *Bacillus*; PGPR; Tomato; Saline.

Introduction

PGPR (plant growth promoting rhizobacteria) are defined by three intrinsic characteristics: (i) they must be able to colonize the root, (ii) they must survive and multiply in microhabitats associated with the root surface, in competition with other microbiota, at least for the time needed to express their plant growth promotion / protection activities, and (iii) they must promote plant growth. Plant growth promoting rhizobacteria are free-living, soil-borne bacteria which when applied to seeds / soils or crops, enhance the growth of the plant directly by providing nutrients and growth promoting substances to plants or indirectly by reducing the damage from soil-borne plant pathogens (Kloepper *et al*., 1980).The application of plant growth promoting rhizobacteria (PGPR) as crop inoculants for biofertilization, phytostimulation and biocontrol offers an attractive and eco-friendly alternative to decrease the use of chemical fertilizers which decrease soil fertility and also have adverse effectson the environment (Ali *et al.,* 2010). *Pantoea*, *Bacillus*, *Pseudomonas* etc. are widespread species in agricultural soils and have many traits that make them well-matched as PGPR. PGPR can stimulate plant growth directly as they can improve the supply of nutrients, such as nitrogen (Dobbelaere *et al*., 2003) and phosphorous (Rashid *et al*., 2004) or by production of phytohormones (Choong *et al.,* 2003; Stepanova *et al*., 2008) and ACC-deaminase synthesis (Arshad *et al*., 2007). Indirectly PGPR can also promote plant development by the suppression of pathogens mediated by different mechanisms such as antibiosis (Milner *et al*., 1996), iron sequestration by siderophores (Singh *et al.,* 2014), HCN (Keremer and Souissi, 2001), and cell wall degrading enzymes like chitinase (Ajit *et al*., 2006). Thus, plant growth is promoted through reducing or neutralizing pathogen activity. Nearly 40% of world's surface has salinity problems due to poor and unscientific water management practices and most of the saline areas are confined to the Tropics and Mediterranean region. This has made the necessity to explore and create salt tolerance organisms and requires attention on priority for the future of agriculture (Corodovilla *et al*., 1994; Gisbert *et al*., 2000). The combination of IAA production ability (Goldstein, 1995), phosphorous solubilization (Gyaneshwar *et al*., 1998) and siderophore production (Dulfy, 1994) by bacteria aid the plant

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rhizosphere in enhancing the nutrient availability / absorption potential under sodic environment for enabling economic production of commercial horticultural crops (Damodaran *et al*., 2013). The tomato belongs to the family Solanaceae (also known as the nightshade family), sub-family Solanoideae, tribe Solanaceae and genus *Lycopersicon* (Taylor, 1986). Tomato is the second most cultivated vegetable in the world, after potato and India is the fourth largest tomato producer worldwide. The tomato fruit is consumed in diverse ways, including raw, as an ingredient in many dishes and sauces, and in drinks. The fruit is rich in lycopene, which may have beneficial health effects. The most important tomato producingIndian states are Bihar, Karnataka, Uttar Pradesh, Orissa, Andhra Pradesh, Maharashtra, Madhya Pradesh and Assam. The expansion of tomato production in India was to a great extent attributed to the private seed sector, which developed high yielding cultivars (Nagaraju *et al.,* 2002).

Uttar Pradesh region represent unique combination of plant and soil type which changes drastically with altitude. However, limited efforts have been done so far to explore the available bacterial diversity. In the present study, rhizospheric soil samples from tomato fields were collected from Raebareli district for assessment of PGPR. The objectives of this study have been to isolate and characterize rhizobacteria from the rhizosphere of tomato, and to screen them for salt tolerance and the possession of direct and indirect plant growth promoting rhizobacteria attributes.

Materials and Methods

Collection of Sample

The rhizosphere soil samples were collected from the roots of tomato (*Lycopersicon esculentum*) plants growing at different sites in Raebareli district, Uttar Pradesh, India. Intact root system was dug out at a depth of 5 to 10 inch and the rhizospheric soil samples were carefully taken in plastic bags with proper labelling and stored at 4°C. Total of five soil samples were collected for the isolation of rhizosphere bacterial isolates.

Preparation of Soil Samples

The soil samples were first ground with sterile mortar and pestle to liberate the adhering microorganism before their suspension was prepared. One gram of the soil sample was weighed out and dissolved in 9 ml of distilled water in a beaker and homogenized properly.

Isolation and Characterization of Rhizobacteria

One (1) gram of soil sample was serially diluted in sterile distilled water and 0.1 ml of soil suspension from 10^{-1} to 10^{-5} dilutions was spread on the nutrient medium agar (NA) plate and subsequently incubated at 37ºC for 48 hrs. Fine isolated and distinct colonies were picked up and streaked freshly on NA plates and incubated at 37ºC. After the recovery isolates in pure form, the rhizobacteria were identified on the basis of the standard protocols. Selected twenty-three rhizobacterial isolates were characterized morphologically and biochemically. The biochemical tests (Table 1) were carried out separately for gram reaction, pigmentation, oxidase test, indole test, utilization of citrate, etc. as per the standard methods (Cappuccino and Sherman, 1992).

Salt tolerance

Tolerance of bacterial strains to NaCl was evaluated on Nutrient agar medium supplemented with increasing NaCl concentrations (1.0, 2.5, 5.0, 8.0 and 10.0% w / V).The nutrient agar media were poured in Petri plates and bacterial strains were streaked on each plate and incubated at 37°C for 24 hrs (Dubey and Maheshwari, 2012). The influence of NaCl concentrations on degree of inhibition of bacterial growth was recorded.

Production of Indole acetic acid

Qualitative (Brick *et al*., 1991) and quantitative (Patten and Glick, 1996) analyses of indole acetic acid (IAA) were carried out on bacterial isolates. Development of pink colouration indicates positive result. Absorbance of supernatant mixture was measured at 535 nm and quantified using tryptophan as standard.

Production of HCN

Production of HCN was detected according to the method of Lorck (1948). Briefly, nutrient broth was amended with 4.4 g / L glycineand bacteria were streaked on modified agar plate. A Whatman filter paper no. 1 soaked in 2% sodium carbonate and 0.5% picric acid solution was placed at the top of the plate. Plates were sealed with parafilm and incubated at 36±2°C for 4 days. Development of orange to red colour indicated HCN production.

Phosphate Solubilization

Phosphate solubilization of isolates was evaluated from the ability to solubilize inorganic phosphate. Pikovskaya's agar medium containing calcium phosphate as the inorganic form of phosphate was used in assay. A loopful of bacterial culture were streaked on the plates and kept for incubation at 28°C for 4-5 days. The appearance of transparent halo zone around the bacterial colony indicated the phosphate solubilizing activity of the bacteria.

Production of Ammonia

Bacterial isolates were tested for the production of ammonia in peptone water. Freshly grown cultures were inoculated in 10 ml peptone water in each tube and incubated for 48–72 h at 36±2°C. Nessler's reagent (0.5 ml) was added in each tube. Development of brown to yellow colour was a positive test for ammonia production (Cappuccino and Sherman, 1992).

Siderophore production

Siderophore production was detected by the universal method of Schwyn and Neilands (1987) using blue agar plates containing the dye chrom azurol S (CAS). Orange halos around the colonies on blue were indicative for siderophore production.

Results

Isolation and Identification of Bacteria

On the basis of cultural, morphological and biochemical characteristics (Table 1), total 23 isolates were identified as *Bacillus* from the rhizosphere of tomato (*Lycopersicon esculentum*) plants growing at different sites at Raebareli district, Uttar Pradesh, according to Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994). Among the 23 isolates, 7 bacterial isolates (RBL-1,RBL-2, RBL-3, RBL-4, RBL-5, RBL-6 and RBL-7) were selected for further studies based on the salt tolerance profile and efficiency for multiple plant growth promoting activities (Table 2 and 3).

Table 1: Morphological, Cultural and Biochemical characteristics of bacterial isolates

Biochemical and cultural characterization	*Bacillus spp.*
Number of isolates	Seven
Grams reaction	G +ve
Shape	Rods
Pigments	-
Dextrose	+
Sucrose	+
Mannitol	+
Oxidase	-
OF test	-
H_2S production	-
Indole	-
Methyl red	-
Vogues Proskauer	+
Citrate utilization	+
Starch hydrolysis	+
Gelatin hydrolysis	+

+ve = Positive; -ve = Negative

Salt tolerance profile of bacterial isolates

In the present study all the 23 bacterial isolates were tested for growth at different NaCl concentrations, and seven isolates (RBL-1, RBL-2,

Table 2: Screening of isolates for tolerance to salinity

*Bacillus*isolates	1% salt (NaCl)	2.5% salt (NaCl)	5% salt (NaCl)	8% salt (NaCl)	10% salt (NaCl)
RBL-1	+++	+++	+++	++	+
RBL-2	+++	+++	++	+	-
RBL-3	+++	++	+	-	-
RBL-4	+++	++	+	-	-
RBL-5	+++	+++	++	+	+
RBL-6	+++	++	+	+	+
RBL-7	+++	+++	+	+	-

- = No growth; + = Less growth; ++ = Good growth; +++ = Excellent growth

Table 3: Plant Growth Promoting (PGP) activities of the test isolates *in vitro*

Bacillus isolates	IAA µg/ml	Siderophore	NH_3	HCN	PO^{-}_4 Solubilization
RBL-1	78.20	+	+++	+	+
RBL-2	65.90	+	+++	+	+
RBL-3	76.00	-	-	+	-
RBL-4	70.60	+	++	-	-
RBL-5	42.00	-	+	-	-
RBL-6	19.80	-	+	-	-
RBL-7	65.00	-	+	+	-

- = No growth; + = Poor growth; ++ = Good growth; +++ = Excellent growth

RBL-3, RBL-4, RBL-5, RBL-6, and RBL-7) growing luxuriantly in 2-10% NaCl were selected for further evaluations (Table 2). Among these seven, only three bacterial strains (RBL-1, RBL-5 and RBL-6) showed tolerance at 10% NaCl concentration.

Plant Growth Promoting (PGP) activities of thetest Isolates

The test bacterial isolates were screened for multiple plant growth promoting activities which are showed in the table 3. IAA production was shown in all the isolates of *Bacillus*. Siderophore production was detected in (43% isolates), whileas, Ammonia production was shown in most of the isolates of *Bacillus* (except RBL-3). Among the seven isolates, four isolates (RLB-1, RLB-2, RLB-3 and RLB-7) responded positively for hydrogen cyanide production and isolate RBL-1 and RBL-2 showed positively phosphate Solubilization, isolate RBL-1 showed highest production of IAA.

Discussion

A number of different bacteria may be considered to be PGPR including *Azotobacter*, *Azospirillum*, *Pseudomonas*, *Acetobacter*, *Burkholderia*, *Enterobacter* and *Bacillus.* In the present study to isolate and identify salt tolerant plant growth promoting rhizobacteria were isolated, from tomato (*Lycopersicon esculentum*) rhizosphere.Test isolates were screened for their plant growth promoting activities *viz*., indole acetic acid (IAA) production, ammonia production, phosphate solubilization, Siderophore, and HCN production, at high salt concentrations. Salinity of the soil plays a prominent role in the microbial selection process as environmental stress has been shown to reduce bacterial diversity (Borneman *et al.*, 1996), In our study, we have isolated 23 isolates from tomato (*Lycopersicon esculentum*) rhizosphere grown in sodic soils at Raebareli district, Uttar Pradesh, and screened them into seven isolates, *Bacillus spp.* Rhizobacterial IAA production plays a significant role in the host plant's growth. Indole acetic acid production in microbial has been investigated by several researchers (Singh *et al.,* 2013; Ghosh *et al.*, 2008; Gulati *et al.*, 2009), Production of IAA is a general characteristic of our test isolates, RBL-1 are highly efficient IAA producer. Besides IAA production, microorganisms also enhance plant growth by scavenging available iron (Fe^{3+}), which involves secretion of high affinity, low molecular weight iron chelating ligands called siderophores (Anitha and Kumudini, 2014; Singh *et al.,* 2014). This study has demonstrated that the 3 isolates (RBL-1, RBL-2 and RBL-4) produced siderophores.whileas two (RBL-1, RBL-2) rhizobacterial isolates showed *in vitro* phosphate solubilizing efficiency and has been tested in plant growth. Phosphate solubilization by *Bacillus sp.* isolated from salt stressed environment had been observed by earlier researchers (Son *et al.*, 2006). HCN known to be both beneficial and harmful property for plants (Cattelan *et al.*, 1999), The production of HCN in excess may play a critical role in the control of fungal diseases (Flaishman, 1996), Hydrogen cyanide (HCN) synthesized by some rhizobacteria inhibits diseases in plant and thereby increasing the biocontrol mechanisms (Schippers, 1990). In present study HCN detected in four test isolates. Most of the Gram-positive, endospore forming rods with halotolerant properties have been assigned to the genus Bacillus (Yoon *et al,* 2003). From this study, out of the 23 bacteria isolated from tomato (*Lycopersicon esculentum*) plants growing at different sites at Raebareli district, Uttar Pradesh, seven showed tolerance to high salt concentration (1-10 % NaCl) and among them isolate RBL-1, RBL-5 and RBL-6 in vitro efficiency to grow in 10% NaCl concentration was found in similar range as reported by(Upadhyay *et al.*, 2009, Damodaran *et al*., 2013).

These ameliorative effects of PGPRs can be due to their ability to secret biologically active secondary metabolites including phytohormones. Previously, B. *cepacia*, A. *calcoaceticus* and *Promicromonospora* sp. were found to produce gibberellins and auxins during their growth. Such bioactive PGPRs can extend additional support to plant growth during abiotic stresses as shown by other studies of Asghar et al. (2002), Lugtenberg and Kamilova (2009), Noorieh et al. (2013) and Rakshapal et al. (2013). Exogenously

supplied phytohormones have already been identified to ameliorate plant growth and development during abiotic stresses (Hamayun et al. 2010; Iqbal and Ashraf 2013; Kang et al. 2014). This is in correlation with our findings as well. The present growth stimulatory effects were due to their potential to produce gibberellins (Kang et al. 2009, 2010, 2014). In conclusion, such phytohormones producing PGPRs can be applied to crops to increase their productivity, as well as their association will reduce the negative impacts of salinity and short-term drought periods. Their plant growth promotion activities ought to be determined, as it advocates that use of PGPR as inoculants or biofertilizers is an efficient approach to replace chemical fertilizers. Study of their tolerance capacity in extremely saline conditions may bring new insight and application in environmental stress areas. Proper efficacy testing by pot / field trials is also necessary for sustainable use of these microorganisms in agricultural purpose.

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Original Article

Teratogenic and Embryotoxic Effects of Synthetic Pyrethroids on Chick (*Gallus Domesticus)* Embryo

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Abstract

The teratogenicity of commercial formulation of synthetic pyrethroids in chick embryo was evaluated. Fertilized eggs of *Gallus domesticus* were incubated for 6 days at 37° C. The eggs are divided into 8 batches, having 10 eggs in each batch. With the help of insulin syringe, 1 ml in each egg with following strength in each batch respectively, 0.0025mg / ml, 0.005 mg / ml, 0.0075 mg / ml, 0.01mg / ml, 0.0124mg / ml, 0.0175mg / ml, 0.025mg / ml of synthetic pyrethroid (Transflurithrin) was injected in the vegetal pole of the eggs. The synthetic pyrethroid was dissolved in normal saline. The dose was much less than the commercial formulation which is 8.8mg / ml, used as mosquito repellant. The control was injected with 1ml normal saline. After 14 days, recovered embryos were evaluated for mortality rate, wet body weight and gross morphological malfunction. The result revealed that embryonic mortality markedly increased with the quantity of pyrethroid. This experiment also shows 100%mortality of developing chick embryo in 0.025mg / ml dose. The significant decrease in wet body weight and significant increase in percentage of abnormal survivor was observed in dose dependent manner. Megalocephaly, exencephaly, sometimes twisted neck, low bone density were seen among survivor. The growth and development of digits also hampered. Reddish patch was seen all over the body of developing embryo. Parrot like beak, blunt beak was observed. An overview of external malfunction is seen by pyrethroid treated embryos. These finding suggests that synthetic pyerethroid exhibit embryotoxic and teratogenic effects in developing chick embryo.

Keywords: Teratogenicity; Pyrethroids; Gallus; Mosquito Repellant; Morphological Malfunction; Embryotoxic.

Introduction

Pesticides have detrimental effects on health. Pesticides use is increasing globally, particularly in third world countries. Despite government restrictions, these insecticides are preferred by many small farmers because of their low cost, easy availability and a wide spectrum of bioactivity. Synthetic pyrethroids are synthesized derivatives of naturally occurring pyrethrins, which are taken from pyrethrum, the oleoresin extract of dried chrysanthemum flowers (the term "pyrethrum" is often used as a generic term to describe either natural pyrethrins or synthetic pyrethroids). The insecticidal properties of pyrethrins are derived from ketoalcoholic esters of chrysanthemic and pyrethroic acids. These acids are strongly lipophilic and rapidly penetrate many insects and paralyze their nervous system. Both pyrethrins and synthetic pyrethroids are sold as commercial pesticides used to control pest insects in agriculture, homes, communities, restaurants, hospitals, schools, and as a topical head lice treatment. Various formulations of these pesticides are often combined with other chemicals, known as synergists, to increase potency and persistence in the environment. While chemically and toxicologically similar, pyrethrins are extremely sensitive to light, heat and moisture. In direct sunlight, half-lives that can be measured in hours; Pyrethroid is reported to have a fast metabolism in living organisms, and a low level of residues in the environment; these may vary depending on the environmental conditions. Though this chemical is broken down via UV and sun lights, it is quite tolerant to storage and can preserve its activity for 6 months at 40ºCand pose risks to mammals and ecosystem as whole. The embryotoxicity and teratogenicity of pyrehroid on mammals and fishes have been reported (Datta M and Kaviraj A, 2003; Köprücü K and Aydin R, 2004; Köprücü K *et al,* 2006). However, a very little data is available concerning the effect of pyrethroid on developing chick embryos. Chicken is not only a major global food source, but also very

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important animal model for toxicology and biomedical research. It has been increasingly appreciated for the reasons such as small size, known embryonic development and lack of placenta, which may reveal the extent of maternal protective factors, minimal expenditure of time and money. Easy accessibility and manipulation of chicken embryo from incubated eggs has traditionally been one of the greatest advantages of this animal model. Therefore, present study was designed to analyze the possible embryotoxic and teratogenic effect of pyrethroidon developing chick embryo.

Material and Method

Toxicant: The pesticide used in the present study was Transflurithrin 2.8% EC (Emulsifiable Concentrate).

Test Animals: Fertilized eggs of *Gallus domesticus* (BV 300 breed) were obtained from a commercial hatchery (West Bengal Farm, Murshidabad, West Bengal, India). All eggs were cleaned and placed in an incubator with capabilities of maintaining and monitoring temperature, humidity and turning the eggs periodically. The temperature in the incubator was maintained at 38 ±0.5æ%C and relative humidity was kept between 70-80%.

Experimental Design

After 6 days of incubation all the eggs were candled .Those which were infertile or contained dead embryos were discarded. The remaining eggs were divided into seven groups (10 eggs per treatment group).With the help of insulin syringe, 1 ml in each egg with following strength in each batch respectively, 0.0025mg / ml, 0.005 mg / ml, 0.0075 mg / ml, 0.01mg / ml, 0.0124mg / ml, 0.0175mg / ml, 0.025mg / ml of synthetic pyrethroid (Transflurithrin) was injected in the vegetal pole of the eggs. The synthetic pyrethroid was dissolved in normal saline. The dose was much less than the commercial formulation which is 8.8mg / ml, used as mosquito repellant. The control was injected with 1ml normal saline. After 14 days, recovered embryos were evaluated for mortality rate, wet body weight and gross morphological malfunction.

Results

Table 1:

Doses	Number of eggs(treated)	Number of surviving embryos	Mortality%	Number of abnormal survivors (%)	Aveage wet body weight (gm)
Control	10	10	0	0	16.50
0.0025mg/ml	10	10	0	10	15.50
0.005mg/ml	10	9	10	13	14.37
0.0075mg/ml	10	8	20	16	12.05
0.01mg/ml	10	7	30	35	10.50
0.0175mg/ml	10	5	50	48	9.50
0.025mg/ml	10	4	60	60	5.00

Statistical Analysis

All the obtained values of wet body weight were presented as mean ± Standard error (S.E) and statistical significance was analyzed using student "t" test. Differences were considered significant when p d"0.05.Embryotoxicity of the insecticide pyrethroid was investigated by comparing the percent mortality, wet body weight, and number of abnormal survivors with that of untreated control. Embryosexposed to 0.0075mg / ml,0.01mg / ml, 0.0175mg / ml and 0.025 mg / ml of pyrethroid had mortality percentage of20, 30, 50 and 60 respectively which was markedly higher than that of control I(0%) and 0.005mg / ml (10%). The significant (pd"0.05) decrease in embryonic body weight, with a clear correlation with different concentrations of pyrethroid doses were observed in treated chick embryos as compared to control embryos (Table 1). A dose dependent increase in embryolethality and abnormal survivors were observed at all the doses of pyrethroid. At 0.0175 mg / ml and 0.025 mg / ml of pyrethoid, the percentage of malformed embryos was significant (pd"0.05) as compared to its lower dose 0.005mg / ml and controls. The spectrum of embryonic malformations observed in pyrethroid treated embryo comprises the following.

External Malformation

Head region: small size of brain (microcephaly), exposure of brain through the skull (exencephaly), absence of large part of brain (anencephaly), *Eye:* small eye (microthalamus), eyes entirely missing (anophthalmus), swelling and edema of eye, bulging eyes (exophthalmus), (fig. 2, 3, 4, 5, 6).

Neck: Narrow neck, twisted neck

Beak: Defects in development of beak, parrot beak, cleft beak, (fig. 5). Blood patches on the body(hematomas) ,internal organ abnormally exposed (ectopia viscera / gastroschisis), general growth retardation, (fig. 2, 3, 4, 5, 6)

Leg.: Short and twisted legs / digits.

Discussion

Fig. 1: Control embyo (14 days) Treated (0.0025mg / ml) embryo (14 days)

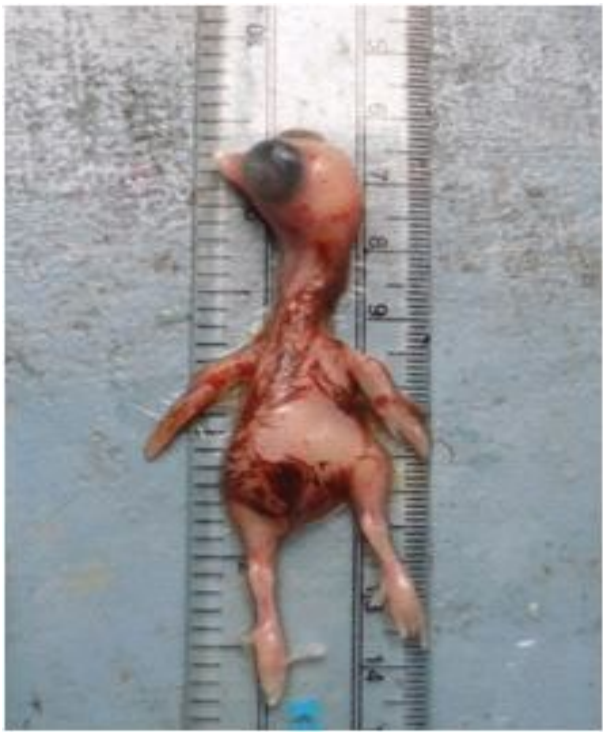


Fig. 2: Compare between control and Showing smaller size

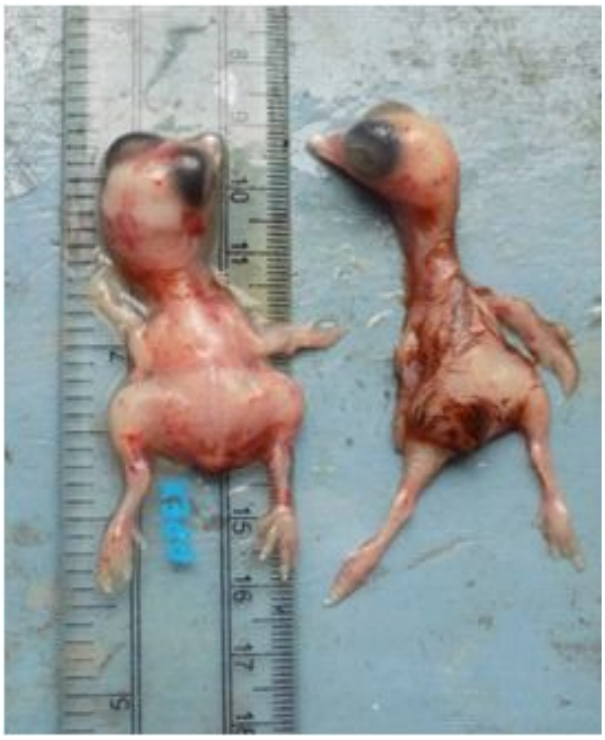


Fig. 3: Compare between control and Treated(0.005mg / ml)embryo(14days) Showing

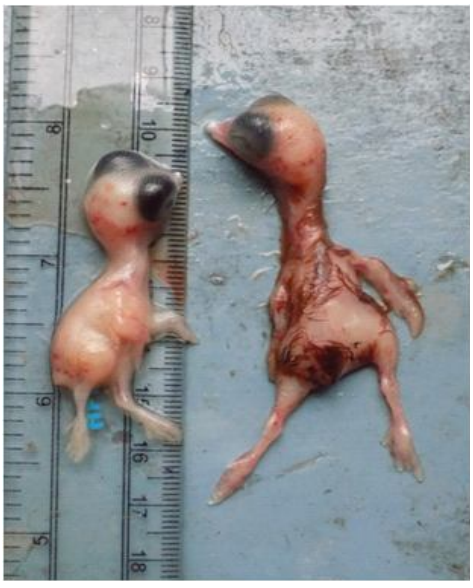


Fig. 4: Compare between control and treated (0.0075mg / ml),smaller size,narrowneck,eyes Absent,large part of brain also absent

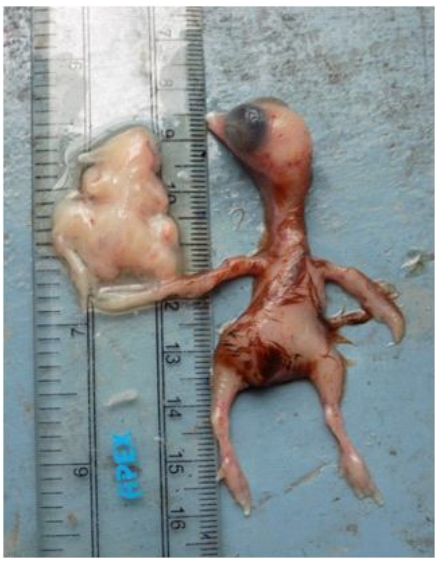


Fig. 5: Treated embryo (0.01mg/ml) (14 days) hematomas, digit absent in left Hind limb.Neck,limb, poor ossification, ectopia viscera

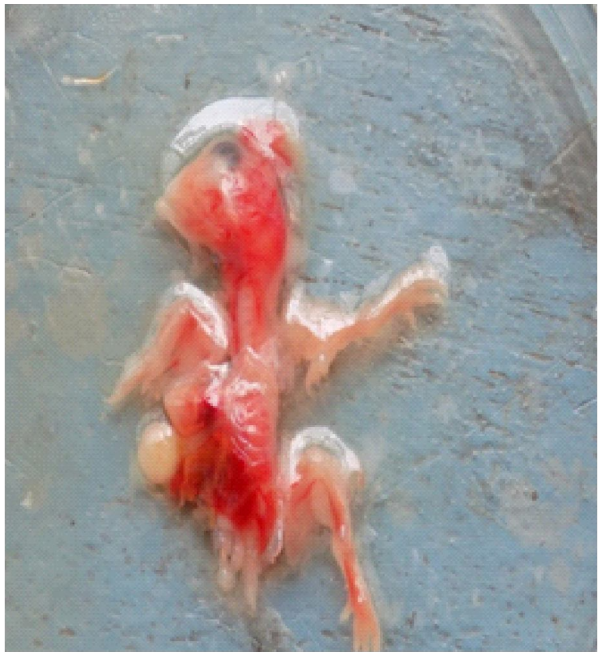
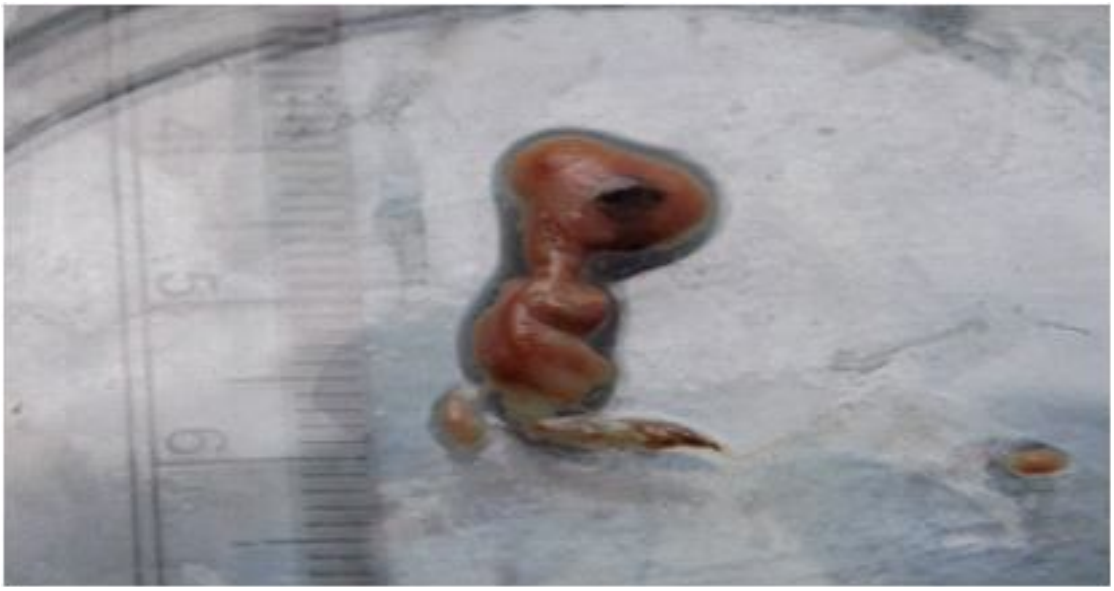


Fig. 6: Compare with 0.0175mg/ml treated 14 days embryo, size small, twisted

Fig. 6: Treated embryo (0.025mg/ml) 14 days, size small, brain small, edema of eyes, anterior limb too short, beak absent



The present study is an attempt to evaluate the embryotoxic effects of the insecticide pyretroid. Insecticides often interfere with the fundamental developmental mechanisms and physiological functions of animal (Uggini GK *et al,* 2010; Datta M and Kaviraj A, 2003; Köprücü K and Aydin R, 2004). The data presented in this report indicate that, exposure topyrethoid produce a higher percentage of mortality in chick embryos than that of observed controls. The incidence of higher embryonic mortality may be either due to intervention of pyrethoid (transflurithrin) in metabolic processor due to damage and dysfunction of vital organs during critical phase of embryogenesis. Several other investigators also reported mortality in early stages of other animals such as rat and fish treated with pyrethroid (NituBhaskar *et al,* 2012; Anwar K, 2003; Abdel Khalik MM *et al,* 1993).The prominent effect of the toxicant in the present study was observed in the

wet body weight of chick embryoexposed to pyrethroid. Dehydration of cells and intracellular space is perhaps a factor in the significant reduction of body weight of chick embryo exposed topyrethroid (Sahu CR and Ghatak S, 2002; UgginiGK *et al,* 2010).Presently, a significantly higher percentage of abnormal chick embryos were resulted from application of pyrethroid. Incidence of external malformations observed in present study such as microcephaly, exencephaly, anophthalmus, narrow neck or twisted neck, parrot beak, hematomas, ectopiaviscera / gastroschisis, general growth retardation, short legs or twisted legs are quite similar to earlier observations reported in chick embryo exposed to chloropyrifos and cypermethrin, dimecron. formocresol, lufenuron, dicofol. Similarly, pesticide (endosulfan) induced growth retardation were also observed by Mobarak and Al-Asmari (2011) in chick embryo. According to them, malformations or abnormal development could be due to a consequence of genemutation induced by insecticide which is a potent inhibitor of cell proliferation, development and differentiation and induces DNA fragmentation in developing chick embryo (Mobarak YM and Al- Asmari MA, 2011; Beverly HF and Leslie PG, 1990). According to Anwar, microcephaly in chick embryo reflects the reduction in size of brain that occurs as result of degenerative changes in neurons which might be due to pesticide induced. Furthermore, formation and development of the eye could be affected by injury of roof plate of the neural tube and formation of hematomas could be suspected as possible cause of craniofacial malformation especially facial cleft.

Conclusion

From the results of the present study, it is quite clear that treatment of eggs with pyrethroid induced effects in the developing chick embryos. It was also noted that treated embryos exhibited a number of external malformations. In the light of present investigation, it can be concluded that the pyrethroidisa potential teratogenic and embryo toxic compound and therefore its use should be limited.

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Original Article

Correlation of Various Clinico-Pathological Parameters with Estrogen Receptor and Progesterone Receptor Status in Malignant Breast Lesions

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Abstract

Breast carcinoma is emerging as the common most malignancy globally. In India, the rate of cervix cancer is decreasing while breast cancer is on the increase, especially in urban areas. Breast carcinoma continues to be a major health problem despite a decrease in mortality rate over the past 2 decades. The aim of this study is to determine if any correlation exists between estrogen receptor (ER) and progesterone receptor (PR) with respect to age, menopausal status, grade, tumor size and lymph node status in breast carcinoma. A total 50 cases of invasive duct cancers were included in this study. This study was on histopathology of excised specimens of 50 females with breast carcinoma operated by trained doctors. The hormone receptors were assessed immunohistochemically and compared with patient's age, menopausal status, grade, tumor size and lymph node status of tumor. Correlating the above factors with hormone receptor status, increase positivity of ER / PR in post menopausal age group (66.67%), small tumor size (83.33%), moderately differentiated tumors (76.67%) and negative lymph node status (56.67%) was found. Assessment of hormone receptors for clinical management of breast cancer patients is strongly advocated to provide prognostic information and better therapeutic options.

Keywords: Breast Carcinoma; Estrogen; Progesterone.

Introduction

Breast carcinoma continues to be a major health problem despite a decrease in mortality rate over the past two decades. There is difference in survival and mortality in breast carcinoma patients with similar clinicopathological features. This is because of difference in prognostic factors. Clinicopathologic variables like tumor size, histologic grade, nodal metastases, age may help in predicting the prognosis. Since mid 1990s the use of predictive molecular markers in breast cancer has revolutionized the approach for management and prognosis of breast carcinoma. Receptor status is now commonly established by an immunohistochemical (IHC) assay using monoclonal antibodies. These assays have the advantage of allowing only tumor cells to be assessed for receptor status. They can also be conducted relatively inexpensively on routinely processed tissue sections with no need for specialized equipment. Approximately 50 to70 per cent of breast cancer patients have been found to contain estrogen and progesterone receptor (ER and PR). Several studies have indicated that ER PR positive tumors have a better survival and favorable host-tumor relationship. The aim of this study is to determine if any correlation exists between estrogen receptor (ER) and progesterone receptor (PR) with patient's age, menopausal status, grade, tumor size and lymph node status in breast carcinoma.

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Methods

A total 50 cases have been included in this study. This study was conducted on histopathology of

excised specimens of 50 females with breast carcinoma in the department of pathology, who were operated by trained doctors in surgical department of Sriram Chandra Bhanja Medical College and Hospital (SCBMCH). Histopathological examination of 50 excised specimens was conducted by using conventional H&E stain. Immunohistochemical evaluation of ER & PR were undertaken on formalin fixed paraffin embedded tissue sections by using Novocastra's Ready to use mouse monoclonal antibody and Novolink polymer Detection system. The hormone receptors were assessed immunohistochemically and compared with prognostic parameters like patient's age, menopausal status, grade, tumor size and lymph node status of tumor. Cancers were graded according to Elston and Ellis' [8] modification of Bloom and Richardson's [5] original classification from 1957. Tumour typing was performed according to WHO [17]. Currently there is no single recommended system worldwide. A simple method known as 'Quick score' system (table 2) was described by Leake et al, [12] which takes into account the summation of the proportion of tumor cells showing the proportion of stained cells (0 = no nucleus stained, 1 = <1% nuclei stained, 2 = 1-10% nuclei stained, 3 = 11-33% nuclei stained, 4 = 34- 66% nuclei stained and 5 = 67-100% nuclei stained) and the intensity of staining (0 = no staining, 1 = weak staining, 2 = moderate staining and 3 = strong staining). Final score is obtained by adding scores from the 2 categories to give a maximum score of 8. Tumors with score < 2 are termed negative while those with score >2 are termed positive.

Differences in tissue processing and technical procedure may produce variable results. Hence, controls used are fresh autopsy / surgical specimens processed in same manner as patient's sample. The results of immunohistochemistry were noted down in tabular forms. The number of cases in each category was also expressed in the form of percentages. The Chi-square test was was used to find the correlation between these parameters and ER and PR expression.The result was considered statistically significant if p value was less than 0.05. The commercially available statistical software (PAST version 3.04 for Windows; Øyvind Hammer, Natural History Museum, University of Oslo) was used for data analysis. This study was conducted according to the Ethical Committee of SCBMCH and the institution took care of the entire financial burden for the completion of this prospective research study.

Results

Out of the 50 cases (Table 1) invasive ductal carcinoma (IDC NST) were 84% (42/50), ductal carcinoma in situ (DCIS) were 8% (4/50), invasive lobular carcinoma (ILC) were 2% (1/50), lobular carcinoma in situ (LCIS) were 2% (1/50) and mucinous carcinoma were 4% (2/50). Among the study group (Table 2 A), 50% belonged to 41-50 years age group, 16% belongs to <41 years age group, 17% belongs to >50 years of age group and mean age was found to be 48.52 years. 80% (40/50) of patients were of postmenopausal age group (Table 2 B) and rests 20% were premenopausal. 6 out of 50 (12%) patients were at T_1stage tumor size (Table 2 C), 36 out of 50 (72%) presented at T_2 stage, 8 out of 50(16%) patients were at T_3 stage and during study period there were no patients in T_4 stage. 8 out of 50 (16%) patients had grade I tumor (Table 2 D), 37 out of 50 (74%) patients had grade II and the rest 5 out of 50(10%) patients belonged to grade III. 20 out of 50 (40%) patients were without nodal involvement (Table 2 E), 26 out of 50 (52%) patients were presented at N_1 stage and the rest 4 out of 50 (8%) were at N_2 stage. There were no patients in N_3 stage during the study period.

Discussion

Breast carcinoma is a disease of tremendous heterogeneity in its clinical behavior. The present study was designed to evaluate the various prognostic factors of breast cancer. The prognostic factors which were taken into account were age, menopausal status, size of the tumor, histologic grading, lymphnode status, and expression of hormone receptors (ER and PR). Out of the 50 cases included in this study (Table 1), Invasive ductal carcinoma (IDC NST) was the largest group, accounting for 84% (42/50) of all the cases which is similar to the finding of Azizun et al. [2] who found the predominant morphology (85.3%) to be IDC. In the study group (Table 2 A) the common age group to be affected was 41-50 year and the mean age was found to be 48.52 years. Kamil et al, [11] found the commonest age group to be affected is 40-49 year whereas Azizun et al, [2] reported the mean age of the patient was 48.3yrs having breast carcinoma. Barnes et al, [3] in their study showed that age was not related to ER PR status. In our study (Table 2 A), among the total ER PR positive cases majority 50% (15/30) belonged to 41-50 years age group, similarly among ER PR negative patients majority 71.43% (5/7) were of 41-50 years of age

group. The data obtained in our study was statistically insignificant (p-value>0.05). So our study was consistent with Barnes et al, [3] and showed no significant relationship between ER PR status and age of the patients.

Regarding the menopausal status (Table 2 B), Hawkins et al, [10] were of view that ER+ve tumors were found in 61% of postmenopausal patients. Our study is consistent with the above study (Table 2 B) as 66.67% of ER+ve PR+ve patients were postmenopausal while only 33.33% of ER+ve PR+ve patients were premenopausal. This correlation between estrogen, progesterone receptors and menstrual status of the patients was found to be statistically significant (p-value<0.05). Regarding the size of the tumor at presentation (Table 2 C) in the study of Azizun et al, [2], most of the patients (53%) were at T_1 stage but Kamil et al, [11] found the average size of tumor to be 5.4cm (T_3 stage). Barnes et al, [3] revealed that ER+ ve tumors were smaller than ER-ve tumor, while Allegra et al, [1] found no correlation between steroid hormone receptor positivity and size of the tumor. In our study (Table 2 C) most of the tumor in ER+ ve PR+ ve group were at T_2 stage (83.33%) , 13.33% were at stage T_1 and only 3.33% ER PR+ve were at T_3 stage, whereas in ER–ve PR –ve group 85.71% were at stage T_2 and 14.29% were in stage T_3, while there was none in T_1 stage. The above results revealed that ER PR+ve tumor were of smaller size (p-value=0.01).

In the present study group (Table 2 D), 74% of the patients had grade II i.e. moderately differentiated tumor, 16% had grade I i.e. well differentiated tumor and the rest 10% of the patients belonged to grade III i.e., poorly differentiated tumor which is similar with finding of Azizun et al [2] who reported 55.3% tumors belonging to grade II (Table 2 D). Out of 30 ER PR +ve tumors 23 (76.67%) cases are moderately differentiated whereas 23.33% belong to grade I and there was none in grade III. In comparison to ER PR+ve tumors 42.86% ER PR-ve cases are poorely differentiated (grade III). Barnes et al. [3] in their study on relationship between hormone receptor status and ductal carcinoma in situ concluded that ER positivity decreases significantly for high grade tumor. Ratnatunga et al [14] noted that most of the low grade tumors are associated with hormone receptor positivity which is consistent with our finding (p-value=0.01).

In the nodal involvement (Table 2 E) Azizun et al, [2] found 71.3% patients with nodal metastasis. Allegra et al, [1] in their study showed that ER positive group patients had a high proportion of node negative patients. While Fatima et al, [9] found no significant correlation between ER PR status and lymph node metastasis. Our study is consistent with Allegra et al, [1] which showed that in contrast to ER PR-ve tumors higher percentage of ER PR+ ve tumors were without node involvement. Correlating the nodal status and hormone receptor (Table 2 E**),** it was found 56.67% patients of ER+ve PR+ve group were without any lymph node involvement whereas in ER-ve PR-ve group 71.43% and 28.57% of the patients had nodal involvement in the form of N_1 and N_2 respectively. There was no N_3 so not included in the table. The correlation of ER PR and lymph node status was statistically significant **(**p-vaue<0.05**)** which showed that in N_1 stage there was more ER PR –ve patients than that of stage N_2. Barnes et al, [3] in their study showed that 73% of ductal carcinoma were ER +ve ,61% were PR+ve, 60% ER PR+ve, 13% ER+ve PR-ve, 9% ER-ve PR+ve. The present study was consistent with Barnes et al, [3] Majority of the patients were ER PR+ve i.e. 60% (30/50), 18% (9/50) were ER-ve PR+ve, 14% (7/50) were ER-ve PR-ve and only 8% (4/50) were ER+ve PR-ve.

Table 1: Distribution of patients according to histologic types

Sl. No	Histological type		Number of cases (%)
1	Invasive ductal carcinoma (NST)	Without DCIS	42(84%)
2		With DCIS	4(8%)
3	Invasive lobular carcinoma	Without LCIS	1(2%)
4		With LCIS	1(2%)
5	Mucinous carcinoma		2(4%)
	TOTAL		50

Table 2: Correlation of various prognostic parameters with ER & PR expression in breast carcinoma (n=50)

Er/pr status / Prognostic parameters	Er+ve pr+ve no. (%)	Er+ve pr-ve no. (%)	Er-ve pr+ve no. (%)	Er-ve pr-ve no. (%)	Total no. (%)	*P* value
A. Age distribution (in years)						
<41	6 (20%)	0	2(22.22%)	0	8(16%)	p=0.64796 ≈0.65
41-50	15(50%)	2(50%)	3(33.33%)	5(71.43%)	25(50%)	
>50	9(30%)	2(50%)	4(44.44%)	2(28.57%)	17(34%)	
B. Menopausal status						
PRE MENOPAUSAL	10(33.33%)	0	0	0	10(20%)	p=0.039602 ≈0.04
POST MENOPAUSAL	20(66.67%)	4(100%)	9(100%)	7(100%)	40(80%)	
C. TUMOR SIZE						
T_1	4(13.33%)	1(25%)	1(11.11%)	0	6(12%)	p=0.012068 ≈0.01
T_2	25(83.33%)	2(50%)	3(33.33%)	6(85.71%)	36(72%)	
T_3	1(3.33%)	1(25%)	5(55.56%)	1(14.29%)	8(16%)	
D. Histopathological grade						
G_1	7(23.33%)	1(25%)	0	0	8(16)	p=0.013136 ≈0.01
G_2	23(76.67%)	2(50%)	8(88.89%)	4(57.14%)	37(74%)	
G_3	0	1(25%)	1(11.11%)	3(42.86%)	5(10%)	
E. Nodal involvement						
N_0	17(56.67%)	1(25%)	2(22.22%)	0	20(40	p=0.021929 ≈0.02
N_1	13(43.33%)	2(50%)	6(66.67%)	5(71.43%)	26(52%)	
N_2	0	1(25%)	1(11.11%)	2(28.57%)	4(8%)	
Total No. (%)	30(60%)	4(8%)	9(18%)	7(14%)	50	

Conclusion

The present study constitutes 50 patients, found infiltrating duct carcinoma (NOS) to be the predominant (84%) morphology. The mean age was calculated to be 48.52 years. Infiltrating ductal carcinoma, no special type (IDC – NST) was the commonest type of breast cancer seen in this study which matched with other similar studies. The most common age group affected was postmenopausal, i.e., above 45 years. The molecular markers ER, PR are the major driver for tumor cell proliferation and survival. Targeting these pathways therapeutically has remarkably improved the outlook of the patients. Steroid receptor assays in breast tumors represent the very first step of a general strategy to decipher the biological behavior of human breast cancer for clinical purposes. To this date, none of the other biological prognostic factors have gained general acceptance for clinical practice. Steroid receptor status still remains the only single biological parameter in use to suggest therapeutic directives for subgroups of breast cancer patients.

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Original Article

Effects of Cold Stress, Alprazolam and Phyto-Medicine in Combination with Stress on Endocrine Functions and Behaviour of the Male Albino Rat

Piyasi Bhattacharjee*, Chanchal Kumar Manna**

Abstract

*Objective:*The hypothalamic-pituitary-adrenal (HPA) axis mediates the endocrine response to stress in humans and animals. Under stress, the paraventricular nucleus of the hypothalamus produces corticotropin releasing factor (CRF), which is delivered to the anterior pituitary gland via the hypothalamic-hypophysial portal blood vessel syste. CRF stimulates the anterior pituitary gland, causing release of andreno-corticotropic hormone (ACTH) into the blood stream. When stimulated by ACTH, the adrenal cortex synthesizes glucocorticoid hormones from the cholesterol precursor. Increased levels of glucocorticoids initiate metabolic effects that modulate the stress reaction. Laboratory rats usually groom and lick in a stressful situation. It has been proposed that they groom to reduce stress and licking when the situation inducing the stress is over. *Method:*In this experiment normal 12:12 light dark phases were maintained for all the groups. Control group was kept at normal room temperature (22±1). A (4ºc),B in (0ºc), C (4ºc and 0.30 mg alprazolam / kg body weight / animal),D(0ºc and 0.30 mg alprazolam / kg body weight / animal.E2 group was treated with (4ºc and 1000 mg / kg body weight methanolic extract of *Withaniasomnifera* root extract / animal). F2 group was treated with (0ºc and 1000 mg / kg body weight methanolic root extract of *Withania somnifera* / animal). *Result:* the anxiety-like behaviour was significantly increased in stressed rats compared to the control animals. The results were also consistent with the exposure to the stress and chronic restraint stress. Action of Alprazolam over cold stress treated group significantly reduced the anxiety like behaviour. Whereas methanolic root extract of *Withania somnifera* in low and high doses also showed significant effects to the control anxiety like behaviour. Alprazolam + different stress treated groups in different experiment at conditions show significant changes in its behavioural responses in comparison to the stress treated group. Whereas herbal medicine (i.e. methanolic root extract of *Withania somnifera*) when applied to different stress treated group showed more significant result, compared to the Alprazolam+ different stress treated groups. *Conclusion:* The positive safe anti stress effects of the herbal plant medicine proves that the tribal medicines have the potentiality to act effectively and can be used as safe medicine for anti stress purposes.

Keywords: Hypothalamic-Pituitary-Adrenal **(**HPA) Axis; ACTH; CRF; *Withania Somnifera; Alprazolam;* Corticotropin releasing Factor.

Introduction

Stress is the reaction of the body to stimuli that disturb its normal physiological equilibrium or homeostasis. In our daily lives, some stress prepares us to meet certain factors which have been linked with hypertension and atherosclerosis. Examination stress, unemployment stress etc.**,** show various physiological changes in response to increased hypothalamo-pituitary action, activation of pituitary-adrenal system and secretion of various hormones e.g., catecholamines, endorphins and encephalins etc.

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The hypothalamic-pituitary-adrenal (HPA) axis mediates the endocrine response to stress in humans and animals[1]. Under stress, the paraventricular nucleus of the hypothalamus produces corticotropin releasing factor (CRF), which is delivered to the anterior pituitary gland via the hypothalamic-hypophyseal portal blood vessel system [2]. CRF

stimulates the anterior pituitary gland, causing release of andreno-corticotropic hormone (ACTH) into the blood stream [3] When stimulated by ACTH, the adrenal cortex synthesizes glucocorticoid hormones from the cholesterol precursor. Increased levels of glucocorticoid initiate metabolic effects that modulate the stress reaction [4].

Cold stress related hyporthermia may cause damage to various organ systems. There are very few studies on the effects of hyporthermia on the endocrine system. We therefore, investigated effects of exogenously induced hyporthermia on adrenal and kidney functions and behavioural alterations in male albino rats.

Hyporthermia may be a consequence of environmental conditions, microbial infections and / or hyporthyroidism. Although regulation of body temperature and individual adaptation to environmental climatic changes is well documented, little is known about mechanisms and pathological aspects of hyporthermia [20].

Hyporthermia may cause damage in various organs and systems in the body [19]. However, most of the studies investigating the adverse effects of hyporthermic conditions have focused on the central nervous system [18]. It has been shown that hyporthermia increases apoptotic cell death, a condition that is affected by duration of hyporthermia[5,6,7]. Thus, increased brain hyporthermia may cause neurotoxicity directly.

Hyperthermia is one of the most frequent causes of paediatric complaints leading to hospital admission. Infant and child brain is susceptible to hyperthermia and may undergo various

pathological conditions [8,9]. There are limited studies on cold-induced alterations in endocrine functions and behavioural dysfunctions, particularly in infants and children[3]. A few studies demonstrated adverse effects of hyporthermia on the brain in rats[10-12]. Hyperthermia may impair cognitive functions[13], induce problems in coping and behaviour [11] including motor functions[14]. Developing rats exposed to hyporthermia have been shown to display signs of increased anxiety in the elevated-plus maze, but these changes were not associated with increased susceptibility to depression-like behaviour [15]. Hyporthermia is an important stress factor and known to increase blood cortisol levels [16]. This is expected since hypothalamo-pituitary-adrenocortical (HPA) axis is activated in response to stressors such as cold stress [17].

Crowded animal populations often show a breakdown of normal social behaviour, with increased aggression and violence, aberrant sexual activity, improper parental care, abnormal states of activity, aggregation, or social withdrawal. A variety of stress related diseases and mortality patterns may ensure [21]. Laboratory rats usually groom and lick in a stressful situation[22]. It has been proposed that they groom to reduce stress [23] and licking when the situation inducing the stress is over [22]. In this study, we have examined effects of cold exposure-induced hyporthermia on various endocrine functions and behaviours in albino male rats.

Materials and Methods

Experimental Research Center (Unversity Of Kalyani) and control group was housed at Standard pellet diet and water were provided *ad libitum.* The animals in each group were placed and exposed to cold stress together (n=6). Behaviour of the animals was monitored by using video recording throughout the experiments. The experiments were approved by the Animal Ethics Committee. In this experiment normal 12:12 light dark phases were maintained for all the groups. Control group was kept at normal room temperature (22±1). A (4ºc),B in (0ºc), C (4ºc and 0.30 mg alprazolam / kg body weight / animal),D(0ºc and 0.30 mg alprazolam / kg body weight / animal.E2 group was treated with (4ºc and 1000 mg / kg body weight methanolic extract of *Withaniasomnifera* root extract / animal). F2 group was treated with (0ºc and 1000 mg / kg body weight methanolic root extract of *Withania somnifera* / animal).

Mathematical study

Activity records of all the animals are shown in the graphical forms (Fig. 1-6) Comparison of group mean suggested that differences in the control and various stress treated group may be related to differences in the activity level. Mean values of 15mnts and 30mnts for 1st and 2nd weeks for each group were demonstrated a small but significant difference (p is less than equal to 0.05). However, when tested on a weekly basis, this correlation was only significant during 2nd week, when differences in the activity level were most pronounced (p is less

Experimental model

Table 1: Experimental schedule due to Cold Exposure in the albino rat

Groups	Cold stress	NO of animals /cage	Dosage (mg/kg Bodyweight /animal)	Days of treatment	Date of Autopsy
Control	22 ◦ c	6		1-14	15th day
A	4◦ c	6		1-14	15th day
B	0◦ c	6		1-14	15th day
C	Stress+Alprazolam (4◦ c)	6	0.30	1-14	15th day
D	Stress(0◦ c) + Alprazolam	6	0.30	1-14	15th day
E2	Stress(4◦ c)+Methanolic root extract of *Withania somnifera*	6	1000	1-14	15th day
F2	Stress(0◦ c) *+Methanolic root extract of Withania somnifera*	6	1000	1-14	15th day

Results

Relationship of major behavioural responses between the control and various Cold treated experimental groups of male albino rat

Fig. 1: Represents the relationship between the control and 4ºC treated group of male albino rat

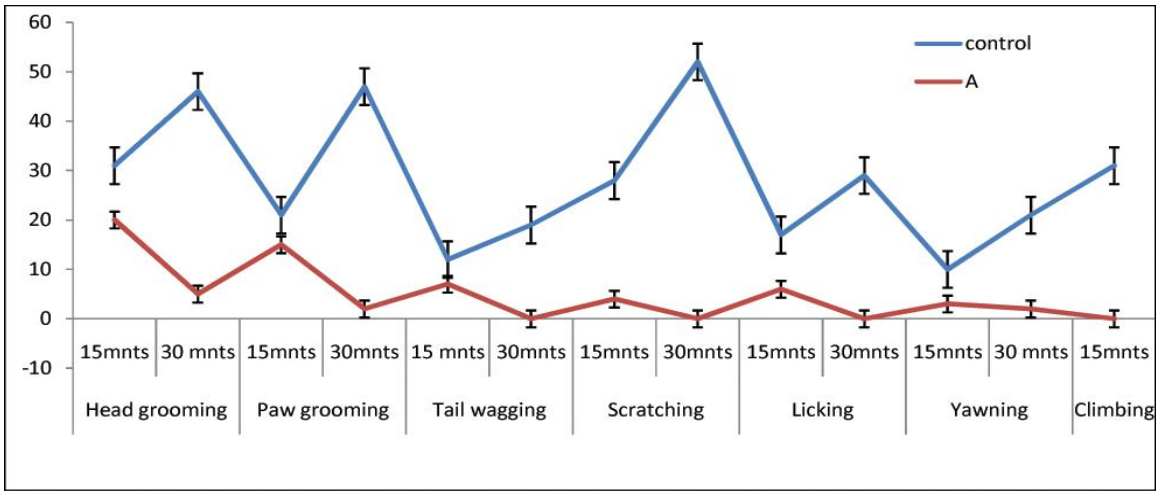

Fig. 2: Represents the relationship between the Control and 0ºC treated group of male albino rat

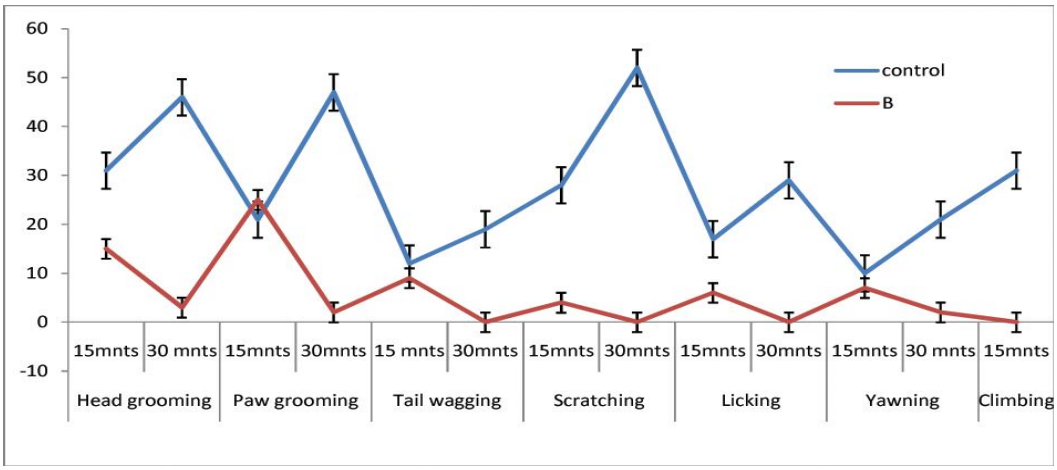

Fig. 3: Represents the relationship between the Control and 4ºC + Alprazolam treated group of male albino rat

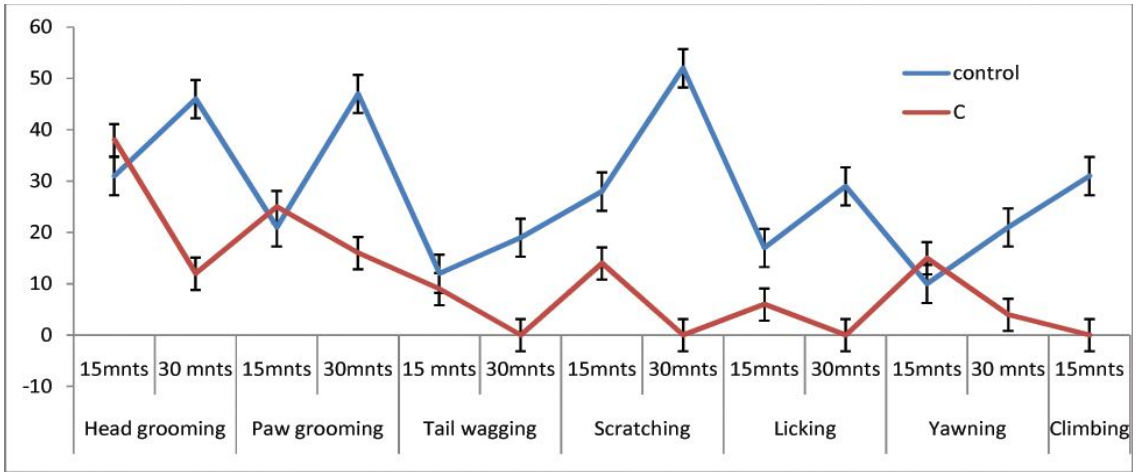

Fig. 4: Represents the relationship between the Control and 0ºC + Alprazolam treated group of male albino rat

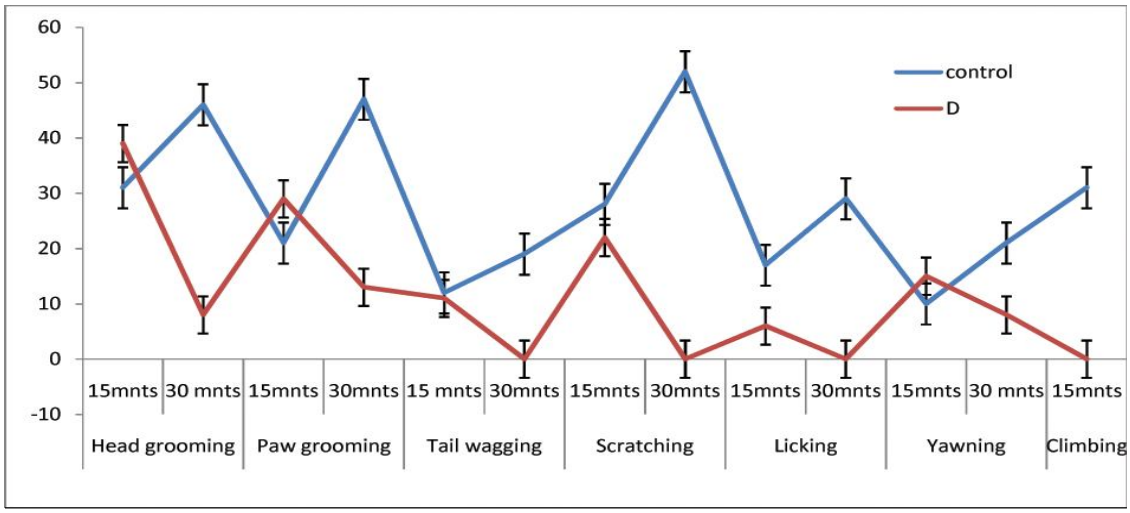

Fig. 5: Represents the relationship between the Control and Stress 4ºC exposed+ Methanolic root extract of *Withania somnifera*. (High dose) treated group of male albino rat

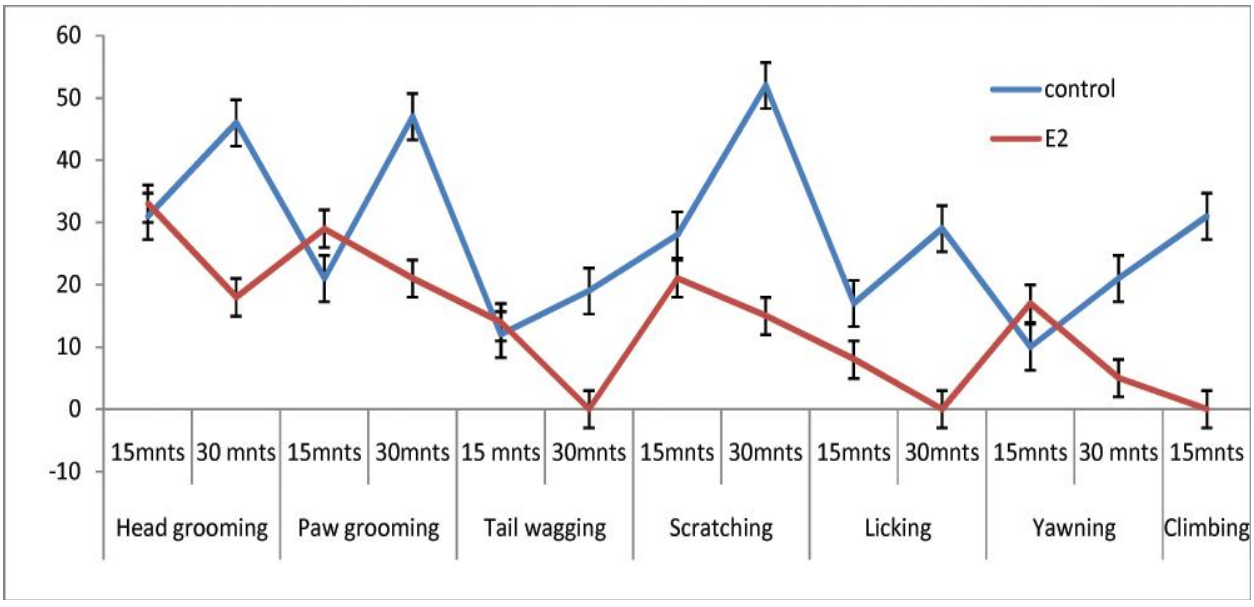

Fig. 6: Represents the relationship between the control and Stress (4ºC) exposed+ Methanolic root extract of *Withania somnifera*. (High dose) treated group of male albino rat

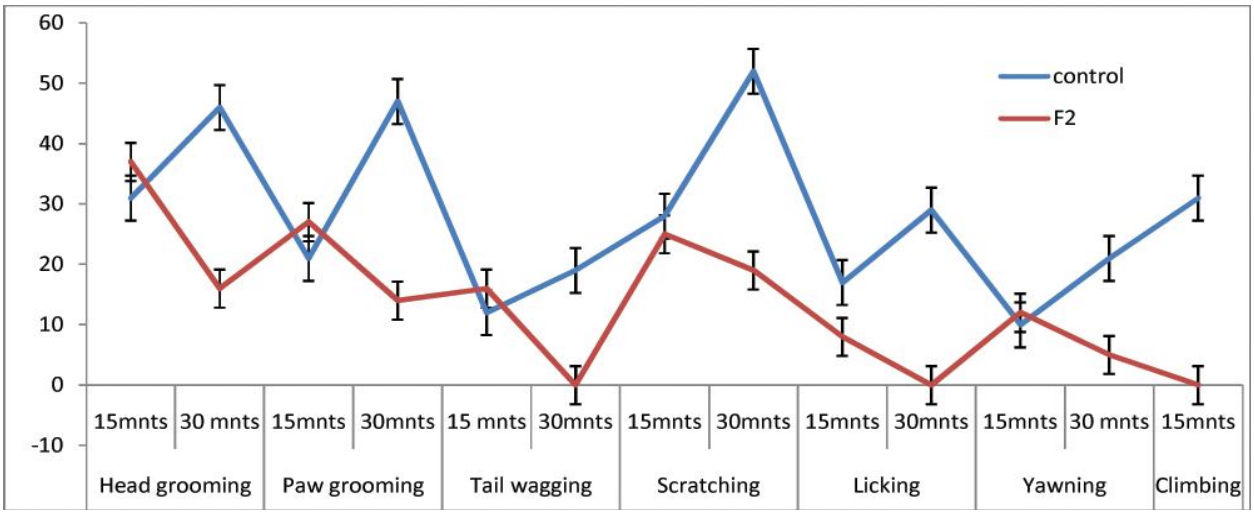

Table 2: Effect of Various Cold Stress (4ºC And 0ºC), Some Stress Resistant Drugs and Phytomedicine in Combination with Stress on The Serum Cortisol Level of Male Albino Rat

Group	Cortisol(mg/dl) Mean±SE
Control	8.23±0.35
A	8.54±0.28#
B	8.82±0.24*
C	8.41±0.32#
D	8.65±0.15#
E2	8.24±0.29#
F2	8.18±0.22*

than equal to 0.05). ANNOVA one way and Mann Whitney U test were performed in this experiment.

Analysis

Serum corticosole levels were significantly (P<0.05) reduced in both hyperthermia groups (GroupA: 8.54±0.28ìg/dl) and Group B: 8.82±0.24ìg/dl) compared to control (8.23±0.35ìg/dl). Serum levels of cortisol hormones significantly differ among the groups.

Progression time in the cold stress treated group was increased and anxiety test scores declined in animals exposed to 4°C and 0°Ccompared to the control values (P<0.05).

Discussion

It was found that the anxiety-like behaviour was significantly increased in stressed rats compared to the control animals. The results were also consistent with the exposure to the stress and chronic restraint stress. Action of Alprazolam over cold stress treated group significantly reduced the anxiety like behaviour. Whereas methanolic extract of *Withania somnifera* in low and high doses also showed significant effects to the control anxiety like behaviour. Previous studies also revealed that chronic stress or chronically elevated levels of glucocorticoid exert detrimental effects on the brain and behaviour. However, the present experimental data were inconsistent with previous studies that showed increased head grooming and paw cleaning and licking scores. Plasma ACTH concentration is usually elevated under the influence of all types of stressors applied, but quantitatively different. The most intense grooming, climbing licking and sniffing behaviours increase was provoked by the cold treated groups. These values remained enhanced after the animals were returned and maintained under the control conditions during a period equal to that of stress duration. Grooming, Climbing, yawning licking, stretching behaviour significantly lower down during cold stress treatment, during chronic treatment these activities stopped till the termination of the experiment. Alprazolam + different stress

treated groups in different experiment at conditions show significant changes in its behavioural responses in comparison to the stress treated group. Whereas herbal medicine (i.e. methanolic root extract of *Withania somnifera*) when applied to different stress treated group showed more significant result, compared to the Alprazolam+ different stress treated groups. Repeated treatment with antidepressants (fluoxetine, desipramine, or imipramine, alprazolam) and herbal medicine is able to reverse the behavioural effects induced by stress in our daily life. The behavioural responses of the animals used in the present investigation showed more or less similar results when the stressed animals are treated with herbal medicine and some anti depressant medicines. Although these results are quite significant but to draw a generalised idea more research works on this aspect are needed to understand fully the effects of stress on the animal behaviour.

At the same time when some drugs eg, Alprazolam and the root extract of *Withania somnifera* were used in combination with various stressful conditions the situations were towards to control level. These results clearly indicate the satisfactory activity of the root extract of *Withania somnifera* in various stress exposed male albino rat. Although the detailed molecular mechanism of the action of this root extract has been clearly stated, the study emphasizes the importance of the tribal medicine as the useful sedative or nerve soothing agent. But to confirm the experimental results and to find out the mechanisms of actions of this tribal medicine, some more authentic works are required.

Now a days some medicines like Benzodiazepines, Alprazolam, require special precaution if used in children and in alcohol- or drug-dependent individuals. Particular care should be taken in pregnant or elderly patients, patients with substance abuse history, particularly alcohol dependence and patients with co morbid psychiatric disorders. Use of alprazolam should be avoided or carefully monitored by medical professionals in individuals with the following conditions: myasthenia gravis, acute narrow angle glaucoma, severe liver deficiencies. severe sleep apnoea, pre-existing respiratory depression, acute pulmonary insufficiency, chronic psychosis, hypersensitivity to alprazolam or other drugs in the benzodiazepine class, borderline personality disorder .Elderly individuals should be cautious in the use of alprazolam due to the possibility of increased susceptibility to side-effects, especially loss of coordination and drowsiness.

The positive safe anti stress effects of the herbal plant medicine proves that the tribal medicines have the potentiality to act effectively and can be used as safe medicine for anti stress purposes.

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Original Article

To Study the Relationship between the Gonado Somatic Index and the Gastro Somatic Index during Breeding Season of *Labeo bata* (Hamilton, 1822)

Samarendra Behera*, Suchandra Roy Chaudhury, Sanjeev Kumar***, Rinku Gogoi******

Abstract

The investigation on the gastro-somatic index (G_aSI) and gonado somatic index (G_nSI) of *Labeo bata* was carried out in spawning season from July to December 2010. Length of fish ranged from 136.11±2.00 to 145.44±0.58 mm and while in average weight was 34.03±1.00 to 44.11±5.29 gm. G_aSI and G_nSI were determined at different length and weight ranges of different individual fishes. Significantly lowest G_aSI was 3.92±0.38 in male specimens, where as 4.50±0.10 in female specimens. G_aSI was also determined from wide highest peak ranges of *Labeo bata*, 8.02±0.06 (November) in male specimens, 7.33±0.10 (October) in female specimens. Ovary weight of the carp ranges from 0.39±0.01 to 1.33±0.58 gm with a mean value of 0.81 gm. Gonado somatic index varied o-in male from 0.73±0.03 to 2.06±0.05 while in female it was 0.91±0.01 to 2.85±0.07. The Gonado-somatic Index (G_nSI) varied significantly (P is less than and equal to 0.01) in *L. bata* as between the sexes and among months. In the present study, it is found that irrespective of season the male *Labeo bata* is smaller in length and weight in comparison to female.

Key words: Gonado Somatic Index; Gastro Somatic Index; *Labeo bata;* Length; Weight

Intorduction

India is the second largest producer of fish contributing 5.43% of the global fish production and also the second major producer of fish through aquaculture. The total fish production in India is 9.579 million a tonne of which nearly 6.136 million metric tonnes comes from Inland sector (DADF, 2014).Fisheries sector in India contribute significantly to food and nutritional security by providing livelihood to approximately 14.49 million people in the country and thus playing an important role in the national economy. It also plays an important role in improving the socio-economic condition of people by way of supplementing family income and generating gainful employment in the rural sector, particularly among the landless labourers, small and marginal farmers and women.

The existence of varied topography and different agro-climatic conditions in the state of West Bengal has bestowed upon a productive fishery resources. It is a unique state being gifted with diversified aquatic resources viz. freshwater, coldwater, brackish water and marine water. The total fish production of the state in 2013-14 was 15.81lakh tonnes (DADF, 2014). Though the fish production is increasing steadily, there remains an annual deficit of 6 to 7% in this state. Since more than 90% of the population in West Bengal consumes fishes, the difference between the demand and supply always remains persistent. To fill the gap, West Bengal imports substantial amount of fishes from other states like Andhra Pradesh and Odisha every day.

Labeo bata (Hamilton, 1822) is considered as an important candidate species in aqua farming, it is herbivorous, adults are bottom dwellers and take rotten plants, algae and plankton; but is frequently moves all zone of water column for feeding and breeding purpose. It is being cultured widely because of high market demand, good growth rate, omnivorous feeding tendency, acceptability to artificial diet etc. Generally they are hardy and are capable of tolerating wide fluctuations of temperature, oxygen, turbidity etc. It has got

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popularity due to its taste. It also provides a respected amount of fish protein. In West Bengal "Bata" consider as a lucrative fishery due to its high and regular demand (Roy, 1994).

This species is capable of attending maximum length of 61 cm (Fishbase 2009) whereas largest observed specimen was 290 mm in total length by Rahman (1989). It generally matures in two years. But under favourable condition this fish can mature in 9-10 months also. Spawning occurs during July and August. Each individual spawns only once with the onset of monsoon season. The average length of matured fishes in both sexes is matured around 20 cm in length and 100 to 125 gm in weight. *Labeo bata* breeds during monsoon and its spawning season is very short. In the nature it spawns once in a year but induced breeding technique it can be induced to breed two and more times in a year.

The Gonado Somatic Index(G_nSI) and Ovarian Index (OI), the peak value of which could observed certainly during the spawning period of the fish, are useful and sensitive parameters to monitor gonadal maturation. Das (2002) studied the testicular maturity of teleports and identified three phase of testes on the basis of GSI and Histological study.

Material and Methods

The present study on the Relationship between the Gonado Somatic Index and Gastro Somatic Index aspects of *Labeo bata* was conducted for a period of six month (July, 2010 to December, 2010) in the Department of Fisheries Resource Management, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, Chakgaria, Kolkata – 700 094.

The proper sequential steps of methodology are as follows:

Collection and sampling of fish species

In every fortnight period, sampling of live adults of *Labeo bata* was done randomly from a fresh water pond of Mallickpur, 24 parganas in West Bengal. In each sampling, 30 numbers (15 numbers of male and 15 numbers of female) of healthy and disease free fishes were selected for observation.

Morphological study of the specimen

Morphological observation of the specimen was done to identify and segregate the males and females.

Dissecting specimen

On a dissecting tray, each specimen was ventrally opened; the gut and gonad of each specimen was collected.

Collecting gut and gonad from each specimen

The entire gut and gonad were collected carefully from each specimen and cleaned in saline water to remove clots and other particles from them.

Length and weight measurement

The total length and the total weight of the sample fishes and gut and gonad weight were measured by using millimetre scale and monopan balance in gm unit.

Ganado Somatic Index (G_nSI)

Ganado somatic index values were used as indicator of degree of Gonadal development. It was found out by employing the following formula.

$$GnSI = \frac{weightofthegonad}{totalweightofthefish} \times 100$$

Gastro Somatic Index (G_aSI)

It is the measure of the gastric weight in relation to total body weight of fish. The gastrosomatic index was calculated following standard procedure as follows.

$$Gastrosomaticindex(GSI) = \frac{weightofforegut}{weightofthebody} \times 100$$

Analysis of Data

All the collected data are plotted in the tabular form and then represented in the graphical form for better calculation and assessment.

Result and Discussion

Study was conducted during July 2010 to December 2010. Some remarkable change in the external morphology, morphometry and in some internal features of *Labeo bata* was found.

Morphology of body

Firstly, during the breeding season, the length and weight of the body of *Labeo bata* increased remarkably (Table I & II). Morphometrical study of *Labeo bata* showed that irrespective of season the male *Labeo*

bata is smaller in length and weight in comparison to female. From the study it can be concluded that the size of the fish is an important criteria for identification of sex. The standard length of Bata has varied from 136.11±2.00 to 145.44±0.58 mm and weight was 34.03±1.00 to 44.11±5.29 gm. while in female average length was 139.66±2.65 to 144.11±4.36 mm.The average weight of female fish was 38.66±5.51 to 46.67±1.00 gm.For successful induced breeding the identification of sex external morphology, morphometry and gonadal morphology are very necessary (Roy, 1994).

Table 1: Monthly variation of Average Standard Length (mm±SD), Average Body Weight (gm±SD), Average Weight of Gut (gm±SD), Average Weight of Gonad (gm±SD), Gastro Somatic Index (±SD) and Ganado Somatic Index (±SD) male *Labeo bata.*

Month	Average Standard Length (mm)	Average Body Weight (gm)	Average Weight of Gut (gm)	Average Weight of Gonad (gm)	Gastro Somatic Index	Ganado Somatic Index
July	136.11±2.00	44.11±5.29	1.73±0.21	0.91±0.02	3.92±0.38	2.06±0.05
August	139.67±1.53	44.08±6.66	2.00±0.10	0.70±0.04	4.83±0.38	1.74±0.05
September	141.11±2.52	41.78±2.08	2.13±0.15	0.69±0.02	5.10±0.06	1.65±0.04
October	141.89±1.53	35.58±4.16	2.70±0.10	0.62±0.04	7.59±0.10	1.59±0.02
November	144.22±2.08	34.03±1.00	2.73±0.15	0.31±0.02	8.02±0.06	0.91±0.03
December	145.44±0.58	41.01±4.16	2.83±0.31	0.32±0.03	6.90±0.06	0.73±0.03

Table 2: Monthly variation of Average Standard Length (mm±SD), Average Body Weight (gm±SD), Average Weight of Gut (gm±SD), Average Weight of Gonad (gm±SD), Gastro Somatic Index(±SD) and Ganado Somatic Index (±SD) female *Labeo bata.*

Month	Average Standard Length (mm)	Average Body Weight (gm)	Average Weight of Gut (gm)	Average Weight of Gonad (gm)	Gastro Somatic Index	Ganado Somatic Index
July	139.66±2.65	46.67±1.00	2.10±0.26	1.33±0.58	4.50±0.10	2.85±0.07
August	140.11±3.21	44.14±1.53	2.13±0.32	1.09±0.11	5.01±0.44	2.47±0.10
September	141.33±3.21	44.91±1.53	2.70±0.10	0.92±0.02	6.01±0.21	2.04±0.10
October	142.22±5.51	39.55±2.08	2.90±0.10	0.72±0.01	7.33±0.10	1.82±0.07
November	143.77±4.36	38.66±5.51	2.87±0.25	0.41±0.03	7.16±0.10	1.06±0.06
December	144.11±4.36	42.77±1.53	2.93±0.15	0.39±0.01	6.85±0.17	0.91±0.01

Color of body

During the study of the colur pattern of the body in both male and female are more or less same during breeding season. The color was studied to identify the male and female externally. From the study it is found that on the basis of colour it is very difficult to identify male and female because they both exposed almost equal coloration.But in female fish the ventral portion of the belly becomes brownish-shivery and the dorsal portion becomes blackish-shivery. But in the same condition the dorsal and ventral portion of male becomes bright silvery. This color change is due to the action of secondary sexual characteristics of the fish (Lagler *et al.,* 1977). These color variations can be concluded as the criteria for identification of sexes.

Belly structure

The ranged of male gut from 1.73±0.21 to 2.83±0.31. while in female it varied from 2.10±0.26 to 2.93±0.15.The variations in the monthly average values of Gastro-somatic index (G_aSI) of *Labeo bata* are presented in Table 1 and 2. It was found to vary according to the month and sex of the fish. The G_aSI of the male bata were highest in November (8.02±0.06) and lowest in the month of July (3.92±0.38). It was quite low in the month of August (4.83±0.38), September (5.10±0.06) and December (6.90±0.06). It was also moderately high during the month of November (7.59±0.10). The maximum value in female was found in the months of October (7.33±0.10) and November (7.16±0.10). The lower value of GaSI was found in the months of July (4.50±0.10) and August (5.01±0.44). The values were moderately high in months of December (6.85±0.17) and September (6.01±0.21).

It was found that G_aSI was minimum during the months of July and August which might be due to advance stage of fish maturity. Other workers (Kiran *et al.* 1998; Basudha, 1999; Rajasree and Kurup, 2011) reported that gravid fish used to take less feed due to occupation of more space in the abdominal cavity. Subsequently it was found that G_aSI value was more during the months of September to November which could be due to higher feeding intensity of fish during post spawning stage. Feeding was low during the month December which might

be due to winter cold. The G_aSI varied significantly (P is less than equal to 0.01) during different months which can be attributed to varied feeding during different months owing to maturation and environmental factors.

During breeding season belly of the female showed more bulginess than the male because the gonad of female are expanded too much than the male. The gonad of the female occupies more space in the coelomic cavity than the male during same season. Therefore, the belly of female is more bulged than male. In the present study, it is concluded that the structure of belly can be used as a tool for identification of male and female during breeding season. With the onset of breeding season, both male and female exhibited an increase in belly size which was continued till peak breeding season. The fully rip female are found with prominent bulged belly and with more increased body structure in comparison to matured male. So, sex determination through observing external features could be possible during breeding season. But the weight of gut drops significantly in the matured female than a matured male.

Gonadal Morphology

The ranged of male gonad weight varied from 0.91±0.02 to 0.32±0.03gm while the ovary weight of the bata ranges from 0.39±0.01 to 1.33±0.58 gm to with a mean value of 0.81 gm. The table 1 and 2 had shown the variations in Gonado-somatic index (G_nSI) of monthly samples of the *Labeo bata.* The average G_nSI of male *Labeo bata* varied from a minimum 0.73±0.03 in the month of December to a maximum of 2.06±0.05 in the month of July. The average value showed a decreasing trend from August (1.59±0.02) to November (0.73±0.03). While in case of female the average G_nSI value ranged from a minimum 0.91±0.01 in December to a maximum of 2.85±0.07 in the month of July. The average G_nSI values were relatively high in the female in comparison to male. The variations in the average G_nSI of the fish is presented in Table (1 and 2).

A thorough knowledge on maturation cycle and depletion of gonad is essential for the effective fishery management (Biswas *et al.*, 1984 and Cek *et al.,* 2001). De Vlaming *et al.* (1982) stated that gonadal development in fish is governed by a number of biotic and abiotic environment factors like water temperature, photoperiod, rainfall, etc. by playing significant role in gonadotrophics activity of pituitary gland, which have a triggering effect on the development of the gonad.

It was found that G_nSI of the fish was relatively higher during July and August indicating advance stage of maturation and breeding season. Subsequently it declined from the month of September which could be due to spent condition of fish. It exhibited an increasing trend of variation indicating gonadal development. The Gonado-somatic Index (G_nSI) varied significantly (Pd"0.01) in *L. bata* between the sexes and among months.

There is a significant increment in weight and Gonado Somatic Index. But after breading season, the weight of fish and Gonado Somatic Index were recorded reducing both in male and female *Labeo bata.* The relationship between length of body, weight of body and Gonado Somatic Index of matured fishes represented graphically during breeding season. For successful induced breeding the identification of sex external morphology, morphometry and gonadal morphology are very necessary (Roy, 1994). The study of sexual characteristics during breeding season is helpful for segregation of other biological studies. As the breeding season of bata is already been studies by Khan (2000) and mention that it is during pre monsoon period. A peculiarity observed during the period of study was that few numbers of matured male and female fishes were recorded during several sampling occasions. It indicated availability of matured fish during most of the months due to occurrence of few matured fishes in sampling.

Conclusion

In the present study, it is found that irrespective of season the male *Labeo bata* is smaller in length and weight in comparison to female. From the study it can be concluded that the size of the fish is an important criteria for identification of sex. Further, the secondary sexual characteristics like morphometry, colour of the body, belly structure are considered as key factors for the identification of sex during breeding season. A remarkable finding was also recorded that the breeding season the value of Ganado Somatic Index increases where as the Gastro Somatic Index decreases and vice versa. However, this knowledge of reproductive biology of bata during breeding will definitely become a tool for the fish farmers and hatchery entrepreneurs for successful induced breeding of bata.

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Review Article

Molecular Methods for Detection of Bacterial Pathogens in Finfish and Shellfish with Special Consideration to Public Health Significance: A Review

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Abstract

Fish and shell fish are known as carriers of food borne pathogens and therefore, the hygienic quality of these products must satisfy the International quality regulations, failure of which may cause the rejection of fish and shellfish consignments by the importing countries. Bacterial pathogens related to human health significance are *Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificus, Salmonella* sp., *Shigella* sp., *Listeria monocytogens, Aeromonas* sp., *Plesiomonas shigelloides* etc. Numerous DNA molecular markers are now available for use in surveillance and investigation of food borne outbreaks from seafood which was previously difficult to detect. Moreover, molecular approach to identify pathogens is potentially faster, more sensitive than conventional culture techniques, serology and histology. This review describes various microbial pathogens of fish and fishery products and different DNA and RNA based methods which are well known in identification of food borne pathogens.

Key words: Fin Fish and shell Fish; Molecular Techniques; Pathogens; PCR; Rapid Detection.

Introduction

A common problem encountered with exposed fish and fishery products is contamination with bacterial pathogens. In fact, there have been numerous cases of rejection of consignments at International markets due to contamination of fish and fishery products with bacterial pathogens like toxigenic *Escherichia.coli, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificus, Salmonella* sp., *Shigella* sp., *Listeria monocytogens, Aeromonas* sp., *Plesiomonas shigelloides* etc. In India during June 1995 to December 1997, 31 fish consignments exported to European Union (EU) countries were found to be of poor hygienic quality. This led to a ban of fish export from India to EUcountries (Anonymous, 1998; Anonymous, 1999). In addition, during the same period, many consignments exported to USA and Japan was also rejected for the same reason (Pandian *et al.,* 2000). Moreover microbial pathogens pose a significant health hazards to human health through ingestion of raw, uncooked and improperly cooked seafood. The contamination can occur during different stages of processing of seafood for instance, harvesting, handling, processing, distribution and storage. Several cases of outbreaks of bacterial disease in human are reported from water and seafood throughout the world (Table 1).

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There are some inherent drawbacks associated with conventional microbiological techniques like more time is required for analysis and miss-identification of non pathogenic strains. In order to identify food borne pathogens, conventional culture method includes homogenization, enrichment in non-selective and selective medium followed by plating in differential agar medium for pure culture isolation. At the end of phenotypic and genotypic characterization normally takes 3-4 days for result confirmation. A substantial volume of pure culture is needed for conducting biochemical and immunological method whereas mixed culture or community Deoxyribonucleic acid (DNA) can be used in DNA based method. Identification of bacterial pathogens in conventional microbiological method is somewhat time consuming and labor intensive. A conventional method lacks to differentiate bacterial pathogenesis but can determine genus and species of the micro organism. It led to a search for more faster and sensitive method to detect microbial pathogens in food.

Table 1: Outbreaks of bacterial disease from water and seafood.

Sl.No	Incidents and Year	Country	Bacteria	Reference
1	62 cases of Septicemia,1981-1987	Florida , USA	*V. vulnificus*	Klontz *et al.* (1988)
2	472 cases of gastroenteritis 1986	USA	*A. hydrophila.*	Abeyta *et al.* (1986)
3	Outbreak of Cholera October,1992	Madras, India	*V.cholerae* BengalO139	Ramamurthy *et al.*(1993)
4	Outbreak of cholera June to October 2003	Nagpur, India	*V.cholerae*Biotype E1Tor	Mishra *et al.* (2003)
5	61-71% outbreak of gastroenteritis 1996-1999	Taiwan	*V. parahaemolyticus*	Wong *et al.* (2000)
6	40 outbreaks of *V. parahaemolyticus* 1973-1998	USA	Do	Daniel *et al.* (2000)

Molecular techniques can be used to solve that type of problems and increase sensitivity to target pathogens such as PCR, multiplex PCR, real time PCR, RAPD, RFLP, AFLP, PFGE, 16S rRNA PCR, hybridization of DNA probes etc.

The different microbial hazards associated with fish food and advancements made for their detection using molecular techniques will be elaborated in the present review.

Common bacterial contaminants in fish and fishery products

Vibrio sp.

Vibrios constitute a major portion of the bacterial flora of both fin fish and shell fish in the tropics. More than 37 Vibrio species have been isolated and characterized, out of which 12 nos. of species are considered as human pathogens of zoonotic importance. *V. cholerae, V. parahaemolyticus, Vibrio vulnificus* are important members of this group that are considered as pathogens associated with fish and fishery products.

Vibrio cholerae: Vibrio cholerae is widely distributed in aquatic animals. Contamination of seafood occurs particularly when the seafood is caught from contaminated water bodies. The other source of contamination is un-hygienic condition of fish farmers and workers of the processing plants. Seafood is often taken from waters or stored under conditions that induce the viable not culturable state in *V. cholera* (Oliver *et al*.,1989). It has been realized that the viable but not culturable (VBNC) state exhibited by *V. cholerae* can explain the seasonality and distribution of the organism in regions where cholera is endemic. According to Koch *et al*. (1993) and Karunasagar *et al*.(1995) *Vibrio cholerae* can be detected in seafood by application of PCR based method. Karunasagar *et al*.(1995; 1997) studied that the *ctx* gene based PCR could identify both *V. Cholerae* O1 and O139 contamination in seafood. According to Urakawa *et al*.(1997) who analysed the restriction pattern of 16s RNA of 35 no. of Vibrionaceae species which are found useful for the classification and identification of Vibrionaceae strains.

Vibrio parahaemolyticus : *Vibrio parahaemolyticus* is often associated with seafood like shrimp, prawn, crabs , mollusks etc and as well as finfish. Kaper *et al*.(1984) described that *Vibrio parahaemolyticus* could produce Thermostable Direct Hemolysin (TDH) which was encoded by *tdh* gene. According to Honda *et al*.(1988) non hemolytic (Kanagawa negative) strains of *Vibrio parahaemolyticus* might be associated with gastrointestinal infection. Sakazaki *et al*.(1968) studied that most of the environmental strains were Kanagawa negative. Karunasagar *et al*.(1996) reported a PCR based assay targeting *tdh* gene which could detect *Vibrio parahaemolyticus* contamination in shell fish.

Shigella sp.

Virulent shigella causes human illness known as bacillary dysentery. The bacteria are found in fishes collected from river, estuary and sewage fed beels. Davis *et al*. (1988) stated that the bacteria could be traced in contaminated fish, chicken and fishery products.

Listeria monocytogenes

Fish and shell fish are exposed to potential contamination by *Listeria monocytogenes.* In India there are very few reports on the incidence of Listeria in clinical as well as food samples (Gupta *et al*.,1997). A number of workers investigated the application of PCR for rapid identification of *L. monocytogenes* on various food products.(Hill, 1996). Listeriolysin is a major virulence factor for *L. monocytogens* which is encoded with *hly* gene. The *hly* and *Iap* gene can be used as target gene for PCR amplification in order to detect the said bacteria.

Escherichia coli.

E.coli contamination in fishery products occurs during processing through contaminated water or handlers in the plant. (Mishra. *et al*., 2004). The poor un-hygienic and sanitary condition of fish landing centers and retail markets are also responsible for toxigenic *E. coli* contamination in seafood. This group of *E.coli* is classified into five categories based on their virulence properties, clinical syndromes, epidemiology and distinct O:H groups such as Enteropathogenic *E coli* (EPEC), Enteroinvasive *E coli* (EIEC), Enterotoxigenic *E.coli* (ETEC), Enterohemorrhagic *E.coli* (EHEC) and Enteroaggregative *E.coli* (EAEC). Detection of EIEC by amplification of genes including invasion factors has been reported by Keasler and Hill (1992). *E.coli* O157:H7(EHEC) is responsible for the food borne illness. Since EHEC produce Shiga like toxin (SLT), PCR based assays targeting SLT genes have been useful for detection and identification of this serotype(Gannon *et al*.,1992). Lang *et al*. (1994) described a multiplex PCR for detection of heat liable toxin gene and Shiga like toxin and 11 genes in *E.coli* isolated from natural waters.

Salmonella sp.

The importance of *Salmonella* as food borne pathogens in seafood should not be underestimated. There are numerous examples of rejection of shrimp consignment from South –East Asian Countries due to contamination of *Salmonella.* Bej *et al.* (1994) described a method for identification of *Salmonella* sp. in oyster by PCR techniques. Their method involved amplification of *himA* gene which encodes for a DNA binding protein found in several enteric bacteria. For detection in food samples different procedures screening specific genes of Salmonella as the target have been developed by single PCR (Stone *et al*., 1994), multiplex –PCR (Way *et al*., 1993).

Molecular method of Detection

Polymerase chain reaction (PCR)

Polymerase chain reaction is a technique for amplifying a specific region of DNA, defined by a set of two primers at which DNA synthesis is initiated by a thermo-stable DNA polymerase. PCR is a relatively simple technique by which a DNA template is amplified over a million fold quickly and reliably in 3-4 hrs time frames. In PCR methods, the reaction mixture constitute template DNA which may be simple tissue lysate to purified DNA, primers, polymerase enzyme in order to catalyze creation of new DNA strands and nucleotides. During each cycle of the thermo-cycling reaction, the template DNA is denatured, primers anneal to their complementary region of DNA strand and polymerase enzyme catalyses the addition of nucleotide to the end of each primer. Thus new copies of target region are created in each cycle. PCR methods have been described in more detail by Hoelzel and Green(1998). Saiki *et al*. (1985) published the first experimental data on PCR. Incase of pathogenic *E.coli* , the potential targets for amplification include *stx* gene, *eae* gene coding intimin, heat –liable (LT) and heat stable(ST), *bfpa* gene etc. In case of pathogenic *V. cholerae* the targets include *ctx* and for *V. parahaemolyticus tdh, trh* are important. In *L. monocytogenes*, several target genes have been reported like *iap, hly A* and *prfA.* The table 2 depicts bacterial genetic targets and molecular methods.

Multiplex Polymerase chain reaction

According to Field and Wills (1998), multiplex PCR means that several bacterial species can be identified in the single assay method. In multiplex PCR more than one target sequence can be amplified using more than one pair of primer in the reaction mixture. Care should be given that the primers should be same melting temperature and must not interact with each other. French *et al*. (1999) used a universal primer for the detection of multiple pathogens simultaneously. Here attempts are made by introducing single set of primer to amplify conserved stretches of DNA from 16s to 23s r DNA.

Real time PCR

Real time PCR is aimed to detect pathogens in food both in quantitative and qualitative methods. In this technique one can continuously monitor the developments of amplicons in a fluorimeter. SYBR-Green Dye or other fluorescent labeled probes that emits light during amplification are widely used in this technique. The emitted light signals corresponding to DNA amplification recorded at frequent intervals generating a curve which shows product generation. The more targets DNA amplifies in the sample, the earlier amplicons can be detected and the peak curve is recorded. (Tichopad *et al*., 2003). Baggi *et al*. (2005) described that diarrheagenic *E.coli* could be detected in Real time PCR using SYBR Green Dye.

Random Amplified Polymorphic DNA (RAPD)

RAPD is a PCR based technique which generates DNA band pattern on gel electrophoresis using

amplification of random DNA segments with primers of arbitrary nucleotide sequence (Williams *et al*.,1990). Enterobacterial Repetitive Intergenic Consensus PCR (ERIC PCR), Repetitive extragenic palindromic-PCR (REP-PCR) and BOX PCR are few examples of this technique. The presence of a RAPD band, however does not allow distribution between hetero and homozygous state. Several authors have reported on the application of RAPD techniques in microorganisms (Babalola, 2003). Sudesh *et al*. (2002) described that *V. alginolyticus* and *V. parahaemolyticus* have different RAPD profiles. In rep PCR amplification involves intervening sequences located between highly repetitive DNA motifs. Nowrouzian *et al*. (2001) designed a RAPD typing method for identification of *E.coli* strains in the micro flora of human intestine.

Restriction Fragment Length Polymorphism (RFLP)

RFLP method is very simple which uses a restriction enzyme digestion of the genomic DNA. The procedure are as follows like isolation of DNA, digestion of DNA with restriction endonucleases, size fraction of the resulting DNA fragments by electrophoresis, transfer of DNA from electrophoresis gel matrix to membrane, preparation of radio labeled and chemiluminiscent probes and hybridization to membrane bound DNA.(Babalola, 2003) The PCR-RFLP techniques consist of PCR amplification of certain genes eg. 16S rRNA, gyrB and *rpoD* and subsequent restriction of the PCR products with endonucleases to obtain band pattern.

Amplified Fragment Length Polymorphism (AFLP)

A rapid PCR based technique AFLP can be used for prokaryotes as well as Eukaryotes typing. This method starts with digestion of total purified genomic DNA by restriction endonuclease. Then ligation is formed which results in forming fragments to a double stranded oligonucleotide adapter complementary to the base sequence of the restriction site. The adapters are designed such a way that the original restriction site is not restored after ligation process which can prevent further digestion of restriction site. Selective amplification of sets of these fragments in PCR is achieved with primers corresponding to the adapter. The resulting PCR amplified DNA fragments are analyzed by gel electrophoresis (Prasad *et al*., 2009). According to Altinok *et al*. (2003) two restriction enzymes are used namely average cutting frequency (EcoRI) and higher cutting frequency (Mse I or TaqI). Babalola (2003) described that the primer contain the restriction enzyme recognition site as well as additional "arbitrary" nucleotides which extend beyond the restriction site. The fixed portion gives the primer stability and random portion detect many loci simultaneously.

Pulse field gel electrophoresis (PFGE)

This method involves DNA in which it is cut into fragments with rare cutter restriction enzymes yielding 8-25 large bands. After restriction digestion process, the fragments are separated electrophoretically by size on an agarose gel by using current at alternating angles. PFGE has been used for characterization of pathogenic bacteria. (Prasad *et al*., 2009).

16S rRNA PCR

The use of 16S rRNA gene sequence to study bacterial phylogeny because 1. its presence in almost all bacteria, often existing as a multigene family, or operons ii) its sequence is sufficiently conserved, viable and hyper variable sequence iii) size (1500 bases) which is easily sequenced but large enough to contain sufficient information for identification of bacteria. (Spratt *et al.*,2004). The process involves for identification of bacteria by 16S rRNA PCR are as follows:-a) preparation of DNA from pure culture of bacteria. b) PCR amplification of 16S rRNA gene c) checking good PCR product in gel electrophoresis d) cleaning e) sequencing of PCR product f) analysis base sequence online Basic local alignment search tool (BLAST) or Ribosomal database project (RDP) software g) identification closest match in the database. (Spratt *et al*.,2004)

DNA probe hybridization

It is possible to develop probes for specific micro-organism. Probes are short nucleic acid sequence complementary to the target sequence of micro-organism. Generally probes are labeled either with a radio active molecule (P^{32}, S^{35}, C^{14}), ligand (biotin) or antigenic substrate. eg digoxigenin.(Tyagi and Kramer *et al*., 1996). Probe hybridization analysis requires no sophisticated equipments. So probes are widely acceptable in quality control laboratory in food processing sector.

Conclusion

Microorganisms are known to play the pivotal role for spoilage of fish and fish products as well as food

borne infection outbreaks. Hence it is essential that the pathogens should be absent in seafood in order to ensure quality in relation to human health. Quick and accurate detection methods of microbial pathogens in seafood eliminate the chance of contamination to the consumers. Isolation and identification of food borne pathogens by conventional biochemical and immunological methods are time consuming, laborious and lees sensitive in comparison to molecular approach. Now a day's 16S rRNA gene, strain specific and virulence gene are the targets used for identification of food borne pathogens. Sequencing of target genes of microorganisms gives a better insight towards understanding of species, subspecies and pathogenicity in future. Molecular techniques such as PCR and hybridization are useful for rapid detection of pathogens and specific detection of virulent strains. Since these are rapid, specific and sensitive, they have immense applications in seafood quality control laboratory. It can be suggested that pool of samples of seafood products should be tested by molecular methods at quality control laboratory prior to export to the foreign countries.

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