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RESEARCH ARTICLE

Effect of Probiotic, *Lactobacillus casei* and Ascorbic acid Supplementation on Some Physiological Parameters of New Zealand White Rabbits Reared Under Hot Humid Summer.

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ABSTRACT

An experiment was conducted to evaluate the physiological response of New Zealand White rabbits supplemented with ascorbic acid and probiotic, *Lactobacillus casei* during summer season (March to May). Twenty four weaned two month old New Zealand White rabbits were randomly selected from Rabbit unit at Krishi Vigyan Kendra, Kerala Agricultural University, Thrissur and were distributed to four treatment groups with six replicates in each treatment. As per the daily Temperature Humidity Index (THI) values, animals were exposed to stress in the afternoon hours (THI > 27.8) during the entire experiment period. The ascorbic acid (200 mg per kg feed) alone (V) and probiotic, *L. casei* (10^6 cfu per kg feed) alone (L) supplemented animals had significantly lower mean respiration rate compared to the control group (C). Supplementation of ascorbic acid alone (V) or ascorbic acid in combination with *L. casei* (VL) significantly reduced the level of fecal cortisol in New Zealand white rabbits compared to the control group (C). There was no significant difference between the four treatments for rectal temperature and serum cortisol.

Key words: New Zealand white rabbits, Temperature humidity Index, Ascorbic acid, *Lactobacillus casei,* fecal cortisol.

INTRODUCTION

Rabbit research today widely focuses on improving the production taking into account the animal welfare. Rabbit rearing is an important income generating subsidiary occupation among Keralites especially women folks. The most critical limitation to rabbit production in Kerala is their susceptibility to heat stress during summer season. A temperature of 21°C is known as the "Comfort Zone" for rabbits [8]. Temperature humidity index (THI) values above 27.8 results in heat - induced physiological stress in rabbits [9,10]. In general, chronic exposure to extremes of heat leads to decomposition of normal physiological and biological mechanisms with a consequent damage of many organs [5]. Alleviation of heat stress includes physical, physiological and nutritional techniques [8].Supplementation with probiotics and ascorbic acid to alleviate heat stress has been tried in chicken and found to be successful [4]. Immune response of laying hens with dietary supplementation of multi strains probiotic and vitamin C was greater than control birds [3]. At higher temperatures ascorbic acid significantly decreased mortality rate in rabbits [13]. Hence the present research work was envisaged to study the effects of probiotic, *Lactobacillus casei* and ascorbic acid on heat stress alleviation by evaluating the physiological response of rabbits during summer season.

MATERIALS AND METHODS

Twenty four- eight weeks old New Zealand White rabbits weighing 0.91 $\pm$ 0.13 kg were randomly selected from Rabbit unit at Krishi Vigyan Kendra, Kerala Agricultural University, Thrissur. The location of the study is geographically situated at longitude 76^{o}, 05" to 70^{o}, 45" E, at latitude 10^{o}, 20" to 10^{o}, 56" N and at an altitude of 22.25 m above mean sea level and is endowed with humid tropical climate. The study was carried out with the approval of Institutional Animal Ethics Committee (IAEC), College of Veterinary and Animal Sciences, Mannuthy. The animals were allotted to four groups of six rabbits each. The rabbits were housed in individual cages (60X60X45 cm) placed in a semi open shed simulating to back yard rearing. They were fed commercial pelleted feed (once a day), *ad libitum* fodder and water. The pellets were given at 10 AM every day. The basal diet (pellet feed) was fed *ad libitum* and the left over feed was weighed the next morning to find the daily feed intake. The proximate compositions of each batch of feed samples were estimated [2] and presented in Table 1.

A one week acclimatization period was given to the animals before the commencement of study. The study was conducted for thirteen weeks which fall in the summer months of March, April and May. The treatments were control group (C) fed basal diet alone (n=6), Probiotic group (L) fed basal diet along with *Lactobacillus casei* (Unique Biotech, Hyderabad, India) at the rate of 10^{6} colony forming units per g of feed (n=6), Vitamin group (V) fed basal diet along with ascorbic acid (Merck, Mumbai, India) at the rate of 200 mg per kg feed (n=6), and probiotic and ascorbic acid group (LV) fed basal diet along with *L. casei* and ascorbic acid at the same rate as in V and L (n=6). The feed supplements were mixed with the basal diet just before feeding.

Climatological data

The dry bulb temperature (^{o}C) and relative humidity (percentage) in the micro climate was recorded daily at 07.30 and 14.00h. Thermal comfort level of an animal environment was assessed by calculating temperature-humidity index (THI) THI=db °C– [(0.31–0.31RH) (db °C–14.4)] where db °C = dry bulb temperature in degrees Celsius and RH = relative humidity percentage/100. The values obtained were classified as follows: <27.8 = absence of heat stress in rabbits, 27.8–28.9 = moderate heat stress, 28.9–30.0 = severe heat stress and 30.0 and more = very severe heat stress (Marai et al. 2001). The physiological parameters recorded were weekly respiration rate, weekly rectal temperature, monthly serum cortisol and monthly fecal cortisol values.

Collection and storage of fecal samples [15]

Fecal samples were collected at monthly intervals from all the animals in the early morning. They were kept in polythene pouches and stored at -20°C till extracted for Radioimmuno assay (RIA)

Extraction of fecal cortisol for radioimmunoassay [12]

The fecal samples stored at -20°C was crushed in the polythene pouch itself and thawed. Then 0.5 g of homogenized wet feces was extracted with two ml distilled water and three ml methanol after vortexing the mixture for 30 minutes. It was then centrifuged at 2500 rpm for 15 min. A 0.5 ml aliquot of the supernatant was decanted and the feces residue in the centrifuge tube was again extracted with three ml methanol same as before. Again 0.5 ml of the supernatant was taken and mixed with aliquot already taken in the screw capped vial. The fecal extracts were stored at -20°C until RIA analysis .Serum samples were also stored at -20°C until RIA analysis [1,2,5]. Labeled Cortisol Radio Immuno Assay was the method adopted for cortisol estimation from serum samples and fecal extracts [7].

RESULTS AND DISCUSSION

The results on temperature humidity index values suggested that animals were exposed to heat stress in the afternoon hours all throughout the experimental period (THI >27.8). They were exposed to 'very severe' heat stress upto 8th week (except for severe heat stress in the 5th week) and 'severe' heat stress in the 9th and 11th week and 'moderate' heat stress in the 10th, 12th and 13th week of the experiment period (Table 2).

The mean weekly respiration rate of the different treatments revealed that V and L rabbits had lower ($P \leq 0.05$) overall mean respiration rate than C rabbits (Table 3). This is in conformity with the results of Abdel-Samee, (1955)[1]. From the correlation analysis (Table 4) it was clear that all the treatments had a significant positive correlation with the Temperature humidity index (average) values. Among the four treatments the T4 (control group) animals showed the highest correlation with THI.

The mean weekly rectal temperature showed no significant difference ($P>0.05$) between the four treatments. There was no correlation between weekly rectal temperature and Temperature humidity index values. Finzi et al. (1994) [6]reported that the average body temperature in rabbits goes up from morning till night, while environmental air temperature goes up from morning till noon then decreases at night, indicating that body temperature was not affected instantly by changes in air temperature during the day. The study done by Ogunjimi et al. (2008)[11] revealed that a low correlation existed between rectal temperature and thermal comfort level, while a strong correlation existed between respiration rate and thermal comfort level in rabbits. Hence it was inferred that rectal temperature was not an effective indicator of heat stress in rabbits. Mean fecal cortisol level of animals in V and VL groups were significantly lower than C animals ($P<0.05$). The mean monthly serum cortisol values did not differ significantly between the four treatments. Faecal cortisol level could be used as an effective indicator of heat stress in New Zealand White rabbits [14].

In the present study significant difference was observed between control groups and feed supplemented groups for fecal cortisol but not for serum cortisol. Blood collection for estimation of cortisol was done in the morning and the temperature humidity index values in the morning were lower than 27.8 indicating absence of stress in rabbits in the morning while the THI values were high (>27.8) in the afternoon. The lack of stress in the morning hours might be the reason for not showing any significant difference in serum cortisol among the four treatments.

CONCLUSSION

Dietary supplementation of ascorbic acid and probiotic, *L. casei* was found to reduce the heat load in rabbits under heat stress by reducing the respiration rate. Rectal temperature turned out to be an insignificant indicator of heat stress in rabbits. Supplementation of ascorbic acid alone or ascorbic acid in combination with probiotic had a significant effect in reducing fecal cortisol level in rabbits.

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Table 1. Proximate composition of rabbit feed.

Chemical composition	Grass (% as DM)	Concentrate pellets (% as DM)
Crude protein	9.74±0.02	18.08±0.07
Crude fiber	31.29±0.14	5.23±0.21
Ether extract	1.75±0.01	2.59±0.32
Ash	11.66±0.11	13.21±0.36
Nitrogen free extract	45.57±0.23	60.89±1.25
Acid Insoluble ash	6.18±0.12	1.42±0.22
Moisture	86.10±1.23	5.49±0.12

Table 2. Temperature Humidity Index (THI) values in the rabbitry between March, April and May.

Weeks	THI morning	THI afternoon	THI Average
1	24.6 ±0.3	30.9±0.2	27.75±0.1
2	26.5±0.1	32.0±0.3	29.29±0.2
3	26.1±0.2	31.4±0.4	28.76±0.2
4	25.6±0.5	30.2±0.5	27.92±0.5
5	26.4±0.2	29.6±1.2	27.98±0.6
6	27.0±0.2	31.5±0.1	29.25±0.1
7	26.9±0.5	31.2±0.1	29.06±0.2
8	26.4±0.6	30.7±0.2	28.54±0.3
9	25.3±0.1	29.7±0.5	27.52±0.3
10	25.1±0.4	28.6±0.4	26.86±0.4
11	25.7±0.5	28.9±0.5	27.31±0.5
12	24.7±0.3	27.9±0.8	26.30±0.5
13	24.7±0.4	28.4±0.2	26.59±0.3

Table 3. Effect of climatic stress on physiological parameters of New Zealand white rabbits.

Parameter	Treatments			
	T1	**T2**	**T3**	**T4**
Respiration rate	112.91±2.17^{a}	112.63±1.18^{a}	115.68±1.13^{ab}	118.09±1.1^{b}
Rectal temperature	38.78±0.33^{a}	38.92±0.47^{a}	38.87±0.36^{a}	39.00±0.42^{a}
Serum cortisol	10.70±0.28^{a}	10.39±0.30^{a}	10.50±0.05^{a}	10.76±0.08^{a}
Fecal cortisol	4.49±0.58^{a}	5.91±0.61^{b}	4.50±0.51^{a}	6.34±0.33^{b}

Mean values bearing different superscript in a row differ significantly ($P \leq 0.05$)

Table 4. Correlation coefficient between Weekly respiration rate of different treatments and Temperature Humidity Index (THI) values in the rabbitry.

Treatment	THI (Morning)	THI (Afternoon)
T1	0.509	0.718*
T2	0.271	0.663*
T3	0.232	0.654*
T4	0.515	0.846**

**Correlation is significant at the 0.01 level *Correlation is significant at the 0.05 level

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RESEARCH ARTICLE

Comparing Personal, Economical and Political Awareness in Pre-University Boys and Girls in Kurdistan Province, Iran.

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ABSTRACT

This article analyzes the personal, economical, and political awareness in pre-university boys and girls in Kurdistan province, Iran. Pre-university is the most vital course of education in the educational centers of the ministry of education in Iran. For this period is so sensitive having complex features and it is a bridge joining high school to university as well. This study enhances the students' personal, financial, and political awareness and prepares them for future life and to some extent helps teachers, counselors and especially parents come up with a good understanding of the students' above-mentioned awareness. The statistical population consists of all students in pre-university course in Kurdistan province studying in 2010-2011. The number of sampling is 270; including 130 boys and 140 girls in pre-university course who were selected by random cluster sampling. To collect the data, we used the researcher-made questionnaires with to questions including those related to the content of the personal, financial, and political awareness. After the implementation of the questionnaires, the participants' answers counting were done manually and then they were analyzed by SPSS software. The findings of this study show that girls` personal awareness in pre-university course is more than that of boys and the boys' financial and political awareness exceeds that of girls.

Keywords: personal awareness, economical awareness, political awareness, pre-university course.

INTRODUCTION

To succeed in every pursuit, having knowledge of the factors and means helping us in achieving our goal easily and fast is of great importance. In other words, adhering to approaches which can help us in treading on the path of success easily and conveniently is one of the necessary requirements of success.Each kind of awareness has its own branches and this gives us the chance to find out other differences among boys and girls besides their facial differences and enables us to know these students better and find the ways to improve both boys` and girls` awareness and reinforce it.

Personal aspect

Physical aspects, health, and sanitation and its` growth and improvement, psychological aspects such as attending to crucial aspects of life, kindness, affection, order and goal, ambition. Aspects related to personal life, in determining a philosophy for life, using a rational approach, memory, transferring speed etc. Aspects related to self-criticism, self-evaluation, self-assessment, criticism tolerance, patience in difficult situation, bravery and moving forward, etc.

Economical aspect

The content of education can encompass the following: the issue of production, consumption, appropriation, vocational and technical training, being cooperative in economical charity campaigns, insurance, working in free time, religious aspects of income and consumption, preserving and developing national properties, the value of the currency, direct and indirect tax revenue, etc.

Political aspect

The issues which can be incorporated in the content of training are as follow; the necessity of having a democratic government, leadership requirements, the life-span of the government, peoples` responsibility toward people and vice-versa. The issue of laws and regulations, execution, executives, organization, ministries and offices, freedom, campaign and unrest, public supervision, militarization, votes and contribution in affairs, national & international ties, etc. Boys and girls, both are a group of creatures who, due to their roles in life, are different from each other in spite of their vivid similarities. The physical, mental and emotional differences between males and females account to one of the masterpieces of creation and regardless of the patriarchal perspective, these differences are not only taken into account as weaknesses but they are prerequisite to creation and necessary for life continuation. One naturally keeps the advantage of being male and sometimes exaggerates in it. Consequently it's the father who becomes a symbol of power in family. The child discovers his father`s outstanding role and notices how the father plans everything to be done and acts as a commander everywhere. Even when the patriarchal effect is not so strong. Yet children consider the father as superior.

According to Frobel, two stages of awareness are necessary for human growth: The first stage of this awareness is the awareness of external things, things by which the child is surrounded. The second stage of awareness is self-awareness which follows the first stage. The purpose of this training is to achieve self-awareness which is realized through contact with the world, reaction to it and knowing it. (Frobel, as cited in Naghibzadeh, p. 160)Awareness or consciousness can never be anything else than conscious existence and the existence of human being is the actual process of their life. This theorem holds true for both social and individual consciousness. Awareness of the existence is fundamentally different from research about nature, existence, and society. Awareness exists before doing any research and doesn`t depend on it. This fact that the results of the research later become a part of awareness, doesn`t destroy the qualitative difference between research and awareness (Theodor Zrman, p. 91). So with this in mind, pre-university course with a specific age range is particularly important. On one hand, it is the enthusiasm period for youth and on the other hand, it is a prelude to entering the university and a new environment. The adolescents and

the young will replace the older member of the society in a near future and for this very reason, what they know will probably be the future dominant awareness of the society.In the present study, we have tried to compare the personal, economical, and political awareness among pre-university boys and girls in various aspects as mentioned above and also to determine the difference between personal, economical, and political awareness of pre-university boys and girls. Therefore, the main problem of this study is answering this question; what differences exist among boys' and girls` personal, economical, and political awareness?Numerous researchers have done studies on comparing awareness of boys and girls in various aspects that some of them will be pointed out here:

Fallah(1997): In a study entitled, the evaluation of the political awareness of Shahed university students` has shown significant differences between male and female that political consciousness and political awareness in men are more than women.Kawsari (2001): In a study entitled with "the comparison of the effects of the effects of control source on personal and social adjustment in high school students" carried out on 133 boys and 163 girls showed that girls show greater personal and social adjustment than boys do.Erfani (200): In a study entitled the analysis of the value system of pre-university students in Kurdistan province in 2000-2001 showed that there are significant differences between boys and girls in terms of economical, artistic and political values. The average of artistic values of girls is more than boys but the economic and political values of boys are more than girls. Besides, there is no significant difference between religious values of boys and girls. Here, the premise of this research is that boys and girls try to learn more about things which are worthy.Heidari (2001): In his study entitled economic education based on Islam, he has concluded that the Islamic approach aims to establish a healthy economy in order to gain personal and social welfare on the basis of social justice and eventually the proximity of God.

Mahroee(1998): In a study entitled," the evaluation of the high school students` political awareness and factors affecting it", showed that the family, school, and society relatively have a great impact on enhancing the students` political view and the society using the media such as radios and TV have the most effective contribution in this regard.Alport & Vernon (1931) showed that women`s aesthetic and social concerns is more than men (as cited in Ganji).Satu (19730 in a research concluded that during the early or mid-teens in social behavior, higher values are swapped. Therefore, there is a significant difference between men and women in terms of values and social awareness.Hoodak (1980): he revealed that the proportion of delinquent youths` social awareness and political liabilities are more apathetic than non-delinquent adolescents.Kevin: regarding social consciousness, he believes that the higher the social class of the person is the greater the probability of participation in social affairs. The level of active participation in social clubs, municipal associations, schools, political organizations is lower among lower classes of the society (1991).Mosgraw (1984) He found that girls emphasize on social issues and integration of personality more than boys.

The Hypotheses

-Personal awareness is different between pre-university boys and girls.
-Economical awareness is different between pre-university boys and girls.
-political awareness is different between pre-university boys and girls.

METHODOLOGY

Data analysis Method

To analysis the date briefly and concisely the following methods were used:
For statistical characteristics of the groups, we used the common methods of descriptive statistics.
To analyses the questions, the classic model was used so that in addition to the percentage of options, two important indicators, including the degree of desirability (average and questions) and the correlation coefficient incandescent spot (the power to recognize each question) were used.

To estimate the validity coefficient of the studied questionnaire, the general formula of Cranach's alphabet coefficient was used.
For data analysis and statistical hypothesis testing, the traditional T-Test was used.

Description	Components
This study is sectional in terms of practical purposes, data quantity and the nature and type of the survey.	Type of research
Statistical society is composed of all the students of pre-university course in Kurdistan province in 2010-2011.	Statistical society
According to sampling method 270 members are selected (130 male, 140 female)	Determine the sample size
Random cluster	Sampling method
The measurement is done through the researcher-made questionnaire consisting of 40 questions.	The assessment tool
The validity of assessment tool is calculated by Cranach's alphabet equal to 0.84	The method of obtaining credit
Validity is obtained by assessing the face validity.	The method of obtaining narrative
Independent T-Test was used.	Data analysis method

RESULTS

Data description: Statistical characteristic of personal, economical, and political awareness of boys and girls are given in Tables 1 & 2.The first findings of the present study show that there is a significant difference between boys and girls personal awareness. The girls` personal awareness is more than boys. Thus, the first hypothesis is confirmed. There was no research done about this hypothesis. As the subcategories of personal awareness consist of health, sanitation, kindness, affection, nutrition, self-criticism, etc. It seems logical that the pre-university girls` personal awareness regarding their age to marry and give birth to children in Kurdistan province is more than boys`.The second findings of the present study show that there is a significant difference between boys' and girls` economical awareness. The boys` economical awareness is more than the girls`.This finding is consistent with Erfani (1999) as boys have more chance to go out and work and in the research society of the present study (Kurdistan province) the traditional doesn`t let girls work out of their homes and gain knowledge and skill about it. Therefore, this finding showing the superiority of boys` economical awareness over girls` seems logical.

The third finding of the present study indicates a significant difference between boys' and girls` political awareness. The boys` political awareness is more than girls`. This finding is consistent with Majid Fallah (1997) Erfani (1999). As in the research society of this study (Kurdistan province) boys are more in contact with organizations, ministries, campaigns and revolutions, military issues and participation in national affairs than girls. Besides families would let boys participate in demonstrations but girls don`t gain such permission. Thus, it seems logical that the pre-university boys` political awareness is more than the girls.

Limitations of the study

- Lack of similar studies in this regard.
- This study includes so many characteristics which may reduce its precision.

- As the questions were numerous, the students may not have paid enough attention while answering them.
- Financial and official problem. As the present study was extensive and the sample population was all over the province, there existed financial and beurocratic problems.
- Another limitation facing this study was selecting an appropriate tool for data collection. Although the validity coefficient of this questionnaire was 0.84. The results of this study should be viewed with discretion.

Research Recommendations

A: Recommendations to future researchers

- As the subject of this study were pre-university students, it is recommended to do research on guidance and high school students studying various fields.Compare personal, economical, and political awareness of students with personal, economical, and political awareness of this parent.Different kinds of awareness of different students from different areas be compared, e.g. rural versus urban students.
- It is suggested that such research be carried out in different provinces of the country to identify students` awareness and generalize the findings.

B: Recommendations based on research findings

- The first findings show that girls` personal awareness is more than boys`. Then administrators, parents and other people in charge are recommended to pay more attention to boys` awareness of nutrition, sanitation, health, kindness, affection, the speed to convey problems, self-evaluation and other issues related to personal awareness on which radio, TV, newspapers, brochures, holding seminars and meetings can have a great impact for sure.The second findings show that boys` economical awareness is more than girls`. It is recommended to pay more attention to girls` economical awareness including awareness of production, consumption, appropriation, skills and awareness, cooperation, charity, currency rate, etc. this will be realized through girls` contribution in families income and expenses, allowing girls to work out on the part of the family, eliminating vocational discrimination for girls, holding meetings and gatherings and so the like.

- The third findings show that pre-university boys` political awareness is more than that of pre-university girls. It is recommended to enhance girls` awareness of democracy, freedom, campaign and unrest, organizations and ministries, foreign affairs, government, parties and other subsets of political awareness. The mass media such as radio, TV, newspapers, magazines concerned with woman, and holding seminars can be of great help in improving this very awareness. Besides, having girls to play a role in family decision making has a great effect on their political participation and this issue has to be paid full attention in families.Improve boys' and girls` awareness, education should begin at an early age.

- Administrators and curriculum designers are recommended to design a lesson unit called personal, economical and political awareness in all periods' .Of school, especially pre-university course attending to cultural, geographical and native differences of each district.Enhancing the staff and parents` personal, economical, and political awareness through holding proper workshops.Encouraging students to do research on personal, economical, and political issues.Establishing economical and political associations at schools, city, province and country to raise the students` awareness is recommended.

CONCLUSION

The following conclusions can be implied from tables 1 & 2.The overall comparison of the average showed that among personal, economical, and political awareness for girls 26.51 was the greatest for personal awareness and 25.47 was highest for boy's personal awareness.Comparison of standard deviation shows that dispersion in factor of

political awareness for girls and boys is more than other factors.The lowest score for girls is that of political awareness and the same is true about boys as well. The maximum score for boys and girls is that of personal awareness.Positive skewness indicates that skewness of distribution towards the normal distribution is to the right and negative skewness indicates that distribution towards the normal distribution except for girls` economical awareness is to the left.Positive elongation showed that the distribution elongation is longer than the normal distribution and the negative elongation indicates that distribution elongation is shorter than normal distribution.

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Table 1. Statistical characteristics of personal, economical, and political awareness score of boys.

Variance	Elongation	Skewness	Ampli-tude	Maxi-mum	Mini-mum	Standard deviation	Average	Statistical index / Components
15.75	0.197	-0.373	21	34	13	3.96	25.47	Personal
18.32	0.082	-0.588	19	28	9	4.04	20.32	Economical
55.14	-1.06	-0.310	26	33	7	7.42	21.65	Political

Table 2. Statistical characteristics of personal, economical, and political awareness score of girls.

Variance	Elongation	Skewness	Ampli -tude	Maxi-mum	Mini-mum	Standard deviation	Average	Statistical index / Components
12.59	0.263	-0.458	19	34	15	3.54	26.51	Personal
13.63	0.980	0.262	23	33	10	3.96	18.67	Economical
26.44	-0.020	-0.269	24	31	7	5.14	19.24	Political

Table 3. The observed value of T (Tob=2.28) at the level of α=0.05 with the degree of freedom (d☞ = 268) is more than T.

Level of significance	Degrees of freedom	T	Standard deviation	Average	Abundance	Gender	Statisticalindicators / Components
0.023	268	2.28	3.96	25.47	130	male	Personal awareness
			3.54	26.51	140	female	

The above table indicates that the observed value of T (Tob=2.28) at the level of α=0.05 with the degree of freedom (d☞ = 268) is more than T in the table. Thus, the hypothesis of zero based on lack of difference between personal awareness of pre-university boys and girls is rejected and we can conclude that girls` personal awareness is more than boys` personal awareness.

Table 4. The results of independent T-Test to compare averages of economical awareness among boys and girls. (n = 270)

Level of significance	Degrees of freedom	T	Standard deviation	Average	Abundance	Gender	Indication / Components
0.001	268	3.50	4.04	20.23	130	male	Economical awareness
			3.69	18.67	140	female	

The above table indicates that the observed value of T (Tob = 3.50) at the level $\alpha = 0.05$ with the degree of freedom of (d☞ = 268) is more than T in the table. Thus, the hypothesis of zero based on lack of differences between pre-university boys` and girls` economical awareness is rejected and we can conclude that boys` economical awareness is more than girls` economical awareness.

Table5.The results of independent T-Test to compare the averages of boys' and girls`political awareness. (n = 270)

Level of significance	Degrees of freedom	T	Standard deviation	average	abundance	gender	Indicator / component
0.002	268	3.11	7.42	21.65	130	male	Political awareness
			5.14	19.24	140	Female	

The above table indicates that the observed value of T (Tob = 3.11) at the level of $\alpha = 0.05$ with the degree of freedom of (d☞ = 268) is more than T in the table. Thus the hypothesis of zero based on lack of difference between boys' and girls` political awareness in pre-university course is rejected and we can conclude that boys` political awareness is more than girls`.

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RESEARCH ARTICLE

Analysis of V335 Ser, V821 Cas, V431 Pup and GG Ori by a Neural Network.

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ABSTRACT

We use an Artificial Neural Network (ANN) to derive the orbital parameters of spectroscopic binary stars.Using measured radial velocity data of four double-lined spectroscopic binary systems V335 Ser, V821 Cas, V431 Pup and GG Ori, we find corresponding orbital and spectroscopic elements via a Probabilistic Neural Network (PNN). Our numerical results are in good agreement with those obtained by others using more traditional methods.

Key words: Artificial Neural Network, spectroscopic binary stars, Probabilistic Neural Network.

INTRODUCTION

Analysis of both light and radial velocity (hereafter V_R) curves of binary systems helps us to determine the masses and radii of individual stars. One historically well-known method to analyze the V_R curve is that of Lehmann-Filhés [1].Some other methods were also introduced by Sterne [2] and Petrie [3]. The different methods of the V_R curve analysis have been reviewed in ample detail by Karami & Teimoorinia [4]. Karami & Teimoorinia [4] also proposed a new non-linear least squares velocity curve analysis technique for spectroscopic binary stars. They showed the validity of their new method to a wide range of different types of binary See Karami & Mohebi [5-7] and Karami et al. [8].

Artificial Neural Networks have become a popular tool in almost every field of science. In recent years, ANNs have been widely used in astronomy for applications such as star/galaxy discrimination, morphological classification of galaxies, and spectral classification of stars (see Bazarghan et al. [9] and references therein). Following Bazarghan et al. [9], we employ Probabilistic Neural Networks (PNNs). This network has been investigated in ample details by

Bazarghan et al. [9]. Probabilistic Neural Network (PNN) is a new tool to derive the orbital parameters of the spectroscopic binary stars.

In the present paper we use a Probabilistic Neural Network (PNN) to find the optimum match to the four parameters of the V_R curves of the four double-lined spectroscopic binary systems: V335 Ser, V821 Cas, V431 Pup and GG Ori. Our aim is to show the validity of our new method to a wide range of different types of binary.V335 Ser is a double-lined eclipsing binary and consists of primary and secondary components. The spectral class is between A0 and A1 and the orbital period is P=3.4500025 days [10]. V821 Cas is a double-lined eclipsing binary and the spectral type of the primary component is an A1V type main-sequence star. The secondary component appears to be an A4V star and the orbital period is P =1.769813 days [11]. V431 Pup is a early-type eclipsing binary with an evolved components. The spectral type is B1 III and the orbital period is P=9.3634 days [12]. GG Ori is a double-lined eclipsing binary and is a member of the Orion OB1 association. The spectral type is B9.5 and the orbital period is P=6.6314936 days [13]. This paper is organized as follows. In Sect. 2, we introduce a Probabilistic Neural Network (PNN) to estimate the four parameters of the V_R curve. In Sect. 3, the numerical results are reported, while the conclusions are given in Sect. 4.

METHODOLOGY

V_R curve parameters estimation by the Probabilistic Neural Network (PNN)

Following Smart [14], the V_R of a star in a binary system is defined as follows

$$V_R = \gamma + K[\cos(\theta + \omega) + e\cos\omega] \quad (1)$$

where γ is the V_R of the center of mass of system with respect to the sun. Also K is the amplitude of the V_R of the star with respect to the center of mass of the binary. Furthermore θ, ω and e are the angular polar coordinate (true anomaly), the longitude of periastron and the eccentricity, respectively.

Here we apply the PNN method to estimate the four orbital parameters, γ, K, e and ω of the V_R curve in Eq. (1). In this work, for the identification of the observational V_R curves, the input vector is the fitted V_R curve of a star. The PNN is first trained to classify V_R curves corresponding to all the possible combinations of γ, K, e and ω . For this one can synthetically generate V_R curves given by Eq. (1) for each combination of the parameters:

- $-100 \le \gamma \le 100$ in steps of 1;
- $1 \le K \le 300$ in steps of 1;
- $0 \le e \le 1$ in steps of 0.001;
- $0 \le \omega \le 360^{\circ}$ in steps of 5;

This gives a very big set of k pattern groups, where k denotes the number of different V_R classes, one class for each combination of γ, K, e and ω . Since this very big number of different V_R classes leads to some computational limitations, hence one can first start with the big step sizes. Note that from Petrie [3], one can guess γ, K and e from a V_R curve. This enable one to limit the range of parameters around their initial guesses. When the preliminary orbit was derived after several stages, then one can use the above small step sizes to obtain the final orbit. The PNN has four layers including input, pattern, summation, and output layers, respectively (see Fig. 5 in Bazarghan et al. [9]). When an input vector is presented, the pattern layer computes distances from the input vector to the training input vectors and produces a vector whose elements indicate how close the input is to a training input. The summation layer sums these contributions for each class of inputs to produce as its net output a vector of probabilities. Finally, a competitive transfer function on the output layer picks the maximum of these probabilities, and produces a 1 for that class and a 0 for the other classes [15,16]. Thus, the PNN classifies the input vector into a specific k class labeled by the four parameters γ, K, e and ω because that class has the maximum probability of being correct.

RESULTS AND DISCUSSION

Here, we use the PNN to derive the orbital elements for the four different double-lined spectroscopic systems V335 Ser, V821 Cas, V431 Pup and GG Ori. Using measured V_R data of the two components of these systems obtained by Bozkurt [10] for V335 Ser, Cakırlı et al. [11] for V821 Cas, Mayer et al. [12] for V431 Pup and Torres et al. [13] for GG Ori, the fitted velocity curves are plotted in terms of the phase in Figs. 1 to 4. The orbital parameters obtaining from the PNN for V335 Ser, V821 Cas, V431 Pup and GG Ori are tabulated in Tables 1, 3, 5 and 7, respectively. Tables show that the results are in good accordance with the those obtained by Bozkurt [10] for V335 Ser, Cakırlı et al. [11] for V821 Cas, Mayer et al. [12] for V431 Pup and Torres et al. [13] for GG Ori.

Note that the Gaussian errors of the orbital parameters in Tables 1, 3, 5 and 7 are the same selected steps for generating V_R curves, i.e. $\Delta\gamma = 1, \Delta K = 1, \Delta e = 0.001$ and $\Delta\omega = 5$. These are close to the observational errors reported in the literature. Regarding the estimated errors, following Specht [16] , the error of the decision boundaries depends on the accuracy with which the underlying Probability Density Functions (PDFs) are estimated. Parzen [17] proved that the expected error gets smaller as the estimate is based on a large data set. This definition of consistency is particularly important since it means that the true distribution will be approached in a smooth manner. Specht [16] showed that a very large value of the smoothing parameter would cause the estimated errors to be Gaussian regardless of the true underlying distribution and the misclassification rate is stable and does not change dramatically with small changes in the smoothing parameter. The combined spectroscopic elements including $m_p \sin^3 i$, $m_s \sin^3 i$, $(m_p + m_s)\sin^3 i$, $(a_p + a_s)\sin i$ and $\frac{m_s}{m_p}$ are calculated by substituting the estimated parameters K, e and ω in to Eqs. (3), (15) and (16) in Karami and Teimoorinia [4]. The results obtained for the four systems are tabulated in Tables 2, 4, 6 and 8 show that our results are in good agreement with the those obtained by Bozkurt [10] for V335 Ser, Cakırlı et al. [11] for V821 Cas, Mayer et al. [12] for V431 Pup and Torres et al. [13] for GG Ori, respectively. Here the errors of the combined spectroscopic elements in Tables 2, 4, 6 and 8 are obtained by the help of orbital parameters errors. See again Eqs. (3), (15) and (16) in Karami and Teimoorinia [4].

CONCLUSION

A Probabilistic Neural Network to derive the orbital elements of spectroscopic binary stars was applied. PNNs are used in both regression (including parameter estimation) and classification problems. However, one can discretize a continuous regression problem to such a degree that it can be represented as a classification problem [15,16], as we did in this work.Using the measured V_R data V335 Ser, V821 Cas, V431 Pup and GG Ori obtained by Bozkurt [10], Cakırlı et al. [11], Mayer et al. [12] and Torres et al. [13], respectively, we find the orbital elements of these systems by the PNN. Our numerical results shows that the results obtained for the orbital and spectroscopic parameters are in good agreement with those obtained by others using more traditional methods. This method is applicable to orbits of all eccentricities and inclination angles. In this method the time consumed is considerably less than the method of Lehmann-Filhés. It is also more accurate as the orbital elements are deduced from all points of the velocity curve instead of four in the method of Lehmann-Filhés. The present method enables one to vary all of the unknown parameters γ, K, e and ω simultaneously instead of one or two of them at a time. It is possible to make adjustments in the elements before the final result is obtained. There are some cases, for which the geometrical methods are inapplicable, and in these cases the present one may be found useful. One such case would occur when observations are incomplete because certain phases could have not been observed. Another case in which this method is useful is that of a star attended by two dark companions with commensurable periods. In this case the resultant velocity curve may have several unequal maxima and the geometrical methods fail altogether.

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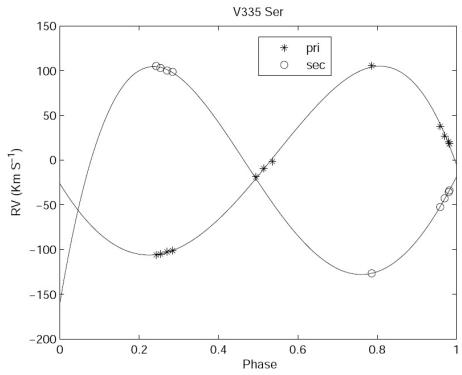

Fig. 1: Radial velocities of the primary and secondary components of V335 Ser plotted against the phase. The observational data [11]. have been measured by Bozkurt [10].

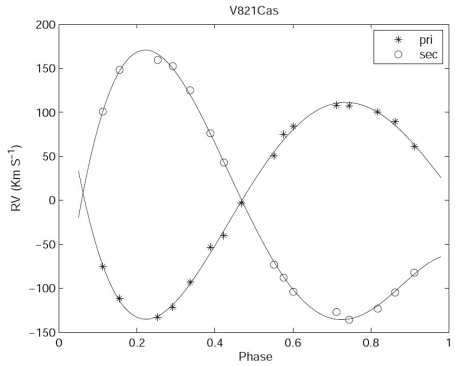

Fig. 2: Radial velocities of the primary and secondary components of V821 Cas plotted against the phase. The observational data have been measured by Cakırlı et al.

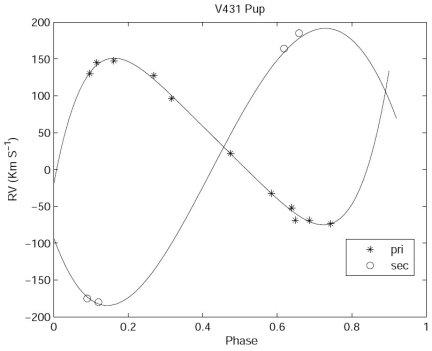

Fig. 3: Radial velocities of the primary and secondary components of V431 Pup plotted against the phase. The observational data have been measured by Mayer et al. [12].

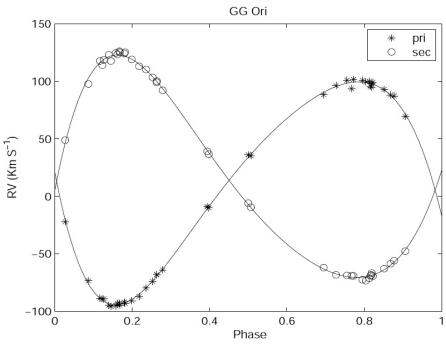

Fig. 4: Radial velocities of the primary and secondary components of GG Ori plotted against the phase. The observational data have been measured by Torres et al. [13].

Table 1: Orbital parameters of V335 Ser

	This Paper	Bozkurt [10]
$\gamma\ (kms^{-1})$	-6 ± 1	-5.97 ± 0.52
$K_p\ (kms^{-1})$	106 ± 1	106.31 ± 0.88
$K_s\ (kms^{-1})$	117 ± 1	116.94 ± 0.88
e	0.138 ± 0.001	0.1379 ± 0.0006
$\omega(^\circ)$	60 ± 5	63.49 ± 0.12

Table 2: Combined spectroscopic elements of V335 Ser

Parameter	This Paper	Bozkurt [10]
$m_p \sin^3 i/M_\odot$	2.0206 ± 0.0544	—
$m_s \sin^3 i/M_\odot$	1.8306 ± 0.0509	—
$(m_p + m_s) \sin^3 i/M_\odot$	3.8512 ± 0.1052	—
$a_p \sin i/R_\odot$	7.1561 ± 0.0685	—
$a_s \sin i/R_\odot$	7.8987 ± 0.0686	—
$(a_p + a_s) \sin i/R_\odot$	15.0548 ± 0.1371	—
m_s/m_p	0.9060 ± 0.0165	0.915 ± 0.038

Table 3: Orbital parameters of V821 Cas

	This Paper	Çakırlı et al [11]
$\gamma\ (kms^{-1})$	-1 ± 1	-0.05 ± 0.01
$K_p\ (kms^{-1})$	119 ± 1	120 ± 2
$K_s\ (kms^{-1})$	149 ± 1	150 ± 2
e	0.126 ± 0.001	0.127 ± 0.007
$\omega(^\circ)$	160 ± 5	155 ± 4

Table 4: Combined spectroscopic elements of V821 Cas

Parameter	This Paper	Çakırlı et al [11]
$m_p \sin^3 i/M_\odot$	1.9158 ± 0.0422	—
$m_s \sin^3 i/M_\odot$	1.5301 ± 0.0363	—
$(m_p + m_s) \sin^3 i/M_\odot$	3.4458 ± 0.0785	—
$a_p \sin i/R_\odot$	4.1278 ± 0.0352	—
$a_s \sin i/R_\odot$	5.1685 ± 0.0353	—
$(a_p + a_s) \sin i/R_\odot$	9.2963 ± 0.0706	—
m_s/m_p	0.7987 ± 0.0123	0.795 ± 0.017

Table 5: Orbital parameters of V431 Pup

	This Paper	Mayer et al. [12]
$\gamma\ (kms^{-1})$	26 ± 1	25.9 ± 0.7
$K_p\ (kms^{-1})$	118 ± 1	118.2 ± 0.6
$K_s\ (kms^{-1})$	149 ± 1	149.2 ± 0.6
e	0.194 ± 0.001	0.193
$\omega(^\circ)$	305 ± 5	300.5

Table 6: Combined spectroscopic elements of V431 Pup

Parameter	This Paper	Mayer et al. [12]
$m_p \sin^3 i/M_\odot$	9.7283 ± 0.2169	9.16
$m_s \sin^3 i/M_\odot$	7.7043 ± 0.1854	7.50
$(m_p + m_s) \sin^3 i/M_\odot$	17.4327 ± 0.4023	—
$a_p \sin i/R_\odot$	21.4146 ± 0.1858	—
$a_s \sin i/R_\odot$	27.0405 ± 0.1869	—
$(a_p + a_s) \sin i/R_\odot$	48.4551 ± 0.3727	48.5
m_s/m_p	0.7919 ± 0.0123	—

Table 7: Orbital parameters of GG Ori

	This Paper	Torres et al. [13]
$\gamma\ (kms^{-1})$	14 ± 1	14.31 ± 0.21
$K_p\ (kms^{-1})$	97 ± 1	97.10 ± 0.30
$K_s\ (kms^{-1})$	98 ± 1	97.28 ± 0.28
e	0.222 ± 0.001	0.2218 ± 0.0022
$\omega(^\circ)$	125 ± 5	122.76 ± 0.39

Table 8: Combined spectroscopic elements of GG Ori

Parameter	This Paper	Torres et al. [13]
$m_p \sin^3 i/M_\odot$	2.3734 ± 0.0746	2.341 ± 0.016
$m_s \sin^3 i/M_\odot$	2.3492 ± 0.0741	2.337 ± 0.017
$(m_p + m_s) \sin^3 i/M_\odot$	4.7226 ± 0.1486	—
$a_p \sin i/10^6 km$	8.6247 ± 0.0909	8.634 ± 0.028
$a_s \sin i/10^6 km$	8.7136 ± 0.0909	8.650 ± 0.026
$(a_p + a_s) \sin i/R_\odot$	24.9113 ± 0.2613	24.833 ± 0.056
m_s/m_p	0.9898 ± 0.0208	0.9982 ± 0.0044

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RESEARCH ARTICLE

Polymorphism in 5'- Non coding Region of Growth Hormone Receptor Gene and its Association with Some Dairy Production Traits in Swamp Buffalo and Yak.

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ABSTRACT

A PCR-RFLP based polymorphism study was conducted in Swamp Buffaloes and Yaks on 5' non-coding region of GH receptor gene using the restriction enzyme (RE) *RsaI*, allowed the identification of two alleles viz., *RsaI* (A) and *RsaI* (B) with frequencies 0.931 and 0.069 respectively. The frequencies of AA (424, 196, 130 and 40bp) and AB (424, 231, 196, 130 and 40bp) genotypes in the population were 0.867 and 0.133 respectively. The statistical analysis of GHR/*RsaI* polymorphism using simple t-test, based on the mean values of AA and AB genotypes revealed that there is no significant difference between the various milk production traits of both the groups. Besides *RsaI* digestion in yaks produced a single digestion pattern with four products of size 424bp, 196bp, 130bp and 40bp respectively denoting monomorphism.

Keywords: Growth Hormone Receptor Gene (GHR), PCR-RFLP and Polymorphism.

INTRODUCTION

Growth is one of the economic traits in the livestock which is controlled by the Growth hormone (GH) or somatotropin synthesized by the anterior pituitary, almost all the economic traits are directly or indirectly related to this. The biological effects of growth hormone (GH) involve a variety of tissues and the metabolism of all nutrient classes: carbohydrates, lipids, proteins, and minerals. Thus these coordinated changes in metabolism of various nutrients plays a key role in the growth performance and milk yield in livestock [2].The action of GH is mediated through the Growth hormone receptors (GHR), a transmembrane protein which belongs to the cytokine receptor

super family [3]. The GHR has been found in all tissues but its level varies from tissue to tissue for example its level is high in liver when compared with other organs.

The present study was conducted on Swamp buffaloes of Assam and Yaks of Arunachal Pradesh both belong to the family Bovidae.The Swamp buffaloes (*Bubalus carabanesis*) are mainly found in the South Eastern countries like Indonesia, Myanmar, Vietnam and Philippines and in India it is mainly found in North eastern states. The majority of buffaloes of Assam are mainly swamp type.Yak (*Poephagus grunniens* or *Bos grunniens*) is a multipurpose species and believed to be indispensable for the highlanders. In India yaks are reared in West Kameng and Twang districts of Arunachal Pradesh, North and East districts of Sikkim, Lahul and Sipti and Kianaur districts of Himachal Pradesh, Ladakh of Jammu and Kashmir and Garhwal Himalaya of Uttranchal. Besides India, yaks are also found in Mongolia, Russia, Tuva, Buryatia, Kirgizia, Bhutan and Nepal. The yaks are well adapted to harsh environment of high land, which came from the long term of natural selection and complex biological process. This capacity to survive in harsh environment is one of the most important traits in the genetic makeup of the yak.Considering the importance of these two distinguished species efforts should also be made for identification of some polymorphic genes and their association with economic traits. The present study was undertaken with the objective of genotyping GHR gene by PCR-RFLP technique and studying the association of performance traits like milk yield (first lactation), fat percentage, milk protein percentage, total solids percentage and SNF with polymorphism in Swamp buffaloes of Assam and Yaks of Arunachal Pradesh.

MATERIALS AND METHODS

The present study was performed on 15 swamp buffaloes (*Bubalus carabanesis*) maintained at the buffalo farm of Network Project on Swamp Buffalo, Department of Animal Genetics and Breeding, College of Veterinary Science, Khanapara, Guwahati and 30 yaks (*Poephagus grunniens* or *Bos grunniens*) maintained at Nyukmadung yak farm, National Research Centre on Yak, Dirang, Arunachal Pradesh.

10 ml of blood was aseptically collected from external jugular vein in vacutainer tube containing EDTA (1mg/ml). DNA was extracted from whole blood within 24 hours after collection using high salt method as described by Montgomery and Sise (1990)[5], with minor modifications. The purity and concentration of DNA samples were estimated using 0.7% agarose gel electrophoresis and UV visible range spectrophotometer (Shimadsu Corporation, Japan). The yield of DNA extracted from 10ml of blood ranged from 318 μg to 648 μg (mean 483 μg) in swamp buffaloes and 328 μg to 739 μg (mean 533.5 μg) in yaks. The O.D ratio was in the range of 1.7-1.9 indicating purity of the extracted DNA from buffaloes and yaks.

The primer sequences used for amplifying 5'noncoding region of GHR gene (790bp) is F: 5'-TGC GTG CAC AGC AGC TCA ACC-3' and R: 5'-AGC AAC CCC ACT GCT GGG CAT-3' for standardization of the PCR a top-down approach was followed to obtain optimum amplification. The PCR reaction was performed in a volume of 50μl containing 5μl DNA(approx.500ng), 1X PCR Buffer (without MgCl2), MgCl2 (1.5 mM), dNTPS (200μM), primers (20pmol each) and Taq DNA polymerase (2.5U/50μl). Amplification was carried out for 35 cycles: 95°C for 30s, 64°C for 60s and 72°C for 60s with initial denaturation of 95°C for 5 minutes and final extension of 72°C for 10 minutes. The PCR products were electrophoresed in 2 per cent agarose gel at 90V for 45 minutes along with molecular marker (100bp ladder) and visualized under Gel doc system (Syngene). A PCR product of 790bp size was amplified for Growth hormone receptor (GHR) gene both in swamp buffaloes and yak. The analysis of milk composition was done by collecting 20 ml milk from each experimental animal, in sterile milk bottles without the addition of any preservatives. The milk composition was estimated using Milk Quality Analyzer (LAKTAN 1-4 Model 220). It was used to estimate various components of milk like fat, protein, Solid Not Fat (SNF) and total solids. The statistical analysis of GHR/*RsaI* polymorphism were done using simple t-test, based on the mean values of genotypes for various milk production traits in both groups.

RESULTS AND DISCUSSION

The 790bp fragment of DNA, 5' non coding region of GHR gene was amplified and digested with *Rsa I* and *Eco R I* in both the species. The PCR-RFLP studies on 5' non coding region of GHR gene in swamp buffaloes, using *Rsa I*, allowed the identification of two alleles viz., *Rsa I* (A) and *Rsa I* (B) with frequencies 0.931 and 0.069 respectively as shown in the Table 1. The frequencies of AA (424, 196, 130 and 40bp) and AB (424, 231, 196, 130 and 40bp) genotypes in the population were 0.867 and 0.133 respectively (Table 1). In both species, the size of GHR gene amplified was 790bp indicating the conservation of DNA sequence in swamp buffalo and yak. However, Maj *et al*. (2004)[4] obtained the amplified product of 836bp size in cattle using the same primer. The observed difference in the product size could be due to species variation. Many researchers have shown polymorphism in 5' non coding region of GHR gene of cattle [1,3,4] but no such reports are available in swamp buffaloes and yaks. However on digestion with *Rsa I* in yaks revealed monomorphism and the digestion of the amplified GHR gene with *Eco R I* failed to show any polymorphism both in swamp buffaloes as well as in yaks (Table 2).The statistical analysis of GHR/*RsaI* polymorphism for the performance traits in swamp buffaloes was done using simple t-test and the analysis based on the mean values of AA and AB genotypes exhibited no significant effect on total milk yield, milk fat percentage, milk protein, SNF and total solids.

SUMMARY

A PCR-RFLP based polymorphism study was conducted in Swamp Buffaloes and Yaks on 5' non-coding region of GH receptor gene using the restriction enzyme (RE) *RsaI*, allowed the identification of two alleles viz., *RsaI* (A) and *RsaI* (B) with frequencies 0.931 and 0.069 respectively. The frequencies of AA (424, 196, 130 and 40bp) and AB (424, 231, 196, 130 and 40bp) genotypes in the population were 0.867 and 0.133 respectively. The statistical analysis of GHR/*RsaI* polymorphism using simple t-test, based on the mean values of AA and AB genotypes revealed that there is no significant difference between the various milk production traits of both the groups. However further research has to be done in large population to obtain a conclusive result.

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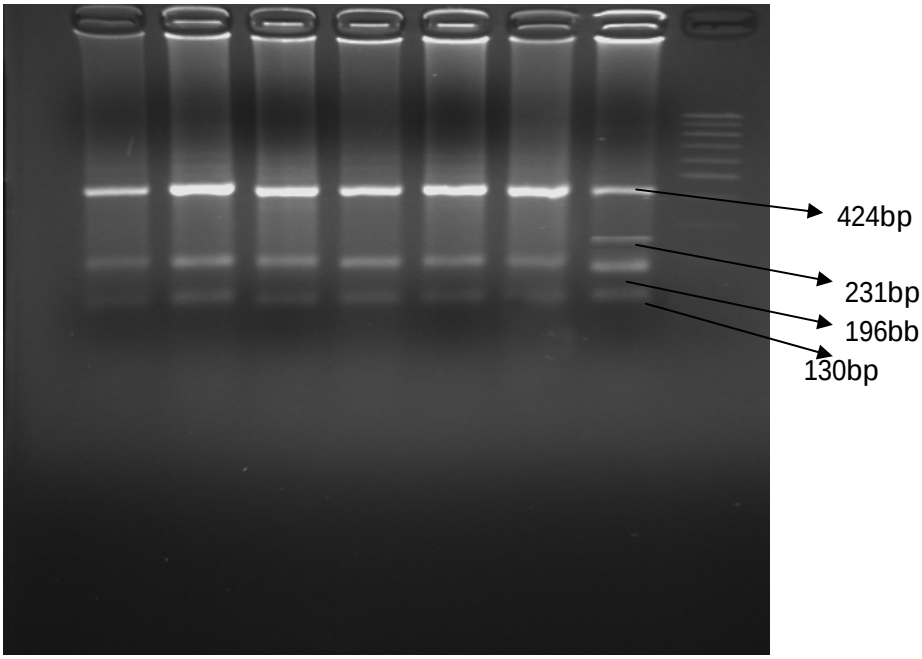

Fig. 1 *Rsa I* digestion of GHR gene in Swamp buffalo shows polymorphism

Lane 1-6:GHR/ *Rsa I* (AA) genotypes 424, 196 and 130bp, Lane 7: GHR/ *Rsa I* (AB) genotypes 424, 231,196 and 130bp and M: denotes 100bp LADDER.

Table 1. Genotype and gene frequencies of GHR/*Rsa I* polymorphisms in swamp buffaloes.

Number of animals	Genotype frequencies		Allele frequencies	
	AA	AB	A	B
15	13(0.867)	2(0.133)	0.931	0.069

*Frequencies in brackets

Table 2. PCR-RFLP of Growth hormone receptor gene.

Species	Restriction enzymes	Fragment size (bp)
Swamp buffalo (*Bubalus carabanesis*)	*Eco R I*	750bp and 40bp
	Rsa I	AA genotype 424bp, 196bp, 130bp and 40bp AB genotype- 424bp, 231bp, 196bp, 130bp and 40bp
Yak (*Poephagus grunniens.*)	*EcoR I*	Single undigested product of 790bp
	Rsa I	424bp, 196bp, 130bp and 40bp

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RESEARCH ARTICLE

Knowledge Level of the Farmer in System of Rice Intensification Cultivation Practices in Tirunelveli District of Tamil Nadu,India.

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ABSTRACT

The System of Rice Intensification (SRI) is a method of increasing the yield of rice produced in farming. SRI is considered to be a disembodied technological breakthrough in paddy cultivation. SRI involves the application of certain management practices, which together provide better growing conditions for rice plants, particularly in the root zone, than those for plants grown under traditional practices. This system seems to be promising to overcome the shortage of water in irrigated rice. The study was conducted in Vasudevanallur block of Tirunelveli district in Tamil Nadu. A total of 120 respondents were selected, and interviewed using a well structured, pretested interview schedule. System of Rice Intensification cultivation practices around Fifty percent of the SRI farmers had medium level of knowledge and particularly cent per cent of the respondents possessed knowledge about the age of seedlings for transplanting, the spacing recommended for transplanting, number seedlings planted in a hill and name of the mechanical weeder. Around 50.00 per cent of the respondents had medium level of knowledge followed by 38.30 per cent and 10.00 per cent who had high and low levels of knowledge in SRI cultivation method respectively.

Keywords: System of Rice Intensification, Knowledge, and Crop management practices.

INTRODUCTION

SRI, the system of rice intensification is a system of production of rice. SRI is considered to be an intangible technological breakthrough in paddy cultivation. SRI involves the application of certain management practices, which together provide better growing conditions for rice plants, particularly in the root zone, than those for plants grown under traditional practices. This system seems to be promising to overcome the shortage of water in irrigated rice. It was developed in Madagascar in the early 1980s by Father Henride Laulanie, A Jesuit Priest, who spent over 30 years in that country working with farmers.

It has since been tested in China, India, Indonesia, Philippines, Sri Lanka and Bangladesh with positive results. In Sri Lanka, SRI cultivation was practiced in 18 districts with encouraging results of doubling the yields. In this method synergic interaction leads to much higher yields. It offers increased land, labour and water productivity. In fact, it is a less water consuming method of rice cultivation, which is suitable to poor farmers who have relatively more labour force than land and capital.

The System of Rice Intensification (SRI) technique has received considerable attention globally including India due to its potential for yield improvement and water saving. The main features of this system include transplanting of young seedlings singly in a square pattern with wide spacing; using more of organic fertilizers; and keeping the paddy field moist with intermittent drying and wetting during the vegetative growth of plants. SRI causes better plant growth and development and economizes the use of seed, irrigation water, labour, plant protection chemicals and fertilizers and hence increases the productivity of land, water capital and labour significantly over conventional method of rice cultivation.

The System of Rice Intensification technique is promoted under World Bank assisted project Irrigated Agriculture Modernized Water Bodies Restoration and Management (IAMWARM) in Tamil Nadu. During 2007-08, 912 demonstrations at the cost of Rs. 36.48 lakhs were organized. In 2008-09, a sum of Rs. 122.04 lakhs was spent for conducting 2034 demonstrations. In the present study knowledge denotes the respondent's level of understanding of SRI practices in the cultivation of Paddy. To measure the knowledge level of respondents, they were asked straight questions in respect of nursery preparation, main field preparation, irrigation management, weed management and nutrient management in line with SRI technologies.

MATERIALS AND METHODS

This research was carried out in Tirunelveli district of Tamil Nadu. Tirunelveli district consists of eleven taluks, Sivakiri taluk was purposively selected this taluk 23 blocks Vasudewanallur block was purposively, as it has larger area under paddy. Out of 26 panchayat villages in Vasudevanallur blck, 4 villages were randomly selected namely Vasudevanallur, Sivagiri, Rayagiri and Ullar. The respondents were selected a list of farmers practicing SRI was obtained from the Assistant Director of Agriculture office of Vasudevanallur Block. There were more than 100 farmers practicing System of Rice Intensification (SRI) in each villages of the block. By considering the sample size of the study, it has been decided to select 30 farmers from each of the four villages, where the highest number of farmers practicing System of Rice Intensification (SRI) extensively. Accordingly the sample has been fixed as 120 SRI farmers. The respondents were selected by employing simple random sampling technique in each village. Ex post-facto design was followed in this study. The data were collected by personal interview with respondents in their farm and home.

RESULTS AND DISCUSSION

In the present study, knowledge has been operationalised as the body of understood information possessed by the respondents on cultivation of paddy under SRI method. The overall knowledge level and technology-wise

knowledge level of the respondents were studied and the findings were presented in this section. The knowledge level of respondents in SRI cultivation technology was measured by using a teacher made knowledge test consisting of SRI techniques. The test included 16 items relating to various SRI techniques. In order to assess the overall knowledge level of the respondents, necessary data were collected and they were categorized into three groups viz., low, medium and high using cumulative frequency method and the results are shown in Table 1.

A glance at the Table 1, revealed that around 51.70 per cent of the respondents had medium level of knowledge followed by 38.30 per cent and 10.00 per cent who had high and with low levels of knowledge in SRI cultivation method respectively. In general it could be concluded that there existed medium to high level of knowledge with majority (90.00 per cent) of the respondents.The appropriate reason for medium to higher level of knowledge on the recommended SRI cultivation practices might be due to their higher literacy, area under rice cultivation, medium level of credit orientation and medium to high level of economic motivation and scientific orientation. The respondents' ambition to increase their farm income; would have motivated them to gain more knowledge on SRI cultivation practices. Further, the agricultural scientists also played an important role in the dissemination of knowledge on SRI techniques through TN-IAMWARM project. This would have contributed for the medium level of knowledge among majority of the respondents. The practice wise knowledge level of the SRI farmers in different technologies of SRI cultivation is as follows.

Practice wise knowledge level of respondents in different technologies of SRI cultivation

The result of respondents' knowledge level in different technologies of SRI cultivation is assessed and the results are depicted in Table 2.

Knowledge level of the respondents in nursery preparation

It is evident from the Table 2 that among the three selected nursery practices of SRI technology in rice cultivation. a vast majority (94.16 per cent) of the respondents had knowledge on seed rate per acre. As the recommendation of seed rate (2 kg) is easy to remember, most of the respondents had acquired high knowledge score.More than fifty per cent of the respondents (52.50 per cent) had knowledge about size of nursery area. The medium level of farming experience of respondents in farming could be the reason for medium knowledge level. Since the mean percentage score on knowledge level of the nursery preparation is 80.27 per cent, it could be inferred that most of the respondents possessed adequate knowledge on most of the nursery preparation technologies.

Knowledge level of the respondents in main field preparation

It is observed from the Table 2, that cent per cent of respondents possessed knowledge about the age of seedlings for transplanting, the spacing recommended for transplanting and number of seedlings planted in a hill. More than (94.00 per cent) of the respondents had knowledge about per hill in one square meter. This may be due to the regular contact of respondents with extension agency.

Cent per cent of the respondents obtained high knowledge score for the 'seedling per hill'. This may be due to its local name viz., "single seedling" which could be easily understood and remembered by the respondents. Since the mean percentage score on knowledge level of the main field preparation is 96.00 per cent, it could be inferred that most of the respondents possessed adequate knowledge on most of the main field preparation technologies.

Knowledge level of the respondents in irrigation management practices

An overwhelming majority (71.66 per cent) of the respondents had knowledge about the time of irrigation in SRI cultivation. Majority of the respondents (47.50 per cent) was found to have knowledge on height of water level to be

maintained in the main field. Since the mean percentage score of the knowledge level on irrigation management practices was 59.58 per cent, it could be inferred that most of the farmers possessed adequate knowledge in the irrigation management practices practiced in SRI cultivation method.

Knowledge level of the respondents in weed management aspects

It is observed from the Table 2, that cent percentage of respondents had knowledge about the name of the mechanical weeder (100.00 per cent). The extension efforts coupled with supply of Cono-weeder by State Department of Agriculture to the individual respondents, would have made them to remember the name of the weeder.

Knowledge level of the respondents in nutrient management aspects

It could be seen from the Table 2, majority of the respondents (62.50 per cent) had knowledge on recommended inorganic fertilizers. This might be due to the possession of more years of experience in rice cultivation. About 46.00 per cent of the respondents had knowledge on purpose of leaf colour chart and 35.00 per cent of the respondents had knowledge on quantity of bio-fertilizers. Nearly 27.00 per cent of the respondent had knowledge on preferred time for taking observation using LCC. Knowledge on the nutrient management aspects would have been contributed by the training given by the extension officials in promoting SRI technologies.

CONCLUSION

Knowledge is an indispensable criterion for the adoption of any innovation, as it enables the farmers to understand completely and clearly the recommended technologies. The rate of adoption of an innovation is directly linked with level of knowledge of user about the same. It can be concluded Around 50.00 per cent of the respondents had medium level of knowledge followed by 38.30 per cent and 10.00 per cent who had high and low levels of knowledge in SRI cultivation method respectively. While analyzing the knowledge it was found that less than one fourth of the respondents had poor knowledge on Size of the nursery area, preferred time for taking observation using leaf colour chart and maintenance of recommended height of water level. Hence, intensive training with demonstrations on these above technologies may be given by the extension personnel of the State of Department Agriculture.

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Table 1. Distribution of respondents according to their knowledge level.

(n=120)*

S.No.	Category	Frequency	Per cent
1	Low	12	10.00
2	Medium	62	51.70
3	High	46	38.30
	Total	**120**	**100.00**

Table 2. Practice wise knowledge level of respondents in different technologies of SRI cultivation .

(n=120) *

S.No.	Items	Number of respondents	Percentage of respondents
1	Seed rate per acre	113	94.16
2	Size of the nursery area	63	52.50
3	Seedling age	120	100.00
4	Spacing	120	100.00
5	Seedling per hill	120	100.00
6	Name of the mechanical weeder	120	100.00
7	Purpose of leaf colour chart	55	45.83
8	Preferred time for taking observation using LCC	32	26.66
9	Recommended bio-fertilizer	42	35.00
10	Per hills one square meter	113	94.16
11	SRI method boots up productive tillers	103	85.83
12	Maintenance of recommended height of water level	57	47.50
13	Time of irrigation	86	71.66
14	Recommended inorganic fertilizer	75	62.50
15	Use of markers in SRI cultivation	120	100.00
16	Percentage of yield increase	113	94.16

* (Multiple responses obtained)

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RESEARCH ARTICLE

Cloning and Sequencing of Exon 5 - Exon 6 Region of *Tapasin* Gene in White Pekin Duck.

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ABSTRACT

MHC class I molecules will get hold of the peptides which are produced by the degradation of cytosolic proteins by proteasomes. These protein antigens are then translocated across the endoplasmic reticulum membrane (ER) by transporter associated with antigen processing (TAP). Several molecular chaperons are involved in this transport. Tapasin (Tpn) is one among those proteins which helps in peptide loading. Present study was under taken to characterize the exon 5 – exon 6 region of *Tapasin* gene in white pekin ducks. Since ducks diverged earlier from Galloanseriforme lineage study on duck *Tapasin* gene may help to understand the structure and organization of primordial avian *Tapasin* gene. Genomic DNA from blood of white pekin ducks were isolated and exon 5 – exon 6 region of the *Tapasin* gene were then amplified using primers designed based on chicken *Tapasin* gene sequences. PCR conditions were standardized so that a single product was obtained. The PCR products were then purified and cloned in TA cloning vector. Recombinant clones were then selected by blue white screening and were confirmed for the presence of insert by colony PCR with the same primer combination and purified plasmids from positive clones were sequenced.

Key Words: *Tapasin* gene, Major Histocompatibility complex class I(MHC class I), Cloning, Vector

INTRODUCTION

Cell – mediated immunity plays an important role in tumour avoidance and viral infection [1]. Cell – mediated immunity is an important component of acquired immune system. Acquired immune system detects the antigenic peptides presented by antigen presenting cells [2]. Antigen presentation involves cytoplasmic degradation of protein antigens by proteasomes. Antigenic peptides are then translocated across the endoplasmic reticulum (ER) membrane by the transporter associated with antigen processing (TAP). Tapasin is a molecular chaperon along with other proteins like oxidoreductase, ERp57 and calreticulin jointly dedicated to form a tether between TAP and Major Histocompatibility complex class I (MHC class I) molecules [2].Tapasin is a 48 kDa protein present on endoplasmic reticulum membrane [3]. The structure of tapasin consisting of a short cytoplasmic tail containing an endoplasmic reticulum retention signal, a transmembrane region, and a large N-terminal intralumenal part [3]. In mammals *Tapasin* gene referred to as TAPBP comprises of eight exons. Coding region is of 12,357 bp length and is located within the extended class II region[4].Like human counterpart chicken *Tapasin* gene includes 8 exons but 2 intron are much smaller than the corresponding human ones. *Tapasin* gene in chicken is located at the centromeric end between class II *B-LBI* and *B-LBII* genes. Structure of *Tapasin* gene showed a wide variation among different species. Present study was undertaken to unravel the structure of *Tapasin* gene in white pekin ducks [5].

MATERIALS AND METHODS

One ml of blood was collected from wing vein of the white pekin duck from a closed flock population. Genomic DNA was isolated by using DNeasy® blood and tissue kit (Qiagen) as per the manufactures protocol. Primers (Forward 5'ACGCTGTCCCCGAAGAACCTGGT 3', Reverse 5' CCAACGGATGAGGCCACAGAGGA 3') were designed for amplification of the region containing exon 5 - exon 6 of white pekin *Tapasin* gene based on chicken specific *Tapasin* gene sequence submitted in NCBI. Total volume of 25µl was used for the reaction. Reaction carried out with 20 pmol each of forward and reverse primer/reaction, 2.5µl 10X buffer without MgCl2, 3.3µl (3.3 mM) $MgCl_2$, 2.5µl (0.2 mM) of deoxyribonucleotides triphosphate, and 1.5U of Taq polymerase on an Eppendorf Mastercycler gradient PCR machine. The PCR cycles were carried out as follows: 95°C for 2 min, 35 cycles of denaturation at 95°C for 1 min, annealing at 61.2°C for 1 min, extention step at 72°C for 2 min and final extension at 72°C for 5 min. The resulted PCR products were visualized after electrophoresis in 2% ethidium bromide-stained 1x Tris-acetate-EDTA agarose gel. The PCR product was extracted from the gel using Gel Extraction Kit (Fermentas, Lithuania).

The gel purified PCR products were cloned using the pTZ57R/T Vector System (InsTAclone™ PCR Cloning Kit, Fermentas, Lithuania). TA cloning system exploits the terminal transferase activity of certain Taq DNA polymerase. Cloning was carried out as per manufactures protocol. Recombinant clones obtained were screened by PCR and restriction endonuclease (RE) digestion for confirmation of the gene insert. Plasmid isolation was carried out with GeneJET™ Plasmid Miniprep Kit (Fermentas, Lithuania).

Isolated recombinant plasmids were then subjected to double digest with the restriction enzymes *Hind* III and *Eco*RI to release the inserted DNA. Reaction was carried out in 20 µl reaction mixture with 2 µl buffer tango, 2.5 µl recombinant plasmid, 1µl *Eco*R1, 1 µl *Hind*III, 13.5µl nuclease free water. The digestion was carried at 37°C overnight in a water bath. These fragments were then sequenced using sequencing facility at Sci genome Pvt. Ltd., Cochin Special Economic Zone, Kakkanad by the dideoxynucleotide sequencing method using an automated DNA sequencer (Applied Biosystems, USA).

RESULTS AND DISCUSSION

Purity of the extracted genomic DNA was checked. The samples showed a ratio of $OD_{260/280}$ above 1.8 and were found to be free of protein contamination. Composition of the PCR mix, temperature, cycle parameters and number of cycles, time of the steps were optimized for the specific and efficient amplification of the product. The $MgCl_2$ concentration was found to be an important parameter for the efficient amplification of white peckin DNA. Sizes of the amplified products were confirmed by agarose gel electrophoresis using 100 bp molecular size markers.

Amplified products were of size between 400-450 bp as expected. The gel purified PCR products were cloned in the multiple cloning site of pTZ57R/T vector system and transformed *E.coli* appeared as white colonies. Positive clones were then screened by colony PCR. Recombinant plasmids were then isolated and insert were then released by double digestion. The cloned amplicons were sequenced by automated dideoxy chain termination method. Complete nucleotide sequence of exon 5, intron 5 and partial exon 6 thus obtained is depicted in Fig 1. The products obtained were of 445bp in length.

The nucleotide sequence of the 445 bp fragment of *Tapasin* gene in white pekin is shown in Fig 1. Primers were designed in such a way that the obtained product includes the sequence of exon 5, intron 5 and a partial sequence of exon 6. This region of Tapasin gene shows a wide variation in its length among different poultry species [5]. Compared to other poultry species white pekin *Tapasin* gene showed maximum variation in its sequence length. Identity search of the nucoeotide sequence with the nucleotide sequence of the chicken available in GenBank with Accession No: AJ004999 shows only 75% identity. This wide variation in nucleotide sequence and sequence length may be due to changes during the course of evolution of waterfowls. In contrast to the chicken there are two clusters of MHC Class 1 genes, the MHC of the duck contains five differentially expressed Class I genes [6,7]. These changes might have occurred in the course of evolution to offer them protection against diverse pathogens that would be encountered than the terrestrial birds. Since tapasin is a protein which plays an important role in the immune system identification of this *Tapasin* gene in white pekin duck will later help us to know the immune status of these birds which diverged Gallinoanseriforme lineage [7].

CONCLUSION

The fragment length of the amplified product, obtained sequence data and its analysis are suggestive of a different evolutionary lineage for ducks.

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1 acgctgtccc cgaagaacct ggttacgggg tgataaacat tcagacgtgc tttgtgtgat

61 cgtacaaggt tcttgaccag actgaaaggg aggtgctgta ttggcatcgg tatgcaggct

121 actcttttgt acgacgtaac actaattttc tacaggtagg gtccgtaccc tctgtgtact

181 gactttctag tatcctaata tcggcttgta gcctggagat atgcagtttg tttctccctt

241 taagctgtgt tattggccac tttactgcaa ataacctgac ttcgcaaatg tggaattgta

301 cctcatcctc ctctgtggcc tcatccttta aaagcctgat caacccggcc atatgcctag

361 gcgccctcat cccattgtga gaatatatgc ttgatggttg tattccctct cttaatcaat

421 catcctctgt ggcctcatcc gttgg

Fig 1. Nucleotide sequence (5'-3') of 445 bp of partial Tapasin gene, comprising of exon 5 – exon 6 in white pekin ducks.

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RESEARCH ARTICLE

Haematological Alteration in *Oreochromis mossambicus* after Exposure to ^{60}Co Gamma Irradiation.

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ABSTRACT

The present investigation was carried out to study the impact of the ^{60}Co gamma Irradiation on the haematological parameters of fresh water fish *Oreochromis mossambicus*. Adult fishes of nearly similar weight (33.45±1.3g) and length (12.27±1.5 cm) were exposed to five different dose levels of 3 mGy, 30 mGy,300mGy,3000mGy and 30000 mGy of 60Co gamma Irradiation for a period of 96 hrs. The fishes were divided into six groups, one group was served as control and the other groups (II-VI) were subjected to different doses of gamma irradiation. After irradiation, blood samples were collected in different time intervals of 12, 24, 48, 72 and 96 hrs. The haematological analysis showed significant reduction in red blood cells (RBCs) count. While total white blood cells (WBCs) count, haemoglobin (Hb) values were significantly increased in the treated groups as compared to the control group. The results of the present investigation suggest that gamma Irradiation affects the Haematological parameters and serves as bio monitoring tool to assess the radiation pollution in the aquatic environment.

Keywords: Haematology, Oreochromis *mossambicus*, Gamma Irradiation, Cobalt-60, Mean Corpuscular Haemoglobin.

INTRODUCTION

Aquatic systems are highly vulnerable to pollution since they act as immediate sinks for the consequences of human activities always associated with the danger of accidental discharges or criminal negligence (Vutukum, 2005). Cooney et.al. (2001) stated that aquatic lives are also affected by the cleanup operations as well as indirectly through physical damage to the habitats in which plants and animals live. Human destructive influence on the aquatic environment is in the form of sub-lethal pollution, which results in chronic stress conditions that have negative effect on aquatic life (Mason, 1991).Fishes are the most at threat from aquatic pollution and together with their long-term exposure in natural habitat they are suitable biomonitors of environmental pollution (Padmini et al.,2004). They also are widely (and increasingly) used as animal models in toxicological research. Several features of fish make them valuable as models in toxicology (Ballatori and Villalobos, 2002; Hinton et al., 2005; Kelly er al., 1998).

The fish *O.mossambicus (*Tilopia) is one of the most important fish species in the fisheries world. It is the second most essential group of food fishes in the world after carps. There is about 100 species and subspecies of tilapia with global annual production of 2532407 tons (FAO, 2008) of which aquaculture contributes 92% of the production (FAO, 2008). Although tilapia species are receiving great attention as they occupy two different market types, being a main fish food in most Asian and African countries and being a high value fish food in Southern United States (Maclean et al., 2002).The International Commission for Radiological Protection (ICRP) stated that "…if man was adequately protected (from ionizing radiations) other living things (non-human biota) were also likely to be sufficiently protected" (ICRP, 1977). International Atomic Energy Agency and International Commission on Radiological Protection (IAEA and ICRP) developed a regulatory frame work for protecting the non-human biota from the ionizing radiation released from various sources like nuclear power plants, industries, etc. (ICRP 2007)**.**

Radionuclides released from nuclear fuel cycles are incorporated into the biogeochemical cycles of freshwater systems, which enter into aquatic systems either by direct liquid discharges from industries or by secondary processes such as erosion, runoff and groundwater infiltration from landscapes **(**Anbumani and Mohankumar, 2012). Depending upon the element and the chemical form, radionuclides accumulated in bottom sediment or remain in the water column in dissolved state. From either location, that can subsequently accumulate in biota and be transferred through the aquatic food chain. The aquatic organisms receives external radiations from water and sediment radionuclides; internal radiations from absorbed radionuclides through skin and respiratory organs (Iger et al., 1994; Kilemade and Mothersill, 2001).

Haematological techniques are the most common methods employed to determine the sub-lethal effects of the pollutants (Larsson cl a1., 1985). Blood serves as an important tool for studying the rapid changes in blood parameters of fishes since it is highly susceptible to environmental fluctuations (Pandey and Pandey, 2001). Fish haematology has been an essential tool for the biologist as a frontline sensitive indicator of vital physiological and biological functions as well as status of nutrition, health, diseases, and stress in response to changing environmental conditions (Devi et al., 2004).The aim of the present study is to assess the haematological effects of ^{60}Co gamma irradiation on freshwater fish *O.mossambicus.* To our best knowledge this work is the first in this species by using 60Co gamma radiation.

MATERIALS AND METHODS

Experimental animals

The fresh water fish *O.mossambicus* were collected from Cauvery River, Tiruchirappalli district, Tamilnadu, South India (latitude 11°29'; longitude 79°50'E) Which is relatively free from pollutants and were acclimatized to the Natural pond (Environmental Research laboratory, Jamal Mohamed College, Tiruchirappalli) for 30 days and transferred into laboratory condition by using in-door fiber tanks, each with (1.5 m×1 m) adequate aeration. The

water temperature was maintained as 28-32°C, Dissolved O_2 (DO) 3.0mg/l, Carbondioxide 14mg/l, Salinity 0-28ppt, Turbidity 25-100mg/l, pH - 6.0-8.5, Alkalinity 50-700mg/l, Total Ammonia Nitrogen (TAN) - 0.5-1mg/l (APHA, 1998). Commercial fish feed was provided daily at 3% of the body weight.

Lethal Dose (LD50) determination

The acclimatized fishes (330 Nos.) were transported to GVN hospital (at Tiruchirappalli) in aerated polypropylene fiber tanks for gamma irradiation process. One group was acted as control (n=10) and the others (three replicates/group) were exposed to 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50Gy gamma radiations respectively from Theratron Phoenix [P-33] Tele-Cobalt unit having a dose rate of 360mGy/min. After irradiation, fishes were returned to indoor polypropylene fiber tanks in the laboratory. The fishes were closely observed for a period of 96 h (every 6 h). The collected datas were analyzed further by probit analysis.

Irradiation process

The acclimatized *O. mossambicus* (180 Nos.) fishes were collected from fibre tanks and placed in five separate polypropylene rectangular tubs (Nos 30 each). The dimensions of boxes were 0.50 × 0.10m (L×B×H) of capacity 3 L water. Five tubs were exposed to five different dose rates of 3, 30, 300, 3000 and 30000mGy with Theratron Phoenix [P-33] Tele Cobalt unit having a dose rate 360mGy/min (Table 1). A control group (n=30) was maintained separately. In order to take the geometrical consideration, a TLD (Thermoluminescence disk) was put in a tubs along with fishes (each box contains three different TLD disk). Dose received by the TLD is measured subsequently.

Blood Collection and Processing

Blood samples were collected in different time intervals of 12, 24, 48, 72 and 96hrs from randomly selected five fishes for each sampling period (Each group - 25 fishes for 5 sampling periods), totally 150 blood samples were collected by caudal vein puncture technique using a heparinized syringe. Blood was sampled by the following method. The fish were caught very gently using a small dip net, one at a time with least disturbance. Each fish was held and wrapped with a clean, dry towel and the posterior half of its body was blotted with a clean coarse filter paper. The blood samples were collected from individual fishes by caudal vein puncture technique using a heparinized syringe (Fig-1).

Haematological Analyses.

Total RBC Count

Total count of RBC was made using an improved Neubaur haemocytometer (Shah and Altindag, 2004). Blood was diluted 1:200 with Hayem's fluid (Mishra *et al*., 1977). Erythrocytes were counted in the loaded haemocytometer chamber and total numbers were reported (10^6 / mm^3) (Wintrobe, 1967).

Total Count of WBC

Total WBC count was done using an improved nebular haemocytometer (Shah and Altindag, 2005). Blood was diluted 1:20 with Turk's diluting fluid and placed in haemocytometer.4 large (1 sq mm) corner squares of the haemocytometer were counted under the microscope (Olympus) at 640 X. The total number of WBC was calculated in mm^3 $x10^3$. (Wintrobe, 1967).

Estimation of Haemoglobin

Haemoglobin (Hb) was estimated using a haemoglobin test kit (DIAGNOVA, Satan India) by Cyanmethemoglobin method.

RESULTS

Acute lethal studies

Lethal dose of *O. mossambicus* was determined by probit analysis. The ANOVA results showed the percentage of mortality depended on the dosage of radiation. There was a significant difference ($p<0.05$) between the dosage and the mortality. LD_{50} of gamma irradiated *O. mossambicus* was identified at 40Gy (Fig. 2).

RBC count (10^6/cumm)

The erythrocytes of *O. mossambicus* due to the exposure of different dose level of 60 Co (3, 30, 300, 3000mGy and 30000mGy) in different blood sampling intervals (12, 24, 48, 72 and 96hrs) were analyzed and recorded .The control group showed normal range of RBC count it was 1.81±0.07, 1.8±0.11, 1.83±0.07, 1.79±0.10 and 1.84±0.12 in the 12hr, 24hr, 48hr,72hr and 96hr respectively.The RBC count in all the five treatment groups of fish was found to be less than the RBC count of the control groups. (Tables 2, Fig3). The RBC count was found to be 1.79±0.07 in fish treated with 3mGy sampling intervals 12hr and it was 1.74±0.08, 1.69±0.07, 1.62±0.10, and 1.56±0.08 in the 24hr, 48hr,72hr and 96hr respectively .In the case of fish treated with 30mGy sampling intervals 12hr it was found to be 1.61±0.07 and 1.53±0.08, 1.51±0.07, 1.45±0.05 and 1.41±0.10 in the 24hr, 48hr,72hr and 96hr respectively .

The RBC count of the fish treated with 300mGy sampling intervals 12hr was found to be1.54±0.05 , while it was 1.49±0.07, 1.42±0.08, 1.37±0.07 and 1.35±0.07 in the 24hr, 48hr,72hr and 96hr respectively . RBC value of fish exposed with 3000mGy sampling intervals 12hr was 1.44±0.07 while it was 1.41±0.07, 1.36±0.07, 1.35±0.05 and 1.27±0.07 in the 24hr, 48hr,72hr and 96hr respectively.In the case of fish treated with 30000mGy sampling intervals 12hr it was 1.24±0.07, while in the 24hr, 48hr,72hr and 96hr group it was 1.17±0.07 , 1.07±0.15, 0.99±0.14 and 0.85±0.15 respectively.

The difference in RBC value of treated fish was very significant ($p < .001$) when compared with the respective controls. The data clearly indicated that the exposed fresh water fish had decreased RBC values to increased dose levels and increase sampling intervals.

WBC count (10^3/cumm)

The normal range of WBC count for the control fish *O.mossambicus* was recorded as 28.43±0.78, 28.66±0.66, 28.65±1.05, 28.40±0.84 and 28.65±0.98 in the 12hr, 24hr, 48hr,72hr and 96hr respectively.The WBC count in case of treated as well as control groups of fish were presented in (Tables 3, Fig4). The WBC count in all the five treatment groups of fish was found to be higher than the WBC count of the control groups.The WBC count was 30.21±1.72 in fish treated with 3mGy sampling intervals 12hr and it was 31.08±0.89, 33.68±1.48, 36.38±1.04 and 37.67±1.78 recorded in the 12hr, 24hr, 48hr,72hr and 96hr sampling intervals respectively.The WBC count of fish treated with 30mGy sampling intervals 12hr was 31.62±1.74 and it was 34.08±0.96, 36.65±1.18, 38.54±0.79 and 39.91±1.098 in the 12hr, 24hr, 48hr,72hr and 96hr sampling intervals respectively.In case of fish exposed with 300mGy sampling intervals 12hr it was 34.91±1.55 , while in the 24hr, 48hr,72hr and 96hr group it was 37.06±1.08, 38.37±0.95, 40.37±1.19 and 42.52±1.38 respectively.In the fish exposed to 3000mGy sampling intervals 12hr was 39.73±1.80 while in the 24hr, 48hr,72hr and 96hr it was 43.88±1.17, 46.05±0.97, 48.84±0.70, and 51.8±2.03 respectively.The WBC was 50.05±1.69 in the fish treated with 30000mGy sampling intervals 12hr and 51.89±0.97, 53.42±0.69, 56.11±1.12, and 62.1±1.79 in the 24hr, 48hr,72hr and

96hr respectively.The difference observed was found to be highly significant (p<.001) when compared with the respective controls. The data clearly indicated that the exposed fresh water fish had increased WBC values to increased dose levels and increased sampling intervals.

Haemoglobin (Hb) (g/dl)

The Hb value in control group was 6.75±0.48 to 6.77±0.14 .The Hb value in all the five different dose level of fish was found to be higher than the control groups (Tables 4, Fig5). The Hb value was 7.20±0.22 in fish treated with 3mGy sampling intervals 12hr 60co exposure while it was 7.54±0.28, 7.71±0.34, 8.15±0.17,and 8.64±0.22 in the 24hr, 48hr,72hr and 96hr respectively.Hb value of fish exposed with 30mGy sampling intervals 12hr was 8.27±0.29 while it was 8.45±0.41, 8.94±0.32, 9.13±0.15,and 9.38±0.12 in the in the 24hr, 48hr,72hr and 96hr respectively.In the fish exposed to 300mGy sampling intervals 12hr was 8.98±0.32 while in the 24hr, 48hr,72hr and 96hr it was 9.21±0.16, 9.21±0.16, 9.46±0.14, 9.95±0.13 and 10.11±0.14 respectively.The Hb was 9.27±0.39 in the fish treated with 3000mGy sampling intervals 12hr and 9.66±0.14, 9.87±0.09, 10.26±0.09, and 10.89±0.10 in the 24hr, 48hr,72hr and 96hr respectively.In case of fish treated with 3000mGy sampling intervals 12hr it was 9.71±0.19, while in the 24hr, 48hr,72hr and 96hr group it was 10.21±0.39, 10.32±0.11, 10.73±0.19 and 11.27±0.13 respectively.

The difference in Hb value of treated fish was very significant (p < 001) when compared with the respective controls. The data clearly indicated that the treated fish had increased Hb values to increased dose levels and increased time intervals after exposure.

DISCUSSION

Fish blood reflected pathophysiological status and its parameters were important in diagnosis of the structural and functional status of fish exposed to toxicants Sampath, K *et al*. (1998). In the control and irradiated group(3, 30, 300, 3000mGy and 30000mGy) haematological parameters were observed at regular time intervals of 12, 24, 48, 72 and 96hrs and recorded (Table 2,3,4 and 5). In this study, RBC count significantly decreased and WBC, Hb, increased significantly especially in high doses. The increased time duration after irradiation also showed the decreased RBC count, WBC, and Hb, count increased.

Red blood cells are the most abundant cells in fish blood. They contained haemoglobin which in turn helped in carrying oxygen from gills to the different body parts (Johal and Grewal, 2004). The present study clearly indicated that the RBC count decreased significantly on exposure to radiation. Such a situation can be an indicator for haemolytic anaemia, as was found in some fish species exposed to paraquat (Salazar-Lugo, 2007). Haemolytic anaemia is a genetic and molecular disease and had also previously been seen in fish in anoxic and low pH conditions previously. This disorder caused rupture of the erythrocytes, and an increase of free haemoglobin in blood, and damage in the tissues and organs of the fish, and death, if the condition continued (Hárosi et al., 1998; Pia Koldkjær and Berenbrink, 2007). Therefore, haemolytic anaemia was reported to be an important parameter in the evaluation of fish health (Hárosi et al., 1998). Decreased value of RBC count observed when O. mossambicus was subjected to varying sub lethal concentrations of ethanol for 21 days suggested an anaemic condition occurring in the ethanol treated *O. Mossambicus.* In another study Wahbi et al., (2004) and Zaki et al., (2008) reported the decrease in the RBC that results in severe anaemia in fish exposed to heavy metals and herbicide respectively.

Leucocytes or white blood cells (WBCs) are the defence cells of the body which provide protection to the organism against environmental as well as anthropogenic stress. Total number of leucocytes per cubic millimetre (TLC) is a diagnostic feature of many diseases. The present study clearly indicated that the WBC count increased significantly on exposure to radiation. During exposure period WBC counts got enhanced, indicating that the fish can develop a defensive mechanism to overcome the toxic stress. Our studies were in agreement with Lovell and Jantrarotai,(1991); Nanda, (1997); Wahbi, (1998); Hymavathi and Rao, (2000); Lebelo, et al.,(2001); Hassen, (2002) and Joshi, et al., (2002).

Taofik at al. (2008) also observed a similar pattern in the cat fish and rat when exposed to increased crude oil concentration. In another studies Buthelezi, 2000; Nussey et al., 2002 reported that increase in WBC count might be as a result of the prevention of damage caused by zinc in the gill, kidney, and liver tissues.

The Hb value in all the five treatment groups of fish was found to be significantly higher ($p<.001$) than the control groups which might be attributed to the fact that the oxygen carrying capacity of the fish was affected by the radiation. Similar results were reported in Common carp (*Cyprinus carpio*) exposed to diazinon (Ahmed 2011). Due to an insufficient supply of oxygen, respiration was not maintained efficiently. As a result, the demand for hemoglobin content increased. Hence, the increase in Hb values could be interpreted as a compensatory response that improved the O2 carrying capacity to maintain the gas transfer. Eddy and Morgan (1969) observed a mean increase in the Hb concentration from 5.3 to 7.6 g/100ml between control rainbow trout and a group acclimatized to high levels of free carbon dioxide.

The present study revealed that ^{60}Co gamma radiation exposure to fish *O. mossabicus* to dose levels of 3, 30, 300, 3000 and 30000mGy showed effect on fish blood parameters in different time durations (12, 24, 48, 72 and 96hrs) after irradiation. Since blood was the most important body fluid, various physiological changes occurring in the body due to the toxicant would be reflected in the blood. Hence even slight variations in the aquatic environment might be reflected in the fish blood thus making it a sensitive indicator of pollution.

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Table.1. TLD dose measurement; each box contains three TLDs (Thermoluminescence disk).

	TLD Dose (mGy)			
	1	**2**	**3**	**Average dose**
Tube 1	3.1±0.30	2.9±0.29	3.0±0.28	3 ± 0.29
Tube 2	30.0±1.3	31.1±1.2	28.9±1.1	30 ± 1.2
Tube 3	310.6±1.01	300± 1.04	289.4±1.05	300 ± 1.03
Tube 4	2995.4 ± 0.12	3007.5 ± 0.15	2997.9 ± 0.11	3000 ± 0.12
Tube 5	30920.11 ± 0.31	30190.05 ± 0.17	28890.4 ± 0.6	30000 ± 0.36

Table 2. Changes in the RBC,s count of *Oreochromis mossambicus* exposed to different doses of ^{60}Co gamma radiation (RBC10^6/mm^3).

	12hr	**24hr**	**48hr**	**72hr**	**96hr**
Control	1.81±0.07	1.8±0.11	1.83±0.07	1.79±0.10	1.84±0.12
3mGy	1.79±0.07	1.74±0.08	1.69±0.07	1.62±0.10	1.56±0.08
30mGy	1.61±0.07	1.53±0.08	1.51±0.07	1.45±0.05	1.41±0.10
300mGy	1.54±0.05	1.49±0.07	1.42±0.08	1.37±0.07	1.35±0.07
3000mGy	1.44±0.07	1.41±0.07	1.36±0.07	1.35±0.05	1.27±0.07
30000mGy	1.24±0.07	1.17±0.07	1.07±0.15	0.99±0.14	0.85±0.15

Table 3. Changes in the WBC count of *O. mossambicus* exposed to different doses of ^{60}Co gamma radiation.

	12hr	24hr	48hr	72hr	96hr
Control	28.43±0.78	28.66±0.66	28.65±1.05	28.40±0.84	28.65±0.98
3mGy	30.21±1.72	31.08±0.89	33.68±1.48	36.38±1.04	37.67±1.78
30mGy	31.62±1.74	34.08±0.96	36.65±1.18	38.54±0.79	39.91±1.098
300mGy	34.91±1.55	37.06±1.08	38.37±0.95	40.37±1.19	42.52±1.38
3000mGy	39.73±1.80	43.88±1.17	46.05±0.97	48.84±0.70	51.8±2.03
30000mGy	50.05±1.69	51.89±0.97	53.42±0.69	56.11±1.12	62.1±1.79

Table 4. Changes in the haemoglobin count of *Oreochromis mossambicus* exposed to different doses of ^{60}Co gamma radiation.

	12hr	24hr	48hr	72hr	96hr
Control	6.75±0.48	6.82±0.31	6.71±0.44	6.71±0.41	6.77±0.14
3mGy	7.20±0.22	7.54±0.28	7.71±0.34	8.15±0.17	8.64±0.22
30mGy	8.27±0.29	8.45±0.41	8.94±0.32	9.13±0.15	9.38±0.12
300mGy	8.98±0.32	9.21±0.16	9.46±0.14	9.95±0.13	10.11±0.14
3000mGy	9.27±0.39	9.66±0.14	9.87±0.09	10.26±0.09	10.89±0.10
30000mGy	9.71±0.19	10.21±0.39	10.32±0.11	10.73±0.19	11.27±0.13

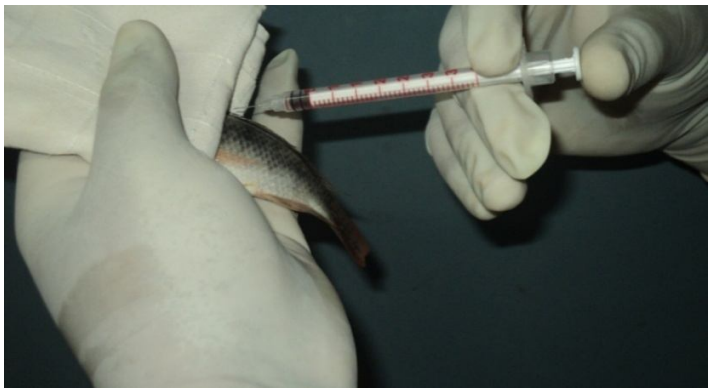

Figure 1: Blood Collection of *O. mossambicus*

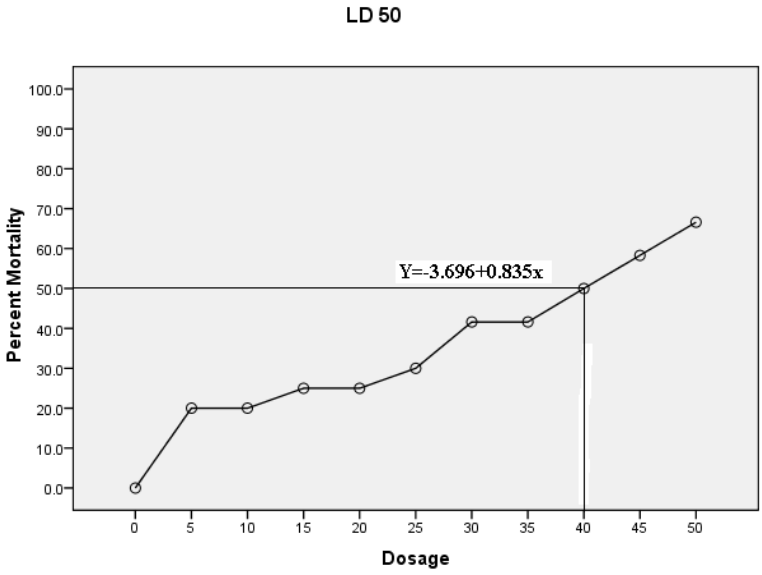

Figure 2: Probit analysis graph showing LD_{50} in *O.mossambicus*

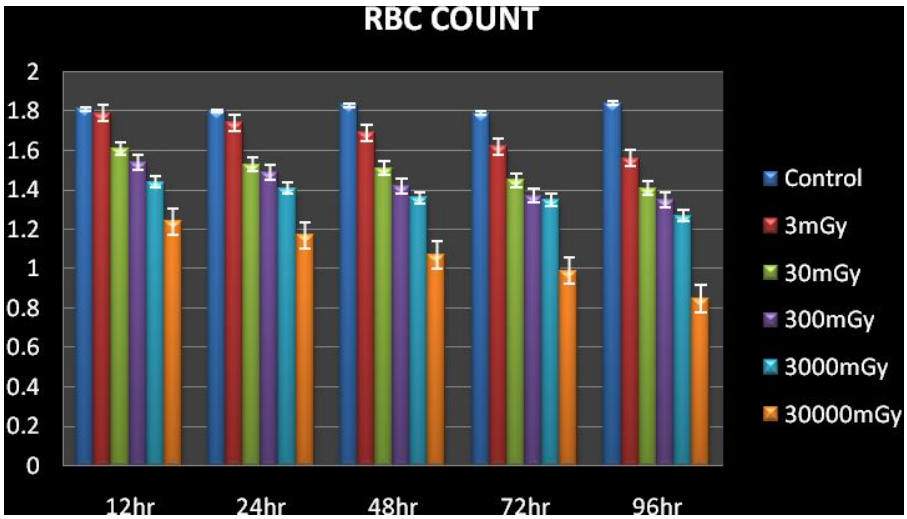

Figure 3: RBC,s count of *O. mossambicus* exposed to different doses of ^{60}Co gamma radiation (RBC106/mm3).

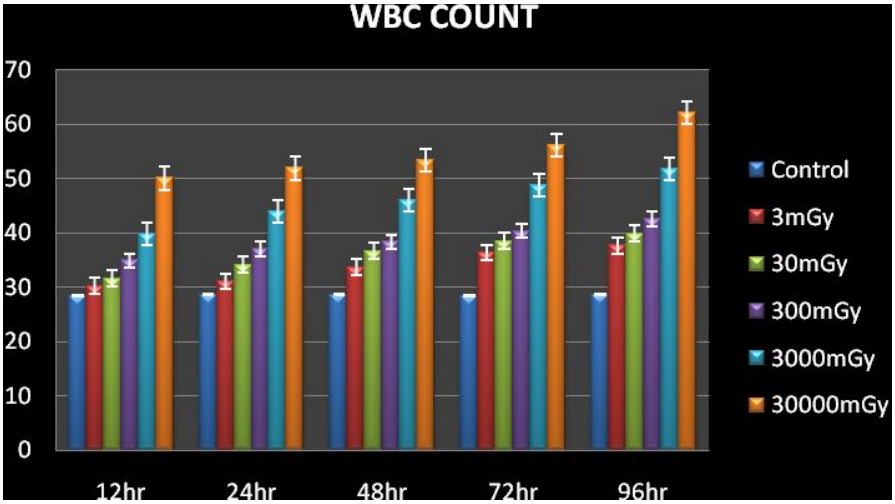

Figure 4: WBC count of *O. mossambicus* exposed to different doses of ^{60}Co gamma radiation.

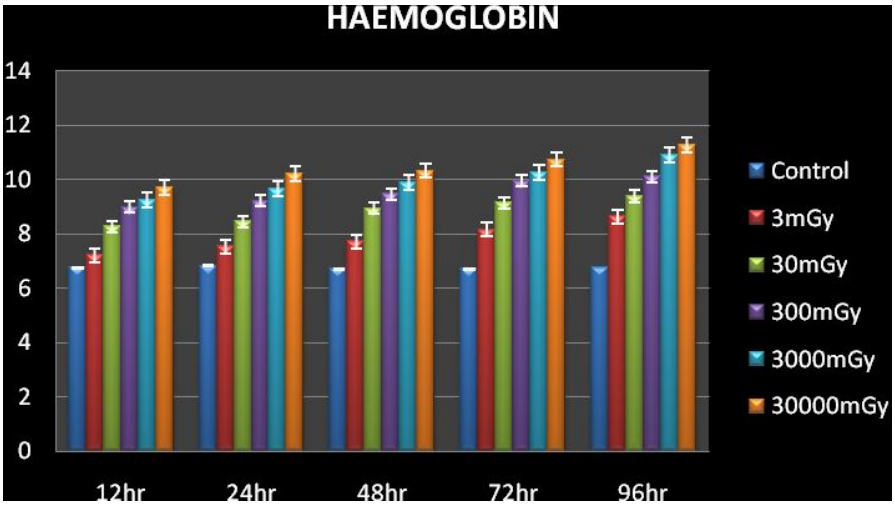

Figure 5: Haemoglobin count of *O. mossambicus* exposed to different doses of ^{60}Co gamma radiation.

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RESEARCH ARTICLE

The Candidate Gene *SPINK5* Association with Summer Eczema in Swedish Horses.

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ABSTRACT

Summer eczema insect bite hypersensitivity reaction is caused by the biting of *Culicoides* sp. midges during the summer season. *SPINK5* gene had been selected as the candidate gene, since the polymorphism in *SPINK5* is associated with atopic phenotypes in humans. PCR based pyrosequencing method were done to genotyping the sick and healthy horses. Forty eight samples were screened, of this 37 were sick and 11 were healthy. Two SNPs were used for the association study of *SPINK5* and summer eczema.

Key words: Summer eczema, SPINK5 gene, SNPs genotyping and pyrosequencing

INTRODUCTION

Summer eczema or allergic dermatitis is caused by allergic reaction to the bite of *Culicoides* sp. Midges, has many local names like Queensland itch, Mexican itch, kasen (Japan), dhobie itch and Spanish itch. The symptoms include intense pruritis and secondary wound infection mainly localized to the mane, tail and withers (Barbet J 1992 & Scott DW 2003). It has a world-wide distribution and several well known breeds of horses were suffering from this disease.

In Sweden, many breeds were affected they include Shetland pony, New forest, Gotland pony, Shire and Icelandic horses. The prevalence of disease is more during the summer season depending upon the presence of the insect. There is no cure; prevention is the best method to get rid of this disease. To avoid the biting of midges so many farmers were keeping their animals in barn during dawn and dusk when the midges were very active. Summer eczema brings huge financial burden to the farmers in terms of treatment and nursing.The disease may be multifactorial with many factors and the environment, epigenetics and genetics etc can play an important role in the manifestation of the disease. Recently many studies were conducted in Shetland pony and Icelandic horses shows

that insect bite hypersensitivity is a heritable disease. Similar symptoms are observed in different species include sheep, cat, dog, camel and human known as Atopic Dermatitis (AD) (Branden & Andersson, 2004).

Serine protease inhibitor Kazal-type 5 (SPINK5) is responsible for Netherton syndrome (Chavanas *et al* 2000), a rare recessive skin disorder that is accompanied by atopy (Smith *et al* 1995). The SPINK5 gene comprises 33 exons and spans approximately 61 kb on human chromosome 5q32. SPINK5 is involved in regulation of proteolysis in epithelia formation and keratinocyte terminal differentiation, and some mutations in the SPINK5 gene cause defects in the skin barrier. Mutation in SPINK5 gene in human causes Netherton's syndrome condition which is similar to eczema. SPINK5 SNPs have been linked with Atopic dermatitis and high total IgE in two Japanese populations (Chavanas *et al* 2000 & Nishio 2003). In the current study SPINK5 is selected as the candidate gene and two SNPs is used genotype to check if there is any difference in allele frequency of sick and healthy horses

A new sequencing methodology called pyrosequencing has been used successfully for confirmatory sequencing and de novo sequencing. This technique has higher accuracy, flexibility and can be easily automated furthermore labelled primer and nucleotides are not used. Pyrosequencing is widely employed for genotyping and resequencing of diseased genes. Pyrosequencing is a faster way to analyze SNPs than traditional sequencing procedure (Brown, 2002).

MATERIALS AND METHODS

Genomic DNA was extracted from whole blood in thirty seven sick and eleven healthy horses which were used as template in PCR amplification. Primers were designed to amplify 50 bp products by using web based primer 3 software and the primers are (Forward 5'TTTCTCCAGAAGCGGAAATC3' and Reverse 3'CACAGGAGAAAAGCGTAGGG5'). A 25 ul reaction was set up as table 1.

Touch-down PCR amplification was performed with the following conditions: initial denaturation for 10 minutes at 94° C, 11 cycles of 30 sec 94° C, 30 sec at 65° C lowering 1° C each cycle and 1 min 72° C, followed by 27 cycles under same conditions expect a constant annealing temperature of 54°C. The PCR amplified products of 50 bp were detected using 1.5% agar gel electrophoresis. Electrophoresis was carried out at 140V for 20 minutes at room temperature until the dye migrate more than two-third of the length of the gel. The gel was visualized under a Gel Documentation system

PCR product was sequenced using the biotinylated PCR primers (F(83) 5'Bio-CAGGTAAGTTG ACAGTGTA AT TGCT3' and R(83) 3'CATTTAGCACTTTTCTACTCTTTAATC5' and F(21)5'TGTTCAGAATTGA AGAA ATA CTTGG3' R(21)5'Bio-GGCTTGGCATTTGATTTTTC') and analysed. PCR plate containing the 25ul PCR product to which 40ul of sepharose beads with BW buffer and 15ul water was added, kept this reaction mixture in a shaker for 20 minutes. Followed by cleaning the vacuum tool by passing 70% ethanol, 0.2 NaOH and washing buffer. Released the sepharose bead into wells containing annealing buffer and sequencing primer. The reactions were carried out in a PSQ HS 96A instrument (Amersham Biosciences).

RESULTS AND DISCUSSION

The PCR products from the two reactions were analyzed in the agarose gel, faint band were observed in the first reaction while in the second no bands were observed. The absence of band can be due to the small product size or over running of the PCR product. However we planned to proceed further with pyrosequencing the success rate varied in two reactions. In the first reaction 17 samples showed the positive result while the rest 31 samples were failed and in the second reaction only 7 samples were passed rest 41 samples were failed we speculated that it may be

due to pipetting error. In this experiment very few animals were screened so it is difficult to draw any conclusive result hence further investigation has to be done with large sample.

The *SPINK5* exons follow a specific pattern unevenly numbered exons have the same length with exception of first and last two they all code for the inhibitory parts of the fifteen domains in *LEKTI* which is believed to be evolved from multiple duplications. While the even numbered exons are shorter and code for amino acids that links the inhibitory elements together. Therefore a SNP located in the uneven exon would have a greater impact on protein function than in even numbered exon. Many researches in humans had already shown that atopic dermatitis has a genetic background, so SPINK5 has been selected as the candidate gene (Bitoun *et al.,* 2002). Summer eczema in horses is considered to be a complex disease, involve the interaction of many genes and environment better option is positional candidate gene approach rather than the candidate gene approach.

SUMMARY

Summer eczema in horses is caused by the hypersensitivity reaction, reaction of saliva from various species of biting midges of the genus *Culicoides.* SPINK5 gene is associated with Atopic dermatitis in human and has effect on immunological status of skin so they have been selected as the candidate gene in horses. PCR based pyrosequencing technique was used for genotyping the sick and healthy horses. In this study two SNPs were used but the success rate was low however we got different genotypes in both sick and healthy individuals. Further investigation has to be done in this field with more SNPs and samples to obtain any association.

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Table 1: Reaction mixture

Content	Quantity
Forward primer (10pmol/ul)	0.3ul
Reverse primer (10pmol/ul)	0.3ul
MgCl2 (1,5mM)	2.5ul
PCR buffer (10X)	2.5ul
Taq Polymerase (5U/ul)	0.3ul
dNTP (20mM)	0.3ul
DNA (~20ng/ul)	2ul
H2O	16.8ul
Total	25ul

Table 2. The result of 2 SNPs shown below

Health Status	Spink 21 AT	Spink 21 AA	Spink9(83)GA	Spink9(83)GG
Sick	4	5	2	3
Healthy	5	3	2	-

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RESEARCH ARTICLE

Influence of High Environmental Temperature on Serum Biochemical Parameters of Four Chicken Varieties.

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ABSTRACT

Heat stress is one of the most important issues affecting poultry production in tropical countries like India. Although the production performances and immune responses of chicken under elevated temperature were studied, knowledge about the biochemical parameters is limited only. In the present investigation, we studied the effect of high temperature on some of the biochemical parameters of four different chicken varieties. Eighty birds from each group were subjected to heat treatment by 45^0 C daily 2 hours for two weeks period (fifth and sixth week) of age. After sixth week, serum was collected and different biochemical parameters like, total serum protein, Serum glutamic oxaloacetic transaminase (SGOT), Serum glutamic pyruvic transaminase (SGPT), Potassium (K^+) and Sodium (Na^+) were estimated. We didn't find significant variations in these parameters under elevated temperature. The study also demonstrated less susceptibility of broilers to heat stress than other chicken varieties. The results of present investigation will help to formulate a suitable breeding plan for poultry production in tropical countries.

Key words: Biochemical parameter, Broiler, Layer, Serum glutamic oxaloacetic transaminase, Serum glutamic pyruvic transaminase.

INTRODUCTION

High ambient temperature and humidity are the major stress factors affecting poultry during summer in tropical countries like India. The high temperature result in heat stress and severity of this is dependent on the strain, feathering, and nutrition and production system. Absences of heat dissipating mechanism i.e. sweat glands and high body temperature (41^{o}C) markedly increases bird's susceptibility to heat stress. The major effects of heat stress are increased mortality, reduced feed intake and lower weight gain or egg production [1]. Exposure of broiler chickens to high ambient temperatures caused a series of physiological changes such as elevated body temperature and metabolic status elicited by decreased levels of plasma triiodothyronine [2].

Birds can dissipate heat via the respiratory system during heat stress i.e., panting. This is the major mechanism of cooling in birds. However, severe panting may result in respiratory alkalosis and acid-base balance disturbances in birds. A significant change in mineral balance was observed in connection with respiratory alkalosis, particularly negative balance of K^{+} ions and other electrolytes [3]. The production performance is affected by imbalanced biochemical parameters. However, reports are limited about the biochemical parameters of chicken in relation to heat stress. Therefore, objective of the present investigation was to assess the biochemical parameters of different chicken varieties under elevated temperature.

MATERIALS AND METHODS

Experimental birds

The experiment was carried at the institutional poultry farm, Veterinary College and Research Institute, Namakkal, India. The average minimum-maximum temperature of this area was varied from 16-24^{0}C and 30-35^{0}C, and the average minimum-maximum relative humidity is in the range of 25-53% and 62-83%. Four different chicken varieties viz., commercial broiler (Cobb 400), commercial layer (Hy-Line), Namakkal Chicken-1 (cross bred) and Aseel (pure indigenous breed) were included in this study. Hatching eggs were purchased from different commercial farms and were incubated together as per the standard procedure in order to give same environmental condition even during embryonic stage itself. The study was conducted from day old to 6 weeks of age. Eighty birds from each variety were randomly selected for study purpose. All the experimental birds were reared under same environmental conditions up to fourth week of age. After four weeks, 50% of the birds were exposed to heat stress. Briefly, birds were subjected to increased heat treatment i.e., 45^{0} C daily, 2 hours for two weeks period (fifth and sixth week) using artificial heating systems.

Preparation of serum

At the end of the experiment (after 6 weeks), one male and one female, totally eight birds from each variety were randomly selected and subjected to slaughter. Sufficient quantity of blood was collected into 15 ml tubes from each bird. Blood was allowed to clot for 2-3 h at room temperature then left overnight at 4^{0} C. Next day, serum was harvested by centrifugation at 3000 rpm for 20 minutes. The serum samples were stored at -20^{o}C in sterile microcentrifuge tubes without any preservative till further use.

Serum biochemical parameters

The serum samples were used to estimate different biochemical parameters like Serum glutamic oxaloacetic transaminase (SGOT/AST), Serum glutamic pyruvic transaminase (SGPT/ALT), Total protein, Potassium (K^{+}) and Sodium (Na^{+}). SGOT and SGPT were analysed using modified Reitman & Frankel's colorimetric- DNPH method. The

total protein was estimated by modified Biuret End point assay. The Colorimetric assay was used for estimation of K^+ and Na^+.

RESULTS AND DISCUSSION

Total serum protein

The elevated temperature treatment did not influence the serum protein level significantly (Table 1). In all the varieties, male birds were having higher protein level than the respective females at high temperature. Layer males had a significantly *(P<0.05)* higher serum protein than broilers at normal temperature. Our study demonstrated a numerically higher value of total protein in layers at elevated temperature compared to other varieties. The heat stress resulted in a significant (*P<0.05)* reduction in protein concentration of broilers than layers. Different studies reported varying levels of plasma protein concentration due to heat stress. Khan et al. (2002) [4] mentioned a noticeable decrease in the amount of protein under elevated temperature (40-45^0 C) as compared to the control. Similarly, the study by Zhou et al. (1999) [5] showed decreased plasma protein concentration during heat exposure. However, Yahav et al. (1997) [6] reported significantly increased plasma protein concentration at 35^{0}C.

Serum SGOT (AST)

We studied the effect of temperature on mean serum SGOT/AST level (U/L) of different chicken varieties at 6th week of age (Table 2). The result showed elevated temperature had no significant effect on serum glutamic oxaloacetic transaminase (SGOT) level. Males and combined sexes of broilers had higher values, when compared to other three varieties. Our study is in agreement with the findings of Hartlova et al. (2002) [7], who demonstrated heat stress had no effect on serum AST.

Serum SGPT (ALT)

There was no significant difference in SGPT level between the chicken varieties under elevated temperature (Table 3). Our finding is accordance with Hartlova et al. (2002) [7]. Ozbey et al. (2004) [8] also reported no change in serum SGPT level of Japanese quails under heat stress.

Serum sodium (Na^+) and potassium (K^+)

No significant changes were observed in serum Na^+ (Table 4) and K^+ (Table 5) levels due to heat treatment. However, the male birds showed more reduction in concentration of K^+ than females under high temperature. All the varieties except layer showed a numerical higher Na^+ level under the elevated temperature. However, Toyomizu et al. (2005) [**9**] reported that plasma K^+ concentration was increased at elevated temperature. The same study showed plasma Na^+ concentration was constant in both control and heat treatment groups. High ambient temperature significantly reduced plasma Na^+ and K^+ levels in heavy broiler chicken (Olanrewaju, et al., 2010) [10]. Similar trends were also observed in the study of Khan et al. (2002) [4].

In conclusion, we studied various serum biochemical parameters of different chicken varieties. Although, the elevated temperature caused reduction in production parameters (unpublished data), much variation in the biochemical parameters of four chicken varieties was not observed. The study also demonstrated less susceptibility of broilers to heat stress than other chicken varieties. The results of present investigation will help to formulate a suitable breeding plan for poultry production in tropical countries.

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Table 1. Effect of temperature on mean total serum protein level (g/l) (±S.E.) of different chicken varieties at 6th week of age

Birds (n=32)	Treatment	Broiler	Layer	Crossbred	Aseel
Male	ET	31.6±4.8^{ab}	37.0±2.8^{ab}	33.4±2.3^{ab}	30.3±6.4^{ab}
	AT	22.7±1.6^{b}	44.1±6.4^{a}	29.1±2.9^{ab}	34.7±2.1^{ab}
Female	ET	27.2±1.8^{a}	37.0±3.4^{a}	29.1±2.9^{a}	27.2±1.6^{a}
	AT	25.8±2.1^{a}	31.4±2.6^{a}	33.0±3.6^{a}	32.5±2.1^{a}
Total birds	ET	24.2±1.4^{b}	37.0±2.0^{a}	31.3±1.9^{ab}	28.7±3.1^{ab}
	AT	24.2±1.4^{b}	37.7±3.9^{a}	31.1±2.2^{ab}	33.6±1.5^{ab}

ET- elevated temperature, AT- ambient temperature. $^{a-b}$ Means within a row of respective category with no common superscript differ significantly (*P<0.05*)

Table 2. Effect of temperature on mean serum SGOT level (U/L) (±S.E.) of different chicken varieties at 6th week of age.

Birds (n=32)	Treatment	Broiler	Layer	Crossbred	Aseel
Male	ET	36.0±5.8[a]	12.3±1.0[a]	17.6±2.5[a]	22.9±8.4[a]
	AT	29.0±13.6[a]	11.4±3.0[a]	15.0±3.3[a]	14.1±2.5[a]
Female	ET	31.7±4.3[a]	19.3±6.8[a]	18.4±4.3[a]	13.2±2.6[a]
	AT	36.0±18.0[a]	23.7±10.1[a]	13.2±5.2[a]	16.7±3.0[a]
Total birds	ET	33.8±3.4[a]	15.8±3.5[a]	18.0±2.3[a]	18.0±4.5[a]
	AT	32.5±10.5[a]	17.6±5.4[a]	14.1±2.9[a]	15.4±1.9[a]

ET- elevated temperature, AT- ambient temperature. [a-b] Means within a row of respective category with no common superscript differ significantly (*P<0.05*)

Table 3. Effect of temperature on mean serum SGPT level (U/L) (±S.E.) of different chicken varieties at 6th week of age.

Birds (n=32)	Treatment	Broiler	Layer	Crossbred	Aseel
Male	ET	7.7±2.7	11.6±2.7	7.7±1.6	6.8±1.9
	AT	8.7±1.9	6.8±1.0	5.8±1.1	4.8±1.0
Female	ET	7.7±2.7	7.7±1.6	6.8±1.9	8.7±4.8
	AT	5.8±1.1	5.8±1.9	6.8±1.9	6.8±1.9
Total birds	ET	7.7±1.8	9.7±1.6	7.2±1.1	7.7±2.4
	AT	7.2±1.1	6.3±1.0	6.6±1.24	5.8±1.0

ET- elevated temperature, AT- ambient temperature

Table 4. Effect of temperature on mean serum sodium (Na^+) level (mmol/l) (±S.E.) of different chicken varieties at 6th week of age.

Birds (n=32)	Treatment	Broiler	Layer	Crossbred	Aseel
Male	ET	146.5±1.4[a]	146.5±2.9[a]	143.9±1.2[a]	145.3±2.6[a]
	AT	148.2±1.4[a]	147.1±0.8[a]	143.9±3.3[a]	138.2±3.8[a]
Female	ET	150.3±4.1[a]	147.1±1.6[a]	144.2±1.0[a]	143.0±1.9[a]
	AT	145.0±2.1[a]	148.8±3.4[a]	142.4±2.4[a]	139.8±2.5[a]
Total birds	ET	148.4±2.1[a]	146.8±1.5[ab]	144.0±0.7[ab]	142.9±1.6[ab]
	AT	146.6±1.3[ab]	148.0±1.6[a]	143.1±1.9[ab]	139.9±2.1[b]

ET- elevated temperature, AT- ambient temperature. [a-b] Means within a row of respective category with no common superscript differ significantly (*P<0.05*)

Table 5. Effect of temperature on mean serum potassium (K^+) level (mmol/l) (±S.E.) of different chicken varieties at 6th week of age.

Birds (n=32)	Treatment	Broiler	Layer	Crossbred	Aseel
Male	ET	3.0±0.3^{ab}	2.5±0.4^{b}	2.6±0.7^{b}	2.4±0.3^{b}
	AT	6.2±1.9^{a}	4.1±0.5^{ab}	4.2±0.4^{ab}	3.1±0.3^{ab}
Female	ET	3.4±0.4^{a}	2.6±0.4^{a}	6.4±2.8^{a}	2.7±0.4^{a}
	AT	3.4±0.3^{a}	3.8±0.1^{a}	4.5±0.3^{a}	2.8±0.2^{a}
Total birds	ET	3.2±0.2^{a}	2.5±0.2^{a}	4.5±1.5^{a}	2.6±0.2^{a}
	AT	4.8±1.0^{a}	4.0±0.2^{a}	4.3±0.2^{a}	2.9±0.2^{a}

ET- elevated temperature, AT- ambient temperature. $^{a-b}$ Means within a row of respective category with no common superscript differ significantly (*P<0.05*)

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RESEARCH ARTICLE

Development and Validation of an Indirect IgG ELISA for Detection of Leptospiral Antibodies in Canine, Bovine and Human Sera.

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ABSTRACT

An indirect IgG Enzyme Linked Immunosorbent Assay (ELISA) was standardized using a heat extracted antigen prepared from cultures of *Leptospira biflexa* serovar Patoc. One hundred and four canine sera, 74 bovine sera and 154 human sera samples, collected from different places in Central Kerala were subjected to Indirect IgG ELISA and Microscopic Agglutination Test (MAT) and the results were statistically analyzed. The ELISA detected a prevalence of 53.84 per cent in dogs, 64.86 per cent in cattle and 62.33 per cent in human beings. The relative sensitivity of ELISA, in detecting leptospiral antibodies, was high when compared to MAT in all the three species. The Indirect IgG ELISA proved to be very effective for rapid screening of the population for leptospirosis.

Key words: Indirect IgG ELISA, Heat extracted antigen, Relative sensitivity, MAT

INTRODUCTION

Leptospirosis is a re-emerging zoonosis, of great public health importance, especially in the tropical third world countries. These countries are endemic for the disease and the human and animal population harbour antibodies to *Leptospira.* Control measures for leptospirosis begin with epidemiological surveillance programmes which include seroprevalence studies in man and animals .Specific IgM antibodies are detected in the sera of patients from the second day of infection and may persist upto 12 months of infection whereas IgG antibodies appear from the seventh day and peaks at second and third months [1]. IgG titres after reaching a peak, decreased much more slowly than IgM titres. Moderately increased IgM titres in conjunction with low IgG titres are found exclusively in the first two months of the disease [2]. Hence detection of IgG antibodies in the sera of these animals indicates present as well as

past infection and hence is useful for epidemiological studies. The ELISA is a highly sensitive serological test for detection of antibodies in human and animal blood. The current study involves the development and validation of an indirect IgG ELISA for detection of leptospiral antibodies in canines, bovines and human beings and comparison of its efficacy with MAT, the gold standard test for leptospirosis.

MATERIALS AND METHODS

The Indirect IgG ELISA was standardised as per [2] with minor modifications. The heat extracted antigen for ELISA was prepared by growing *Leptospira biflexa* serovar Patoc in 100 ml volumes in EMJH liquid media for 10-12 days. The culture was killed with formalin, heated in a boiling water bath for 30 minutes, cooled and centrifuged at 10,000 × g for 30 minutes. The supernatant obtained constituted the heat extracted antigen. The optimum concentration of the heat extracted coating antigen (10^9 leptospires /ml), optimum dilution of antihuman, anti dog and anti bovine IgG HRP conjugates (1:10,000) and optimum test sera dilutions (1:40 for canine and human sera, 1:80 for bovine sera) were arrived at by preliminary checker board titration. Hundred microlitres of the antigen was pipetted into 96 wells of the microtitre plate and left to evaporate for three days at room temperature. Indirect ELISA was carried out on 104 canine sera samples, 74 bovine sera samples and 154 human sera samples. *O*-phenylenediamine dihydrochloride was used as chromogen.. The optical density was read at 492 nm in a Multiscan Ascent ELISA reader and data interpreted as per [3].Microscopic Agglutination Test was performed as per [4] as reported earlier in [5].The results obtained from the IgG ELISA were analyzed for percentage agreement and relative sensitivity and specificity to MAT by Kappa (κ) statistics as per [6].

RESULTS

ELISA

Sera showing OD values above 1.260, 1.264 and 1.240 were taken as positive in dogs, cattle and human beings respectively. The ELISA detected a prevalence of 53.84 per cent in dogs, 64.86 per cent in cattle and 62.33 per cent in human beings (fig 1)**.**

The heat extracted antigen could be stored for more than two weeks at 4^0C without any deterioration in antigenicity. But once coated on to microtitre plates, it could be stored only for a maximum period of one week at room temperature, following which results became aberrant.

MAT

A titre of 1: 80 & above was considered as positive in dog, cattle & human sera. The MAT detected a prevalence of 36.36 per cent in dogs, 47 per cent in cattle and 54.54 per cent in human beings (fig 1)**.**

Statistical Analysis

Percentage of agreement between the two tests showed kappa values above 0.9 ($\kappa > 0.9$) for each species tested. The overall relative sensitivity and specificity of IgG ELISA to MAT was calculated as 94.87 per cent and 70.45 per cent respectively (Table 1a) .The relative sensitivity of IgG ELISA to MAT in dog, cattle and humans were 88.8, 100 and 95.23 per cent respectively. The relative specificity of IgG ELISA to MAT in dog, cattle and humans were 64.70, 68.42 and 77.14 per cent respectively (Table.1b).

Comparison of results of ELISA and MAT

Comparison of results of ELISA and MAT is given in Table.2. Out of the 332, canine, bovine and human sera samples, 148 were tested positive by ELISA and MAT where as 124 sera samples tested negative by the two tests. Eight MAT positive samples tested negative by ELISA while 52 ELISA positive samples tested as negative by MAT. In cattle, none of the ELISA negative sera gave positive results by MAT where as in dogs and humans four of the ELISA negative sera gave positive results by MAT.

DISCUSSION

The present study was carried out to assess the efficacy of an Indirect IgG ELISA in detecting leptospiral infection in human beings and animals. The results of ELISA were compared with MAT (Table.2). The ELISA could detect leptospiral antibodies in 52 sera samples that were negative by MAT while only eight of the ELISA negative sera samples gave positive results by MAT. The heat extracted antigen used in ELISA is reported to contain four antigenic fractions of different specificities. This antigen is also supposed to contain serogroup or type specific fractions apart from the broadly reactive genus specific fractions [2].The MAT negative, ELISA positive sera samples were detected due to the presence of non-agglutinating leptospiral antibodies which were detectable by ELISA and not by MAT, which could detect only agglutinating antibodies [3]. Somatic antigenic components played a role in ELISA while MAT reaction was mainly based on agglutination of surface components present on live organisms [7]. All these factors could have contributed to the better sensitivity of ELISA compared to MAT in this study.

The IgM antibodies to *Leptospira* are produced in the body during acute phase of infection and it is not known for how long these antibodies may persist in the animal's body. Hence those sera which had tested negative by IgG ELISA probably contained IgM antibodies and hence were acute phase sera. Often it is not possible to demonstrate specific IgG antibodies in the first few weeks of infection. [2, 8]. The IgG titres and agglutination titres, after having reached a peak during the second and third months, decreased much more slowly than IgM titres. Hence residual IgG antibodies may persist in the body for a longer time [2] and hence detection of IgG antibodies using ELISA can facilitate seroprevalence studies for epidemiological surveillance [2, 9, 10].

In this study MAT detected leptospiral antibodies in eight of the sera samples which were negative by ELISA. Poor sensitivity and specificity for the IgG ELISA using heat extracted antigen when compared to sonicated antigen and desoxycholate extracted antigen was reported by [11].Fifty two of the MAT negative sera samples were tested positive by ELISA. The MAT was carried out using nine serovars of *Leptospira* and hence it is possible that the infecting serovar had not been included in the battery of leptospiral serovars used as antigen. Nevertheless the possibility that the sera gave false positive results by ELISA due to crossreacting antibodies cannot be ruled out [3]. It has been reported that in many sera, IgG antibodies from long-past infections could still be detected by the homologous antigen but seldom by the heterologous antigens. Therefore, in an epidemiological survey the significance of screening for IgG may lie in detecting serogroup specificity in residual antibodies [2]. The IgG antibody titres more or less paralleled the MAT antibody titres. Hence IgG antibody could contribute more to the MAT than IgM antibodies, though both ELISA and MAT are considered to detect different types of antibodies [10]. Indirect IgG ELISA using different antigenic preparations for detecting leptospiral antibodies have been reported by several authors [12, 13].

The results of the two tests were analyzed by Kappa statistics.The percentage of agreement between MAT and ELISA showed Kappa values above 0.9 for all the species tested. This indicates perfect agreement as per [6].The overall relative sensitivity and specificity of IgG ELISA to MAT was calculated as 94.87 per cent and 70.45 per cent respectively. The relative specificities of IgG ELISA to MAT were similar in dogs, cattle and human beings, ranging from 64 to 77 per cent. The relative sensitivity of IgG ELISA to MAT was 100 per cent in cattle. The high sensitivity of IgG ELISA in cattle shows high prevalence of IgG antibodies against *Leptospira* in sera which indicates chronic

infection or carrier status of the animal. The relative sensitivity of IgG ELISA to MAT in human beings and dogs were 95 and 88 per cent respectively. Hundred per cent sensitivity and 95.6 per cent specificity for IgG ELISA relative to MAT in dogs was reported by [3].The relative sensitivity of MAT was less compared to ELISA in all the species tested while the relative specificity of MAT was more when compared to ELISA in all species tested.

CONCLUSION

The indirect IgG ELISA using heat extracted antigen proved to be a highly effective test for rapid screening of the population for leptospiral antibodies.The antigen was easy to prepare and could effectively detect leptospiral antibodies in canine, bovine and human sera. The ELISA proved to be the more sensitive and less cumbersome when compared to MAT. Hence the indirect IgG ELISA can be routinely employed as a serological test in the epidemiological surveillance programmes for leptospirosis.

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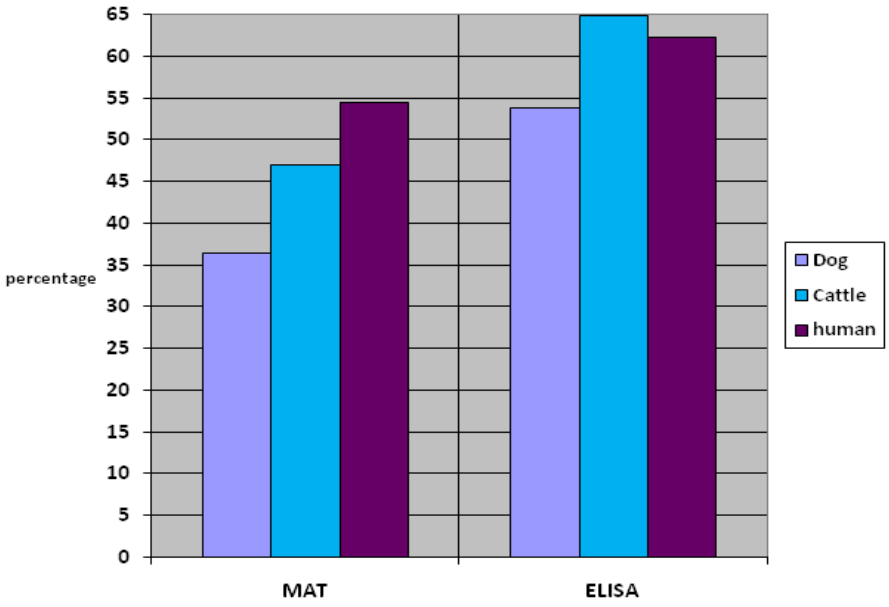

Fig 1.Prevalence of leptospirosis in Dog, Cattle and Man by MAT, and ELISA

Table. 1a. Overall sensitivity and specificity of IgG ELISA to MAT.

Test	Sensitivity(%) of ELISA	Specificity(%) of ELISA
MAT	94.87	70.45

Table. 1b. Relative sensitivity and specificity of IgG ELISA to MAT in dog, cattle and man.

Test	Sensitivity (%)			Specificity(%)		
	Dog	Cattle	Human	Dog	Cattle	Human
MAT	88.8	100	95.23	64.70	68.42	77.14

Table.2 Comparison of results of MAT and ELISA.

Tests	No. of samples			
	Dog	**Cattle**	**Human**	**Total**
MAT+ ELISA+	32	36	80	148
MAT- ELISA-	44	26	54	124
MAT+ ELISA-	4	0	4	8
MAT- ELISA+	24	12	16	52
Total samples tested	104	74	154	332

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RESEARCH ARTICLE

Analyzing the Effective Climatic Components on Outbreak of Skin Leishmaniasis City of Borkhar, Isfahan Province,Iran.

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ABSTRACT

Leishmania is a skindisease generated from a protozoalnamed Leishmania; therefore its scientific term is Leishmaniasis. This disease is categorized in the skin parasite group. The major cores of it in Iran are in Isfahan province are in the cities of Ardestan, Natanz, Kashan, Aranbidgol, Borkhar, Shahinshahr and the city of Isfahan. In this study the objective is to identify some of the geographic components affecting Leishmaniasis in the city of Borkhar. The data here consist of: the number of infected, the plants, and the climatic elements. The Pierson coefficient is generated among the given data through SPSS software. The related geographic map in the GIS software is drawn by applying the IDW method. The subject city has weak vegetation coverage with sporadic pasturelands located in low elevation (semi-desert), a suitable area for promotion of Leishmaniasis. At 27-30°C, 18-25 RH with the lowest precipitation rate the sandfliesswarm the area in the months of August and September in specific. The correlations obtained through SPSS software indicate that there exists anindirect statistical significance between Leishmaniasis and temperature; while there exists a direct statistical significance between Leishmaniasis and both the RH and precipitation.

Key words: climate, Isfahan province, plantation, skin Leihsmaniasis, spatial distribution, topography.

INTRODUCTION

Leishmaniasis is a parasitic disease appearing in the forms of skin Leishmaniasis, mucus-skin, scattered on skin and intestinal. The skinLeishmaniasis is a chronic disease appearing as painless wounds in certain parts of the body, the face in particular. The carrier is the sandfly infected by the parasite which when stings human body makes the person infected (Seaman et al, 1996, Sunder et al, 2000, and Werneck et al 2002). Leishmaniasis belongs to the bloody spermatozoid (back tail) group which are textured and appear in two forms in their life span: The Leishmaniasis or Amastigote form, that is without back tail, which is available in the mammal host body and the shape of Leptomania or Promastigote form, that is with frontal tail found insandfly and farm land (Azizi et al, 2000). The annual worldwide count for skinLeishmaniasis infected is 1.5-2 million cases and intestinal Leishmaniasis is about half a Million. After Malaria this disease is the second in global health arena. The identified core points of Leishmaniasison global scene lie in about 88 countries which are apt to promote this disease to their climatic components (W.H.O, 2010). Although this disease is not as fatal or disabling as others, due to its long lasting wound period that make the face of the infected ugly and having the potential for secondary infections due to using the available medicine with costly and lengthy curing period is a social burden (Ayatolahi and Karimi, 2005). In clinical sense the skinLeishmaniasisappears as “dry”, the urban type and as “wet”, the rural type. The urban type, due to leaving its scar is called “dry”. Here the source is usually an infected person (or in some rare cases infected dogs) known as Anthroponotic cutaneous Leihsmaniasis. The rural type is called “wet” since the scar contains rotten secrete. The major Leishmania caused by chewing animals (mouse) as a source is known as the Zeonotic type. In the wet type the latent period is shorter than that of the “dry” type with a usual life span of less than four months (Shirzady, 2012).

MATERIALS AND METHODS

The features of the study zone

The city of Borkhar is newly developed city; this city was detached from the prior city of Borkhar and May-may in 2007. The total area of this city is 1952.77 Km^3 where 1863 Km^3 is plateau and the rest is elevated. This city is in the north of the city of Isfahan. The city is of arid climate type, with annual precipitation rate of 50-200 mm with semi desert climate in summer and dry winters (Pour Sakhi, 2007). Westwards it joins the Mourchekhort highlands, northwards it joins Borkhar region, southwards to the city of Isfahan and eastwards to Segzi and Kouhpayeh regions. The plateau is about 1600m above MSL with slight gradient south-east wards of 0.5- 1.5 per thousand.

The data regarding Leishmaniasis disease

The data here is obtained from the Provincial Health Center (Epimologic disease presentation center) for the years in 2005-2012 periods separately.

The meteorological data

The concerns here are the temperature, precipitation and RH, the climatic elements obtained from the Mourchekhort Meteorological station for the years counted above.

The procedure

This study follows the descriptive-analytic method where the IDW method is adopted. In order to measure the contribution rate of the geographic components with respect to the epidemiological aspect of Leishmaniasis. To determine the breakout of this disease in the study area the identified patients’ number is divided by the total population of the area per 10.000 persons. By using the obtained ratio the disease spatial distribution is drawn in

Excel software and the disease pattern diagrams and the climatic variables are drawn. Afterwards in the SPSS software the variables with the break out ratio are determined in order to find the causation with respect to their correlation.

RESULTS AND DISCUSSION

The outbreakof most of the diseases as well as Leishmaniasisin addition to the economic, social and cultural issues is subject to the ecologic factors' affect. Among these climatic factors lack of vegetationis the main contributor in sand fly growth as the carrier ofLeishmaniasis.The conditions in the study zone are fit for studying the chewing animals and multiplication of the sand flies (Mozafari, 2011). The main core points where Leishmaniasis is more advanced in Isfahan province have low vegetation. After drawing the vegetation map of the study zone it was revealed that most of the areas contain sporadicvegetation and pasture lands (Fig. 2).

The topographic map of Borkhar (Fig. 3) reveals the fact that its low elevation is an effective geographic factor in Leishmaniasis outbreak. Of course age, gender, occupation, population awareness and the residential area's locations have their share in the outbreak rate in different districts of Borkhar. The spatial distribution of Leishmaniasis is illustrated in Fig. 4.The rate of Leishmaniasis outbreak in the study area in spring and the beginning of summer is low, that is at the peak of the heat the number of cases are low (Fig. 5). The higher rate is evident at the end of summer and reaches its peak in September and follows a gradual decrease as the temperature, RH and precipitation rate increase towards the end of fall. The peak temperature is in July at 28.96°C with a RH of 26%. The patients' number averages are 20.33 in summer and 109 in fall. The Leishmaniasis type in this city is (wet) and its latent period lies within 2-12 weeks, therefore it can be claimed that the stinging of the sand fly that carry the disease must have occurred at least two-three months prior to the disease peak season, that is in the months when the temperature is high and the RH and precipitation are low.

CONCLUSION

The city of Borakhar is one of the major cores of Leishmaniasis in Isfahan province due to sporadic vegetation which is the major contributor in promoting this disease. There is a close resemblance among the findings in this study and the outbreak rate of Leishmaniasis in Yazd-Ardekan plateau region. The major common factor in both regions is the sporadic vegetation and sometimes no plantation in both the regions. The analysis of the correlation rate between the climatic elements and the inflicted cases indicate the existence of an indirect statistical significance between Leishmaniasis and temperature at a 0.95 level and the existence of a direct statistical significance with RH and precipitation. At 27-30°C, RH of 18-25% and the lowest rate of precipitation the sand flies begin their attacks. The findings in this respect correspond to that of the findings obtained from the study conducted in Ardekan regarding stinging season of these sand flies.

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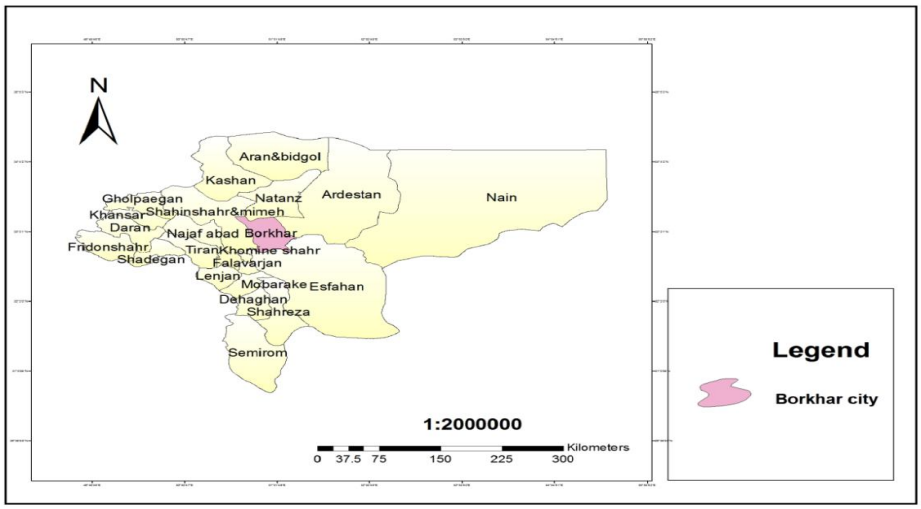

Fig. 1- Borkhar city plan Drawing: by Autho(zahramirzakhani)

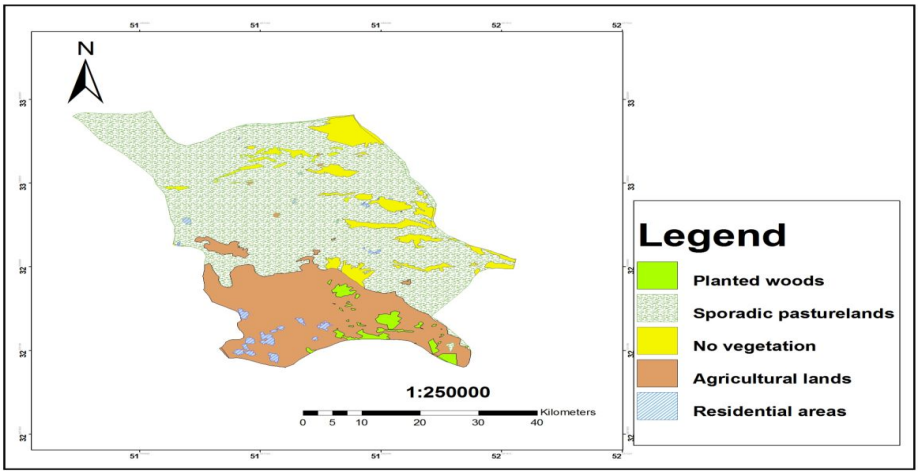

Fig. 2- Plant coverage map Drawing: by Autho(zahramirzakhani)

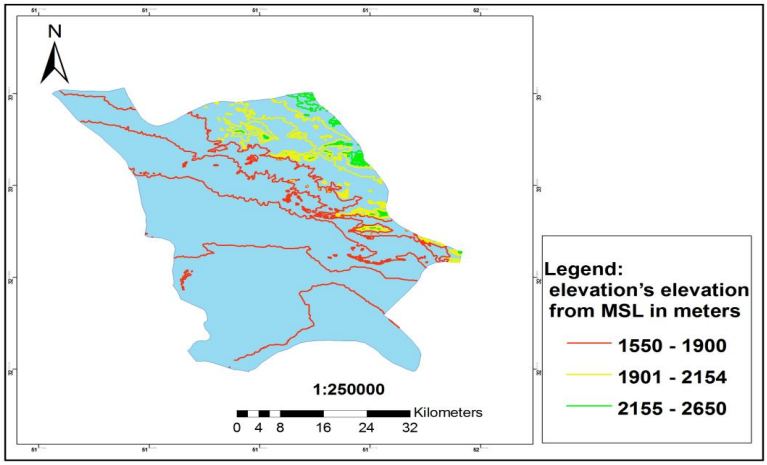

Fig. 3- Topographic map Drawing: by Author(zahramirzakhani)

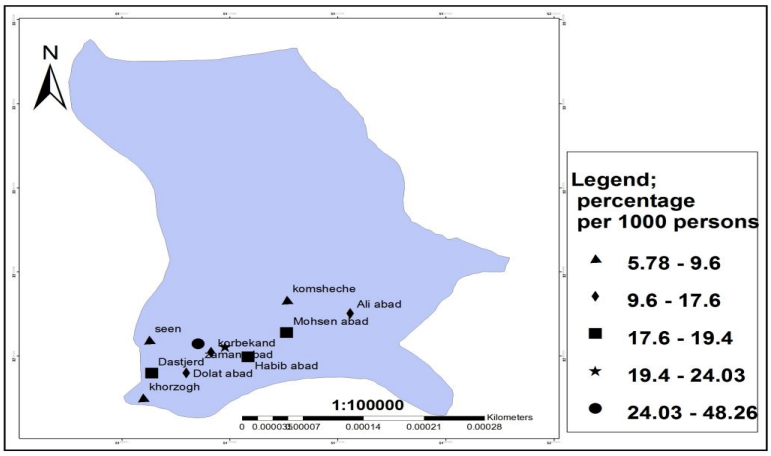

Fig. 4- The spatial distribution map Drawn by Author(zahramirzakhani)

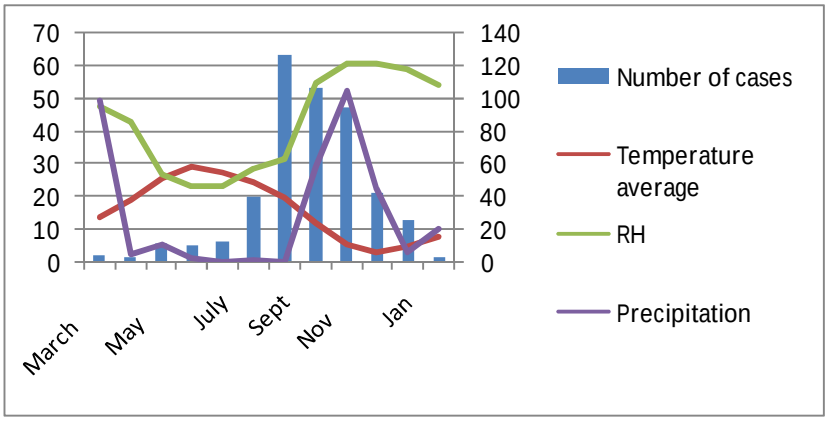

Fig. 5- Monthly changes of Leihsmaniasis rate and its correlation with the climatic elements in Borkhar during 2011-2012.

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RESEARCH ARTICLE

Fertility and Hatchability Studies in Crossbred Chicken in Kerala,India.

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ABSTRACT

Backyard chicken rearing in Kerala is gaining momentum as larger scale investments are meager and land holdings are small. A study was conducted at Centre for Advanced Studies in Poultry Science to compare the fertility and hatchability of eggs collected from three crossbred chicken namely Gramasree, Gramalakshmi and Gramapriya. Fertility and hatchability of eggs set for seven consecutive hatches over a period of ten weeks from January to February were studied. The results of the study revealed that percentage of infertile egg was significantly ($p \leq 0.05$) lower in Gramalakshmi and Gramasree birds than that of Gramapriya, while percentage of dead-in-shells was comparable in all the three crossbreds. The percentage of hatchability on total egg set was significantly higher in Gramalakshmi than Gramapriya while that of Gramasree was intermediate and statistically comparable to the other two crossbreds. The study shows that hatching Gramalakshmi eggs is much more economical than Gramasree and Gramapriya.

Key words: Incubation, Hatchability, Gramasree, Gramalakshmi, Gramapriya.

INTRODUCTION

There has been an increase in the backyard poultry rearing in Kerala during the past decade following the release of high yielding crossbreds Gramasree (with a meat type male line) and Gramalakshmi (with white leghorn as the female line) by University Poultry Farm, Mannuthy. The Project Directorate on Poultry, Hyderabad developed a meat type crossbred namely Gramapriya for this purpose. In the absence of large investments in the poultry sector in Kerala, backyard farming is encouraged by government sponsored schemes supplying low input bird to farmers at six weeks of age. All these three crossbreds are equally popular n Kerala.

The principle objective of a commercial hatchery is to produce maximum number of day old chicks from the eggs set for hatching. Lighter breeds have been found to have higher hatchability than heavier ones [1]. The present study was conducted in the three crossbreds, simultaneously during 10 weeks period from January to February. Hatching eggs of the three crossbreds were incubated, hatched and day old chicks supplied. The infertility percentage, dead in shell, Hatchability on total egg set (HTES) & Hatchability on fertile egg set (HFES) were studied.

MATERIALS AND METHODS

The study was conducted at RFH, Mannuthy during a period of 10 weeks from January to February. Eggs from three different crossbreds namely Gramasree, Gramalakshmi and Gramapriya were studied.Seven batches of egg set over a period of 10 weeks were studied. The eggs were set at 99.5^0F and 55 - 60% relative humidity [2] for a period of 18 days in the setter.On the eighteenth day, the eggs were candled and the infertile eggs were separated. Only the fertile eggs were transferred to the hatcher. Here the eggs were maintained at 99^0F - 99.5^0F and a relative humidity of 60 - 65%. On the twenty first day the hatch was pulled out. The number of healthy chicks and dead in shells were recorded separately. Infertility on transfer and dead in shell at the time of hatch were recorded. The hatchability on total egg set (HTES) was calculated from the number of eggs set. The hatchability on fertile egg set (HFES) was on the number of eggs transferred after candling to the hatcher. These values were later subjected to statistical analysis [3].

RESULTS

Infertility

The percentage of infertility was highest in Gramapriya which was significantly different from the other two and lowest in Gramalakshmi. Gramasree had an intermediate infertility but there was no significant difference between Gramasree and Gramalakshmi.

Dead germ

The dead germ percentage was least in Gramasree and highest in Gramalakshmi and it was comparable between Gramasree and Gramapriya. There was no significant difference in percentage of dead germ between the three crossbreds.

Dead in Shell

The percentage of dead in shells was observed to be highest in Gramasree and lowest in Gramalakshmi. Gramapriya had an intermediate percentage but there was no significant difference.

Hatchability on Total Egg Set

The maximum hatchability on total egg set was observed in Gramalakshmi and lowest in Gramapriya, while Gramasree had an intermediate hatchability percentage.

Hatchability of Fertile Eggs

The hatchability of fertile eggs was observed to be the highest in Gramalakshmi and lowest in Gramasree. It was attributed to the low dead in shell percentage of Gramalakshmi birds compared to the other two.

DISCUSSION

Fertility and hatchability are interrelated traits having variation among breed, varieties and individuals within breeds and varieties [4]. The high hatchability of Gramalakshmi may be attributed to its lower infertility and dead in shell percentage compared to the other two crossbreds. Similarly the low hatchability of Gramapriya was due to the increase in the number of infertile and dead in germs.80.79% fertility and 71.73% hatchability was observed in fertile eggs for desi chicken [5]. Genetic constitution was reported to have some effect on embryonic mortality in flocks provided good feeding, management and optimum condition [6]. Inbreeding will also reduce fertility and hatchability [7]. Studies on fertility in laying strains of chicken showed high heritability [8], which explains accurately the higher hatchability of Gramalakshmi which is a white leghorn cross.

CONCLUSION

The fertility and hatchability studies conducted in the hatching eggs of Gramasree, Gramalakshmi and Gramapriya has shown that Gramalakshmi has the highest fertility and hatchability which indicates that hatching these eggs will be most economical.

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Table 1: Mean (± SE) infertility and hatchability values of crossbred chicken eggs

Breeds	Gramapriya	Gramalakshmi	Gramasree
Infertility	10.15 ± 2.17[a]	6.45 ± 084[b]	7.25 ± 1.41[b]
Dead germ	0.37 ± 0.01	0.79 ± 0.06	0.26 ± 0.02
Dead in Shell	6.05 ± 5.12	4.29 ± 1.01	7.81 ± 2.35
Hatchability on Total Egg Set	83.43 ± 3.66[b]	88.87 ± 1.57[a]	84.68 ± 3.15[b]
Hatchability of Fertile Eggs.	92.83 ± 5.31	94.83 ± 1.65	91.53 ± 2.55

Mean values bearing the same superscript within the column did not differ significantly ($p \leq 0.05$)

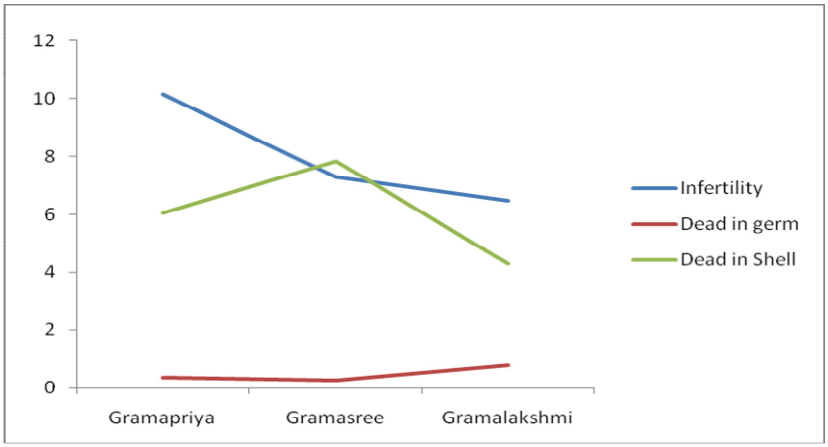

Fig 1: Mean infertility, dead germ and dead in shell

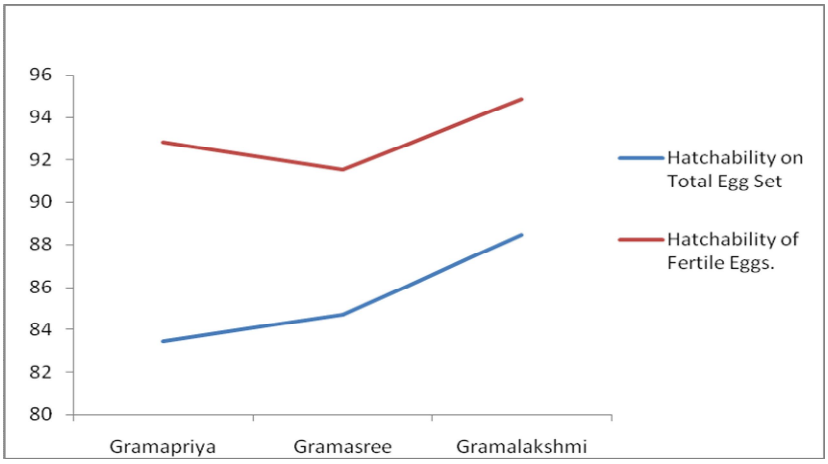

Fig 2: Mean hatchability on total and fertile egg

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RESEARCH ARTICLE

Impact of Textile Bleaching Effluent on Fresh Water Fish *Labeo rohita.*

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ABSTRACT

Textile industry is one of the most important and rapidly developing industrial sectors. It has a high importance in terms of its environmental impact, since it consumes considerably high amounts of processed water and produces highly polluted discharge water in large amounts. The indiscriminate disposal of untreated wastewater in to water courses or on to land invariably pollutes the ecosystem. The sources of pollutants are the natural impurities in the cloth and the processing chemicals which are removed from cloth and are discharged as waste. The Textile bleaching effluents cause harmful effects to living organisms. Periodic monitoring of effluents is highly essential to control pollution. In the present investigation the effect of exposure to textile bleaching effluent at sublethal concentrations (2%, 2.5%) to the freshwater fish, *Labeo rohita*, were studied for 28 days. The toxic effect of the effluent affects the biochemical aspects of the organisms. The levels of Protein, Lipid and Carbohydrates in muscle were decreased significantly. The decline in protein content was due to rapid utilization of body proteins or poor intake of dietary protein by the fish under stress condition. Reduction in carbohydrate content was due to increased utilization of carbohydrate when the fish is under chronic stress. Low level of total lipids recorded in the effluent treated fish suggests that lipids might have been channeled for energy production for other metabolic functions. Clogging of fish gills, Hyperplasia an increased proliferation of cells and Histopathological changes were observed in both epithelia and blood vessels in gills of *Labeo rohita* exposed to textile bleaching effluent. The changes were dependent on period of exposure and concentration of the effluent.

Key words: Textile bleaching effluent, Sub-lethal, Histopathology, Hyperplasia.

INTRODUCTION

The effluent from the textile industry are characteristics of waste water released from sizing, desizing, kiering, bleaching, mercerizing, dye house and printing sections of composite cotton textile mills .The total dissolved solids are high due to the use of chemicals of high solubility and the high suspended solid are due to the precipitation of salts and insoluble impurity separated by grey cloth. The pollutants which accumulate continuously in the body tissues of progressively higher concentrations during exposure are known to have cumulative toxins[8]. If the exposure stopped, a cumulative toxin is released very slowly in the media of the living animals, and then ceases to damage the organs. Accumulation of heavy metal at an ambient level, through the food web may finally reach man and hence is of great importance in the ecological cycle [12]. In addition light absorption hindered by textile dyes creates problems to photosynthetic aquatic plants and algae. The main important pollutants in textile effluent are recalcitrant organic compounds, colour, toxicant and inhibitory compounds surfactants and chlorinated compounds. During processing, 5-20% of the used dye stuffs are released into the process water [19,14] and dye is the most difficult constituent to treat by conventional biological waste water treatment. Pollutants in wastewater from textile factories vary greatly and depend on the chemicals and treatment processes used. Pollutants that are likely to be present include suspended solids, biodegradable organic matter; toxic organic compounds e.g. phenols and heavy metals. The other parameter, sulphates (SO_4) can be naturally occurring or as a result of municipal or industrial discharges. Villegas-Navarro *et al*. (2001)[17] reported that textile effluents exhibit very high toxicity with acute toxicity unit (ATU) in terms of LC_{50} and levels between 22 and 960. Dyes contributed to overall toxicity at all process stages. Also, dye baths could have high level of BOD/COD, colour, toxicity, surfactants, fibres and turbidity, and may contain heavy metals[1]. They generally constitute a small fraction of total liquid effluent, but may contribute a high proportion or total contaminants. Wynne *et al*. (2001)[20] noted that textile effluents are highly coloured and saline, contain non-biodegradable compounds, and are high in Biochemical and Chemical Oxygen Demand (BOD, COD). They reported that the presence of metals and other dye compounds inhibit microbial activity and some cases may cause failure of biological treatment system. Textile effluents are high in BOD due to fibre residues and suspended solids[1]. They can contaminate water with oils, grease, and waxes [7]Dyeing process usually contributes chromium, lead, zinc and copper to wastewater [5]. Copper is toxic to aquatic plants at concentrations below 1.0mg/1 while concentration near this level can be toxic to some fish[13].The textile industries are multi-chemical utilizing concerns of which dyes of various types are of importance. During the dyeing process a substantial amount of dyes and other chemicals lost in the waste water. Estimates put the dye losses at between 10-15% [16]. Though not generally toxic to the environment, dyes colour water bodies and may hinder light penetration thereby affecting aquatic life and limiting the utilization .However, the human eye can detect levels as low as 0.005 mg/l of reactive dyes in a clear river [10]. Objectives of the study are deals with effects of textile bleaching effluent on the environment and find out the growth and survival of the fish.

MATERIALS AND METHODS

The Indian major carp, *Labeo rohita* (Rohu) which belongs to the order Cypriniformes was chosen for this study. It is considered as the tastiest fish. It has a small and pointed head, terminal small mouth with fringed lower lip. It is a column feeder on phytoplankton, plant debris or decaying debris of aquatic plants.**Experimental Design:**The experiment was designed to study the impact of textile bleaching effluent on the medium and fresh water fish *Labeo rohita*. The fish exposed to sublethal concentrations were 2.0% and 2.5%. The duration of the experiment was 28 days. The Physico-Chemical parameters measured were pH, Electrical conductivity, Dissolved Oxygen, BOD, COD, Chloride and Sulphate. Bio-chemical parameters measured were Protein, lipid and carbohydrates in muscle. All measurements were made on 0, 7^{th}, 14^{th}, 21^{st} and 28^{th} day. Histology of gills was studied on 28^{th} day.**Establishment of Culture Systems:** For the present study, effluent was collected from RP textile dyeing industry at Karur. Healthy freshwater fish *Labeo rohita* weighing 4 gms were used. By chronic and sublethal tests, sublethal concentrations 2% and 2.5% of treated effluent were evolved.Three groups of fish as 10 each were introduced to the medium of 3 different tanks of control, 2% and 2.5% treated textile bleaching effluents.Fish were daily fed with 6% pelleted food of the body weight. The medium was renewed every 7 days. The physicochemical parameters of the medium, biochemical composition of the fish muscle and histology of gills were studied.

RESULTS AND DISCUSSION

The effect of textile bleaching effluent in different concentrations 2% and 2.5% on the physicochemical characteristics of the medium revealed that electrical conductivity of the medium was increased gradually on longer period of exposure. Dissolved oxygen level was reduced.BOD, COD, Sulphate and Chloride level showed an increasing trend in different period of exposure. The effect of textile bleaching effluent in different concentrations 2% and 2.5% on the biochemical constituents of the fish showed Proteins, Lipids and Carbohydrates content was decreased tremendously at different periods of exposure.Clogging of fish gills and Histopathological changes were observed in both epithelia and blood vessels in gills of *Labeo rohita* exposed to textile bleaching effluent. Hyperplasia an increased proliferation of cells was observed.Mortality of fish was found in the treated medium on longer period of exposure.The results revealed that the textile bleaching effluent affected the environment and it also toxic to the fish.Results of the experiments carried out to find out the impact of textile bleaching effluent on the water quality and in freshwater fish *Labeo rohita.Labeo rohita* exposed to the sublethal concentrations of textile bleaching effluent 2% and 2.5% exhibited histological, biochemical and some morphological changes when compared to the control groups. In 2% treated and 2.5% treated textile bleaching effluent there was tremendous decrease in the dissolved oxygen with passage of time. The most important measure of water quality is the dissolved oxygen [11,18]. The low level of DO recorded could result in the non-maintenance of conditions favourable to the aerobic organisms. This could lead to anaerobic organisms taking over, with the resultant creation of conditions, making the water body uninhabitable to gill-breathing aquatic organisms.The trends shown by the biological oxygen demand are in tune with that of the dissolved oxygen .The high level of BOD is indications of the pollution strength of the wastewaters. They also indicate that there could be low oxygen available for living organisms in the wastewater when utilizing the organic matter present. Gradual increase in the COD level is noticed in 2% and 2.5% treated textile bleaching effluent medium for longer period of exposure. High COD levels imply toxic condition and the presence of biologically resistant organic substances [13,15].The settle able and suspended solids are high and this will affect the operation and sizing of treatment units. Solids concentration is another important characteristic of wastewater [9]. There is a remarkable increase in the sulphate content in the treated systems.Hydrogen sulphide is formed under conditions of deficient oxygen in the presence of organic materials and sulphate [18]. This could be a possible reason for the high sulphide measured in the effluents analyzed. There is tremendous increase in the level of Chloride on 28th day in 2% treated and 2.5% treated textile bleaching effluent.

The results of the present study clearly reflected that the three principle biochemical constituents, i.e. Proteins, carbohydrates and lipids of the test tissues (muscle and gill) were mobilized under the toxic influence of the textile bleaching effluent. Protein content of muscle, liver, gill and intestine of *Oreochromis mossambicus* was found to decrease with increasing concentration of textile dye effluent [6].Sabita and Yaday (1995) have reported a decline in protein content of muscles and gill in rigor intoxicated *Heteropneustus fossilis*. Vijayamohanan and Nair (2000) observed a significant decrease in protein content of muscles and liver in *O. mossambicus* and *Etroplus maculates* exposed to titanium dioxide factory effluent. All these observations confirm the findings of the present study. The decline in protein content may be due to rapid utilization of body proteins or poor intake of dietary protein by the fish under stress condition [2,4].The present observation indicated that carbohydrate level in tissues of *C. carpio* exposed to different concentration of textile bleaching effluent was considerably reduced. Amudha *et al*. (2002)[3] found reduced level of total carbohydrate content may be due to muscle, gill and liver of *O. mossambicus* exposed to dairy effluent. Reduction in carbohydrate content may be due to increased utilization of carbohydrate when the fish is under chronic stress .This could have happened by rapid glycogenolysis and inhibition of glycogensis through activation of glycogen phosphorylase and depression of glycogen transferase.Low level of total lipids recorded in the effluent treated fish suggests that lipids might have been channelled for energy production for other metabolic functions in which these products lay a vital role during stress conditions. The results revealed that the textile bleaching effluent affected the environment and it also toxic to the fish.

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Table 1. Effect of textile bleaching effluent recorded at different concentrations in protein content (mg/g) of muscle tissue in *Labeo rohita* at different periods of exposure.

Medium	**Period of exposure (days)**				
	0	7	14	21	28
Control	91.5±0.026	73.15±0.021	64.9±0.141	59±0.282	45±0.282
Treated 2%	91.5±0.026	71.18±0.021	60.42±0.014	39.25±0.264	26.1±0.015
Treated 2.5%	91.5±0.026	69±0.017	54.8±0.013	31.5±0.015	19.5±0.020

Table 2. Effect of textile bleaching effluent recorded at different concentrations in Lipid content (mg/g) of muscle tissue in *Labeo rohita* at different periods of exposure.

Medium	Period of exposure (days)				
	0	7	14	21	28
Control	6.75±0.452	6.55±0.021	6.01±0.021	5.8± 0.283	5.13±0.105
Treated 2%	6.75±0.452	6.22±0.026	5.81±0.021	3.52±0.015	3.14±0.078
Treated 2.5%	6.75±0.452	4.92±0.035	3.32±0.035	1.04±0.049	0.77±0.014

Table 3. Effect of textile bleaching effluent recorded at different concentrations in carbohydrate content (mg/g) of muscle tissue in *Labeo rohita* at different periods of exposure.

Medium	Period of exposure (days)				
	0	7	14	21	28
Control	89.04±0.014	65±0.212	44±0.353	39±0.070	36.25±0.098
Treated2%	89.04±0.014	57.01±0.014	33.01±0.025	31.25±0.282	25.02±0.015
Treated 2.5%	89.04±0.014	56.5±0.015	25.06±0.007	23.25±0.035	20.05±0.014

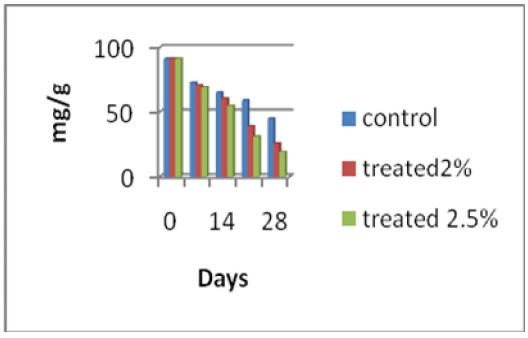

Fig. 1. Effect of textile bleaching effluent at different concentrations in protein content (mg/g) of muscle tissue in *Labeo rohita* at different periods of exposure.

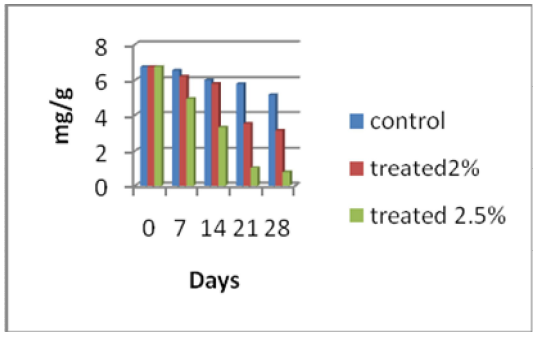

Fig. 2. Effect of textile bleaching effluent at different concentrations in Lipid content (mg/g) of muscle tissue in *Labeo rohita* at different periods of exposure.

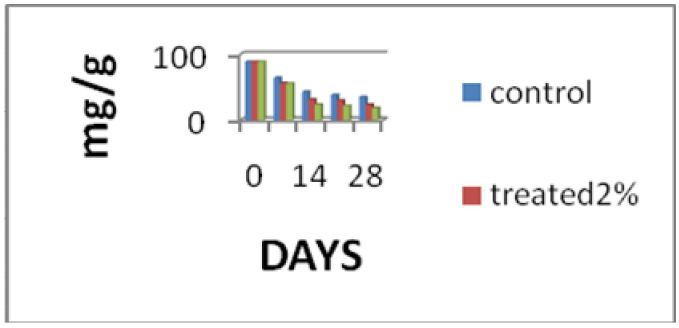

Fig.3.Effect of textile bleaching effluent at different concentrations in carbohydrate content (mg/g) of muscle tissue in *Labeo rohita* at different periods of exposure.

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RESEARCH ARTICLE

The Phylogenetic Analysis of Zinc Finger Domain.

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ABSTRACT

The Zn finger domain belongs into a large protein domain superfamily bind either to the DNA, RNA or protein. The phylogenetic relationship among the different members of *ZBED* family (*ZBED*1, *ZBED*2, *ZBED*3, *ZBED*4, *ZBED*5 and *ZBED* 6) was analysed. The sequences from different species were retrieved by performing extensive iterative database searches on many well known databases. The Neighbor-Joining (NJ) method for the construction of phylogenetic tree based upon the protein and DNA sequence and it uses the information based on the distance between each sequence obtained from different species. The phylogenetic analysis shows that these proteins may have originated from *ZBED*4 or *ZBED*6 either by the process of partial insertion of a reverse transcribed mRNA or possibly a full length *ZBED*-protein has lost its *hATC* by deletion. It is also observed that the BED domain within the same *ZBED* protein of different species is more similar and closely related than the BED domain from different *ZBED* proteins in the same species.

Key words: Zn finger domain, Neighbor-Joining, ZBED protein and phylogeny.

INTRODUCTION

Zinc finger domains are protein domains that can be grouped into a large protein domain superfamily. The Zn finger domains bind either to the DNA, RNA or protein. The first Zinc finger domain was identified in transcription factor IIIA, in *Xenopus* oocyte, which is a RNA polymerase III general transcription factor that binds DNA (Schuh *et al.,* 1986

and Miller *et al.,* 1985). There are many zinc finger motifs, classified depending upon their amino acid residues, structure and binding specificity. The zinc ion in the zinc finger motif provides the zinc finger (ZnF) domain a stable structure for DNA-protein interaction and rarely does it undergo any conformational change during interaction with DNA. In the absence of zinc ion the protein may unfold to a small hydrophobic core. Most of the ZnF proteins have multiple finger like protrusions that interact with the target molecules. There are many structurally well defined ZnF motifs such as classical (C2H2) ZnF motif, GATA-type ZnF motif, LIM-type ZnF motif and for a complete list, see http://www.ebi.ac.uk/interpro/potm/2007_3/ Page1.html.

In eukaryotes, the classical (C2H2) ZnF motif mostly binds with DNA in the major groove spanning around 3-4 bases and found in several of the transcription factors and regulatory proteins which interacts with DNA. These motifs are made up of short beta heparin and alpha helix, and a zinc ion is coordinated in position by two cystiene and histidine residues. The C2H2-ZnF genes which form second largest family in humans, approximately two per cent of total genes belong to this class (Lander *et al.,* 2001 and Bellefroid *et al.,* 1989). These genes mainly encode transcription factors which bind with DNA or RNA with Zn fingers (Theunissen *et al.,* 1992 and Grondin *et al.,* 1996).

Drosophila melanogaster DNA replication-related element binding factor (*dDREF*) and its homologous protein in human (*hDREF*) are the key transcription factors associated with expression of different genes responsible for the DNA replication (Ohshima *et al.,* 2003). However, the amino acid sequence analysis has revealed that *hDREF* belongs to *hAT* transposon family (Esposito *et al.,* 1999). The two well conserved domains at amino and carboxyl terminal of *hDREF* protein are BED zinc finger (**B**oundary **E**lement associated factor and **D**REF) and *hATC* domain, respectively. The BED zinc finger domain is associated with DNA binding (Hirose *et al.,* 1996) whilst *hATC* domain is regarded as dimerization domain (Essers *et al.,* 2000). The DNA-binding ability is the characteristic feature and general function of this BED domain (Hirose *et al.,* 1996 and Hart *et al.,* 1997).

Recently discovered repressor protein, *ZBED*6, contains two BED domains and a *hATC* dimerization domain. These two BED domains when compared with BED domain of other *ZBED* proteins (*ZBED*1, *ZBED*2, *ZBED*3, *ZBED*4 and *ZBED*5) showed less similarity among them, however, there is relatively more similarity between the two BED domains of *ZBED*6 protein indicating the internal duplication event in the past (Markljung *et al.,* 2009). The BED domain in *ZBED*6 when compared between 25 different species of placental mammals showed almost 100 per cent sequence similarity. In this study we investigated the phylogenetic relationship among the different members of *ZBED* family.

MATERIALS AND METHODS

For the phlyogenetic investigation of *ZBED* proteins, all the currently available sequences from different species were retrieved by performing extensive iterative database searches on many well known databases such as National Centre for Biotechnology Information (NCBI) Genbank database, Ensembl and UCSC genome browser. Each new coding DNA sequence of *ZBED* proteins from different species were retrieved and analyzed manually. The acceptance criterion of the retrieved sequences is that they should have a start and stop codon with an open reading frame. The Genbank DNA database which is provided by NCBI since 1992, works in collaboration with other databases such as European Molecular Biology Laboratory (EMBL) and DNA Database of Japan (DDBJ). Ensembl is a joint venture of European Bioinformatics Institute and Welcome Trust Sanger Institute started in 1999, with an idea to study the genome of various species and provide information to researchers in different fields such as genetics, molecular biology and evolutionary biology. Basic Local Alignment Search Tool (BLAST) is a widely used program for searching of query sequences against the DNA and protein databases (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Phylogenetic analysis will shed light on the origin and evolutionary history of many genes in different species. A good reliable phylogenetic tree is an important tool to study the molecular evolution of diversity of genes in different organisms and to understand the mechanisms of evolution in a better way. Phylogenetic relationships are usually

expressed in the form of a tree with many branches. The obtained branching pattern is called topology. Phylogenetic trees are of two types; rooted and unrooted trees. In rooted trees, each node will direct towards immediate ancestor while the unrooted tree will show the relatedness between the species without referring to the common ancestor. There are many different methods to make the phylogenetic tree based upon the amino acid and nucleotide sequence data such as Distance matrix method, Parsimony method and Likelihood method. In the present study we are using the Neighbor-Joining (NJ) method for the construction of phylogenetic tree based upon the protein and DNA sequence and it uses the information based on the distance between each sequence obtained from different species. The Neighbor-Joining (NJ) method has its own advantages over other methods; computational efficiency is higher, large number of sequences can be analyzed and can create both rooted and unrooted trees with high probability to true tree and are statistically consistent with most models of evolution. Nevertheless, NJ has been extensively used in phylogenetic studies and gives a reliable tree.

In MEGA, we can construct the NJ tree by activating the 'meg' file which was previously saved after doing the multiple-alignment with ClustalW. Then select the *phylogeny option* which contains the *Neighbor-Joining* command. There is another tab called '*Model*' which has two options '*Nucleotide|p-distance*' and '*amino|p-distance'* option for nucleotide sequence and amino acid sequence respectively. Finally click the 'compute' after accepting all the default parameters and the tree can be viewed in Tree explorer menu, further modification can be done in tree explorer for a better outlook of the tree.All possible ZBED protein (ZBED1, ZBED2, ZBED3, ZBED4, ZBED5 and ZBED6 sequences from as many species as possible were retrieved by searching the GenBank database.

RESULTS AND DISCUSSION

While doing the specialized BLAST search against the NCBI Domain database revealed that all the *ZBED* family members (*ZBED*1-6) have one or more BED domains but only some of the members have *hATC* domains. *ZBED*1 has a single BED domain, *ZBED*6 has two BED domains and *ZBED*4 has four BED domains, respectively. Moreover, all these three members (*ZBED*1, *ZBED*4 and *ZBED*6) of *ZBED* family have *hATC* domain. When we look at other members of the family such as *ZBED*2, *ZBED*3 and *ZBED*5, they lack *hATC* domain and each has only a single BED domain. The amino acid length of these proteins (*ZBED*2, *ZBED*3 and *ZBED*5) is relatively shorter when compared to other members (*ZBED*1, *ZBED*4 and *ZBED*6) of *ZBED* family.

The phylogenetic analysis shows that these proteins may have originated from *ZBED*4 or *ZBED*6 either by the process of partial insertion of a reverse transcribed mRNA or possibly a full length *ZBED*-protein has lost its *hATC* by deletion.In the present study we use the neighbour-joining (NJ) method developed by the Saitou and Nei (1987), the principle behind this method is the clustering of pairs of operational taxonomical units (OTUs) *i.e.*, the neighbours which in turn minimizes the length of branch at different stages of clustering. One of the virtues of NJ method is its computational efficiency and statistical consistency which fits well into different evolutionary models. When equated with other phylogenetic analysis methods such as minimum evolution (ME), maximum parsimony (MP) and maximum likelihood, NJ method can deal with large amount of data at a time. The phylogenetic analysis revealed that the BED domain within the same *ZBED* protein of different species is more similar and closely related than the BED domain from different *ZBED* proteins in the same species.

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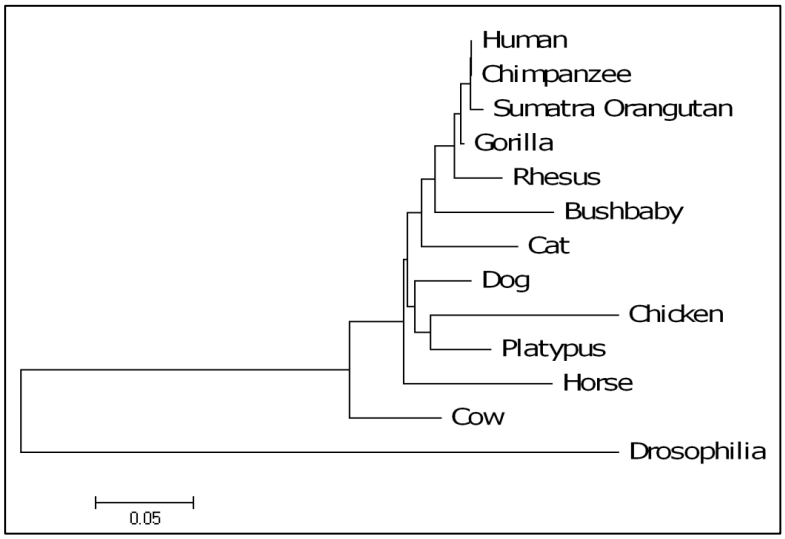

Fig. 1 The phylogenetic tree based on the nucleotide sequence of BED domain o *ZBED*1 in 13 different species

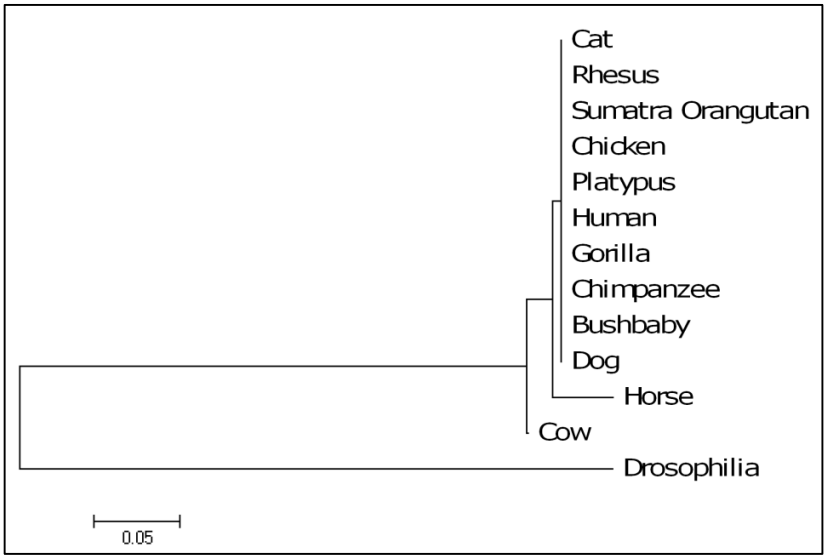

Fig. 2 The phylogenetic tree based on the protein sequence of BED domain of *ZBED*1 in 13 different species

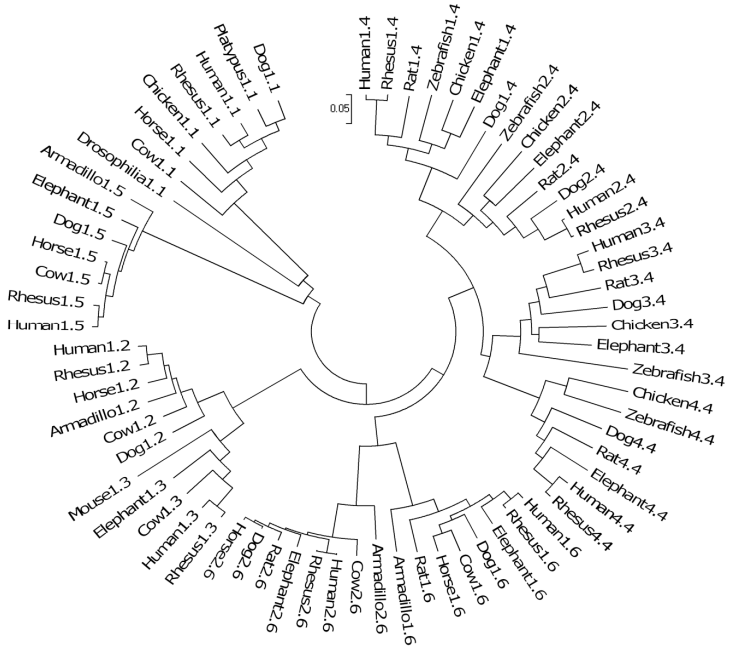

Fig 3. Represent the phylogenetic tree based on the nucleotide sequence of BED domains from different *ZBED* proteins, the first digit represent the position of BED domain in the protein and the second digit represent the member of *ZBED* family.

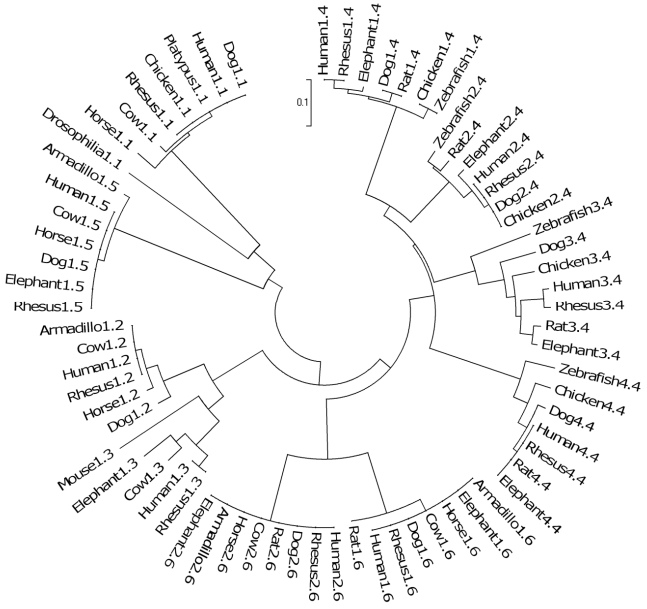

Fig. 4 Phylogenetic tree based on the protein sequence of the BED domain from different *ZBED* proteins, both tree (5A and 5B) look similar but the branching will be more extensive if we use DNA sequence to build the tree.

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RESEARCH ARTICLE

Identification of Brucellosis Incidence in Urban and Rural Settlements in Iran (Kermanshah Province).

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ABSTRACT

Brucellosis is one of the most common joint diseases that are very important. Brucellosis can be seen in many parts of the world, especially in the Mediterranean countries, the Middle East, the Arabian Peninsula, Central and South America, Asia and Africa. This study was examines the incidence of brucellosis in urban and rural settlements, Kermanshah province in the years 2009 to 2012. After entrying Data into Excel software and determining incidence rate and also entering into Spss software and determining difference with T-test, entred into Gis software finally, the focouses of incidence of Brucellosis in urban and rural areas obtained in the map by IDW techniques.Among the urban areas Kermanshah, Qasre-shirin with rate of 118/99 per hundred thousand, is the highest, and the city of Kermanshah, with an incidence rate of 19/2 per hundred thousand is the lowest in the province. In the rural areas, the rate of 267/81 per hundred thousand Dalahocity has the highest rate, and the Paveh city with an incidence of 29/27 per hundred thousand is the lowest in the province.The results of the research show much differences in focuses of incidence of Brucellosis in urban and rural areas of Kermanshah Province.

Keywords: Brucellosis, focuse of incidence, urban settlements, rural settlements, Kermanshah Province.

INTRODUCTION

Common diseases between Livestock and human are still one of the most serious health problem in our country, our country [1] Iran is in the list of the most joint disease Brucellosis, which is very important [2].The importance of this disease ,it is more the province of Alavi's and his co-worker studies indicate increasing disease in recent years[3] and other studies suggest an increased incidence of Brucellosis in 2001 and after that[4]. Fundamentally, Brucellosis belongs to animals, is transmitted to humans and may be affected by any of five species Brucellamelitensis then Brucella Switzerland and less abortus[5] Based on a global measure of the incidence of brucellosis among human in any country depends on very closely with the incidence of brucellosis in cattle in the country. Brucellosis can be seen in many parts of the world, especially in the Mediterranean countries, the Middle East, the Arabian Peninsula, Central and South America, Asia and Africa. Only17countries have officially declared free of brucellosis. In some countries such as the United States of America, the disease is primarily an occupational dangerous while in countries such as Iran, infection is not limited to specific jobs [6&7]Although thedisease exists in all parts of the world, but in countries around the Mediterranean, Middle East, Indian subcontinent, Central andSouth America is more commonly[8] In the Latest reports available from the WHO Eastern Mediterranean Region, 23 countries in the regionin just two countries, Bahrain and Cyprus have not beenreported cases of brucellosis. The most reported cases are in five countries of Iran, Saudi Arabia, Iraq, Syria and Jordan[9] In our countrydue to traditional hunting, live anddirectcontact with livestock farmers and the lack ofsystematic vaccination is cause much disease is high and the economicdamage[10]. During 2003 the incidence of human brucellosis indifferent parts hasbeen 1/5 to 107/5 perhundred thousand different inHamadan 107/5 per hundred thousand and in Kurdistan 83/5 and then West Azarbaijan 71 per hundred thousand and Zanjan 67/1 are the highest incidence,According to statistics Brucellosis is prevalent inmost parts of Iran. In the west provinces the incidence of the disease is more than the rest of the country.Reportedcases of brucellosis from Kermanshah University of Medical Sciences in 2003, the figure was 34 per hundred thousand, higher than the rate of incidence a year for the whole country has been 21 per hundred thousand [11]. The average age of 37 is maximum [12] aged 10 to 19 years and the minimum age under ten years [13]. It was should be said that 87 percent of reported cases Brucellosis are residence in rural areas [14]. This study tries to indicat centers of Brucellosis incidence in Kermanshah province according to rural and urban residences.

MATERIALS AND METHODS

This is across-sectional study performed on 3754 cases of Brucellosis in Kermanshah Province from 2008 to2012. Monthly reports on the number of people suffering from certain forms of health centers in the city will be sent to the Health Center of province. Gather all of the specific forms of Communicable Disease Health Center units are placed into segregated city. This form includes informationsuch as age, gender, location, occupation and people are catching on. This information is used to determine the incidence of each city and the whole province has entered into Excel software. After determining the rate of any city in terms of area disease is calculated incidence of Brucellosis in urban and rural areas of each city. By entering this information into Gis software using deterministic methods and techniques Idw, focous of Brucellosis incidence in urban and rural centers are defined by the output maps. Besides basic information entred into the Spss software that T-test is used to determine differences in the incidence of rural and urban areas.

RESULTS

Patients that suffering from Brucellosis during the periodfrom3754cases,3305 under the 88/3% of casesarein rural areasand 449cases under 11/97% of caseswerein urban areas. Excluding thecityofQasreShirin, where urban cases of disease have been more than rural areas. During the priod cases of infections in urban areas have always been more than in rural areas. Maximum people in 2010 with 1358 cases in rural areas and 194 cases in urban areas. Minimum in 2013 with 525 cases in rural areas and 60 cases in urban areas.Test results aret (t = -9.569andsig = .000) indicate that

the average amount of Brucellosis in both urban(112.2500) andrural(672.0000) there is no significant difference between the two groups in four years with a confidence level differ by 95% in four years, assuming no significant difference implies that H0 is rejected. The mean is zero at the 95% confidence levelon theone hand; this result also shows that the four-year averages of Brucellosis are out differently. In other wordswe can say thatpatients with Brucellosis in urban areas have been more in rural areas in these four years.

Figures 1 and 2 locations with high incidence are observed blue and low incidence red points. Among urban area Qasreshirinwith an incidence of 118/99perhundred thousand, has the highest rate, and the city of Kermanshah, with an incidence of 2/19perhundred thousand has the lowest in the province. It can be said that the incidence of Brucellosis has been in west areas of province and especially in Qasreshirin.In the rural areas, the rate of 267/81 perhundred thousandDalahocity, has the highest rate, and the city of Pave with the lowest incidence rate of 29/27 per hundred thousand in the province. The incidence of Brucellosis centers in rural areas can be said other than the Pave city, Ravansar, Kermanshah who are incidence of less than 100perhundred thousand in the province with a high incidence of 120 perhundred thousand among the main focuses incidence of Brucellosis are in rural areas. The centers are located in West and East.

DISCUSSION

This study was to determine the focus the incidence in Kermanshah in rural and urban areas and wasdetermined to exist a significant difference between the incidence in urban and rural areas, there are also major focousBrucellosis incidence in these areas are different. Studies have identified risk factors for Brucellosis in different parts of the world and behavior such as dairy foods and keeping pet is important to develop [15]. In a study that has been made in Kurdistan has been determined that more than 90 percent of the infected people are rural with less than 40 percent of the total population of province are included villagers[16]. This amount is above amount of our studies. Saudi Arabia is 63/5 percent [17]. The study of Turkey 58/7(18) and in Babol60/8 percent [11] Cases contracted with Brucellosis were resident in rural areas. This is less than the amount of reverberation in our study. The high rate of infection with Brucellosis in rural areas can be expected because in the villages have been kept most of houses the cattle and be main part of job and consumption of unpasteurized daring products more than urban areas and with regard to health care centers in health much daring products have been presented in the city of pasteurized and practical improvement also lack of maintenance livestock in parts of urban that we observe down incidence rate. In a study using GIS in the distribution space regions in Iran by using study of Ecology rural axis in the region – Bardsir, Kerman province has been dealt with to study the space distribution of Brucellosis. The result of this study showed that out of every ten thousand overall year 141/6 on infected with disease. There are the greatest risk in the among villages that have been put north and south Bardsir [19]. Their study shows using ofhealth information we will be able to produce plans for Brucellosis disease danger that these maps will improve quality of control of this disease in Iran. In this study, we have prepared the risk of Brucellosis in rural areas and urban areas that can be used by planners to health.

CONCLUSION

In this study it was clear that to existthe difference between the amount of the Brucellosis in rural and urban areas. Also the center of the occurrence of the Brucellosis or in other words the center of Brucellosis identified in urban areas and in rural areas of Kermanshah and have been submittedthe plans. The center of the main danger or high rate of the appearance is located the urban areas in the west province special town palace and Qasreshirin.

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Table 1: some infected cases in urban and rural places in Kermanshah.

city	2009		2010		2011		2012		Total	
	urban	rural	urban	rural	urban	rural	urban	rural	urban	rural
EslamAbad	**3**	**74**	**9**	**131**	**9**	**59**	**4**	**74**	**25**	**338**
Pave	**0**	**2**	**3**	**18**	**1**	**9**	**0**	**2**	**4**	**31**
Salas	**0**	**34**	**0**	**79**	**2**	**46**	**1**	**16**	**3**	**175**
Jvanrod	**2**	**19**	**4**	**21**	**3**	**6**	**0**	**3**	**9**	**49**
Dalaho	**3**	**56**	**6**	**115**	**12**	**73**	**3**	**52**	**24**	**296**
Ravansar	**4**	**1**	**4**	**9**	**2**	**11**	**6**	**16**	**16**	**37**
Sarpol	**14**	**50**	**30**	**110**	**7**	**77**	**4**	**54**	**55**	**291**
Songhor	**14**	**134**	**19**	**207**	**1**	**78**	**3**	**43**	**37**	**462**
Sahneh	**3**	**60**	**6**	**89**	**2**	**40**	**3**	**34**	**14**	**223**
Qasreshirin	**28**	**14**	**40**	**19**	**13**	**19**	**4**	**4**	**85**	**56**
Kermanshah	**6**	**138**	**29**	**224**	**23**	**152**	**17**	**119**	**75**	**633**
Kangavar	**2**	**18**	**7**	**74**	**2**	**26**	**3**	**20**	**14**	**138**
Gilangkrb	**15**	**113**	**22**	**200**	**9**	**53**	**7**	**41**	**53**	**407**
Harsin	**5**	**28**	**15**	**62**	**10**	**32**	**5**	**47**	**35**	**169**
total	**99**	**741**	**194**	**1358**	**96**	**681**	**60**	**525**	**449**	**3305**

Table 2: Avrege of incidence of Brucellosis in urban resistances of Kermanshah.

City	Incidence	City	Incidence
Kermanshah	2/19	Gilangkrb	56/07
Ravansar	16/3	Pave	3/33
Sarpol	38/4	Jvanrod	4/38
Eslam Abad	7/08	Sahneh	9/44
Kangavar	6/59	Harsin	15/9
Qasreshirin	118/99	Salas	5/62
Songhor	20/1	Dalaho	46/49

Table 3.Avrege of incidence of Brucellosis in rural resistances of Kermanshah.

City	Incidence	City	Incidence
Kermanshah	**91/47**	Gilangkrb	**269/76**
Ravansar	**42/47**	Pave	**29/27**
Sarpol	**148/31**	Jvanrod	**62/52**
Eslam Abad	**138/49**	Sahneh	**141/9**
Kangavar	**127/61**	Harsin	**136/1**
Qasreshirin	**193/87**	Salas	175
Songhor	**255/52**	Dalaho	**267/81**

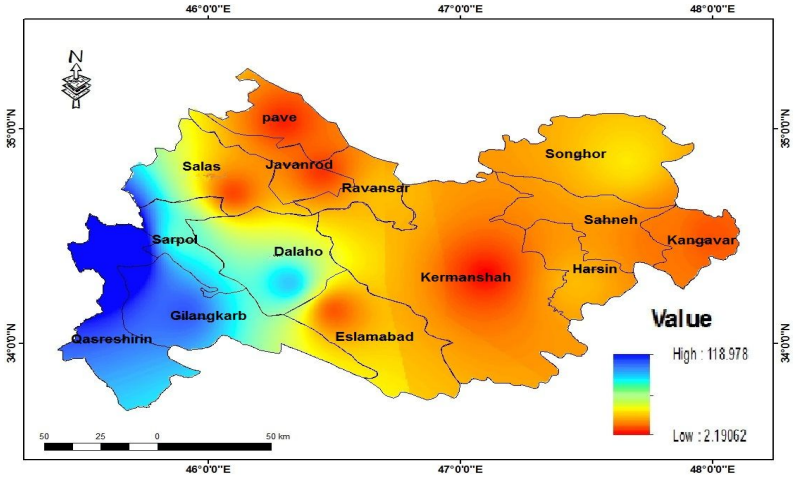

Figure 1: Map of Brucellosis according to average of incidence in urban areas.

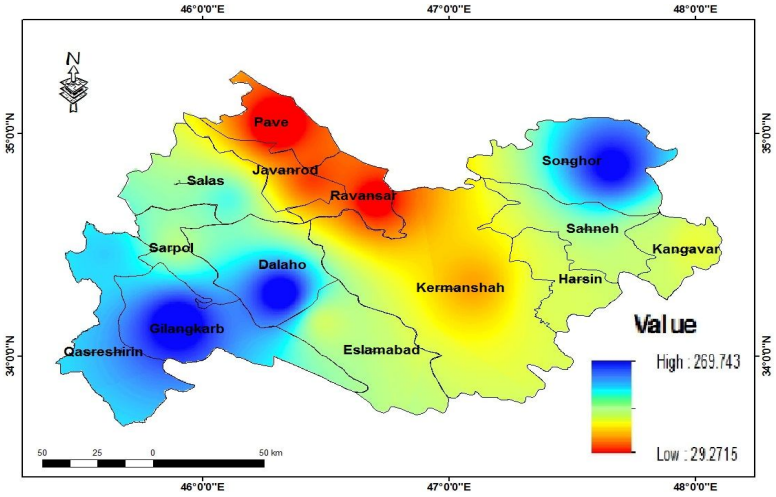

Figure 2: Map of Brucellosis according to average of incidence in rural areas.

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RESEARCH ARTICLE

Resource use Efficiency and Economic Efficiency of Pulpwood Based Agroforestry Models in Tamil Nadu, India.

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ABSTRACT

In India, the growth rate of paper industries was 10 per cent and the demand for pulpwood was 21.92 million m^3 during 2011-12. In Tamil Nadu, the demand from two leading paper industries is 8-9 lakh tonnes of pulpwood. The gap and estimated demand was 1.5 -2.0 lakh tonnes per year and the gap has to be bridged by growing trees in farm lands. The quantum of supply from farms is chiefly decided by resource use efficiency and farm level economic efficiency. Hence an attempt was made in this study to examine the resource use efficiency and economic efficiency of pulpwood based agroforestry with Cobb Douglass production function and Data Envelop Analysis (DEA). The results revealed that seedlings and inorganic fertilizer and machine hours were positively significant in eucalyptus and casuarina production. The mean technical efficiency was higher in casuarina (0.92) than eucalyptus (0.79) whereas allocative efficiency was same (0.82) for both cases. Economic efficiency was also higher in casuarina (0.75) than eucalyptus (0.65). It is concluded that by applying correct dose of inorganic fertilizers, machineries and seedling, the yield of pulpwood from eucalyptus and casuarina could be increased.

Keywords: Agroforestry, DEA, Paper industries, Resource use efficiency and Technical efficiency.

INTRODUCTION

As per an estimate in 2011, the global demand for paper and paperboard was 402 million tonnes per year. There are 7745 paper industries producing 192 million tonnes of pulp. The paper demand has doubled in the last 20 years from 242.79 million tonnes in 1990 to 402 million tonnes in 2011-12. The per capita consumption of paper in India was only 9.3 kg in 2011 as against 42 kg in China, 22 kg in Indonesia, 25 kg in Malaysia, 250 kg in Japan, 325 kg in the USA and the world average of 56.7 kg [3].In India, there are 759 paper industries, out of these 26 are wood-based and face challenges with the supply of forest-based raw material. There is fast growth of pulpwood based industries i.e., 5 per cent per annum in 1990s and 10 per cent in 2010. Each tonne of paper production requires approximately 4.5 tonnes of freshly harvested pulpwood. The pulpwood demand for pulpwood based industries had increased to 21.92 million m^3 in 2010 from 8.76 million m^3 in 2000. This will increase to 34.67 million m^3 in 2015 and 45.80 million m^3 in 2020 (IFO-FAO, 2009). Higher population growth rate, increase in per capita income and higher per capita consumption of paper has led to higher demand for pulpwood.

The present forest resources are inadequate even to meet pulpwood demand for the existing pulpwood based industries. There is a tremendous scope for the farm forestry sector to increase production and widening the growing gap between demand and availability of pulpwood. Majority of paper industries enter into contracts with local communities in the name of the contract based plantation or industrial agroforestry for producing pulpwood [6].

In Tamil Nadu, there are 39 paper industries and out of which two paper industries are popular pulpwood based industries viz., Tamil Nadu Newsprint and Papers Ltd (TNPL), Karur and Seshasayee Paper Board (SPB), Erode. These two industries alone demand 8-9 lakh tonnes of pulpwood, which are mostly derived from casuarina and eucalyptus plantations of Tamil Nadu Forest Plantation Corporation, partly from farm lands and the rest of the supplies from Bagasse and other agricultural residues. The estimate gap between demand and supply gap was 1.5 to 2.0 lakh tonnes per year. It paved way to create raw materials in an alternative method as per the guidelines of 1988 Forest Policy, both the industries have initiated farm and agroforestry plantations through contract farming system with the assured buy back system and price. Moreover, the amount of the pulpwood movement in and out from Tamil Nadu is relatively negligible compared to the total amount of the pulpwood supplied or demanded in the state. The supply from farm lands depends on the efficiency of production [4]. Hence, the study was conducted with objectives of finding resource use efficiency and economic efficiency of pulpwood plantations. The specific objectives are i) to estimate the costs and returns of eucalyptus and casuarina, ii) to find out the resource use efficiency and iii) to find out economic efficiency.

METHODOLOGY

Sampling and Data collection

In Tamil Nadu, there are Seven Agro-Climatic Zones purposefully. Among the seven Agro-climatic zones, Western zone was purposively selected and where two major pulpwood based industries like Tamil Nadu Newsprint and Papers Limited (TNPL) and Seshasayee Paper Board (SPB) were located. Based on area under pulpwood based plantation 3 districts were selected. Using the same criterion one taluk per district and two blocks per taluks were selected. In the second stage, five villages per block were selected. From each selected village five farmers were selected at random.Thus the total sample size was 150. Primary data were collected using pre-tested interview schedule through personal interviews during 2010-11.

Tools of Analysis

Conventional Analysis

Average and percentage analysis were carry out wherever necessary

Functional Analysis

Production function analysis was employed to find out the resource use efficiency in Eucalyptus and Casuarina pulpwood production. The estimated values of the regression co-efficient and R^2 were tested for statistical significance. The Cobb-Douglas production function of the following form was finally specified based on a *priori* expectations.

$$Y = a\ X_1^{b1}\ X_2^{b2}\ X_3^{b3}\ X_4^{b4}\ X_5^{b5}\ X_6^{b6}\ U_t$$

Where, Y -Yield of pulpwood (Tonnes/ha), X_1 -Human labour (Mandays /ha), X_2 -Machine hours (hrs/ha), X_3 - Seedlings (Nos /ha.), X4 - Quantity of Inorganic fertilizer (Kg /ha.), X_5 - Irrigation (hrs /ha), X_6 - Quantity of plant protection chemicals (Litre /ha), U_t -Error term - a, b_1, b_2, …….. b_6 - Parameters to be estimated

Data Envelopment Analysis

In the this study, the DEA method was used because data noise was less of an issue as most of the variable in Eucalyptus and Casuarina pulpwood production were included and because of ability to readily produce rich information on Technical Efficiency (TE), Pure Technical Efficiency (PTE), Scale Efficiency (SE), Allocative Efficiency (AE), and Economic Efficiency (EE).

Given the CRS (Constant Returns to Scale) assumption, the best way to introduce DEA is via the ratio form. For each Decision-Making Unit (DMU) or firm one would like to obtain a measure of the ratio of all outputs over all inputs, such as u^Ty_o / v^Tx_o, where u is an M x 1 vector of output weights and v is a K x 1 vector of input weights. To select optimal weights one specifies the mathematical programming problem

$$\text{Max } (u^Ty_o / v^Tx_o)$$

$$\text{Subject to } u^Ty_j / v^Tx_j \leq 1,\ j= 1,2,....N,$$

$$u^T, v^T \geq 0 \qquad \text{---------- (1)}$$

This involve finding values for u and v, such that the efficiency measure of the i^{th} DMU is maximized, subject to the constraint that all efficiency measures must be less than or equal to one. One problem with this particular ratio formulation is that it has an infinite number of solutions. To avoid this one can impose the constraint $v'x_i = 1$, which provides

$$\text{Max } u^Ty_o$$

$$\text{Subject to } v^Tx_o = 1,$$

$$u^Ty - v^Tx \leq 0$$

$$u^T, v^T \geq 0 \qquad \text{---------- (2)}$$

Where, the notation change from u and v to μ and v reflects the transformation. This form is known as the multiplier form of the linear programming problem.

Using the duality in linear programming, one can derive an equivalent envelopment form of this problem:

$$\text{Min}_{\theta\lambda}\ \theta,$$

$$\text{Subject to } -y_i + Y\lambda \geq 0,$$

$$\theta x_i - X\lambda \geq 0,$$

$\lambda \geq 0,$ ---------- (3)

where θ is a scalar and λ is a N x1 vector of constants. This envelopment form involves fewer constraints than the multiple form (K+ M < N + 1), and hence is generally the preferred from to solve. The value of θ obtained will be the efficiency score of the i^{th} DMU. It will satisfy $\theta \leq 1$, with a value of indicating a point on the frontier and hence a technically efficient DMU, according to the Farrell (1957) definition. The linear programming problem must be solved for N times, once for each DMU in the sample. A value of e is then obtained for each DMU.

The CRS (Constant Returns to Scale) linear programming problem can be easily modified to account for VRS (Variable Returns to Scale) by adding the convexity constraint: $N1'\lambda = 1$ to (3) to provide:

Min $_{\theta\ddot{e}}$ θ,

Subject to $-y_i + Y\lambda \geq 0,$

$\theta x_i - X\lambda \geq 0,$

$N1' \lambda = 1$

$\lambda \geq 0,$ ---------- (4)

where θ is a scalar and λ is a N x 1 vector of constants, whereas N1 is an Nx1 vector of ones. The value of θ obtained will be the efficiency score of the i^{th} Decision Making Unit (DMU). It will satisfy $\theta \leq 1$, with a value of 1 indicating a point on the frontier and hence a technically efficient DMU, according to the Farrell (1957) definition.

Following cost minimization Data Envelopment Analysis was run

Min $_{\lambda,xi^*}$ w_{i2} x_i^*

Subject to $-y_i + Y\lambda \geq 0,$

$x_i^* - X\ddot{e} \geq 0,$

$N1'\lambda = 1$

$\lambda \geq 0,$ ---------- (5)

where w_i is a vector of input prices for the i-th DMU and x_i^* (which is calculated by the LP) is the cost minimizing vector of input quantities for the i-th DMU, given the input prices w_i and the output levels y_i. The total Cost Efficiency (CE) or Economic Efficiency of the i-th DMU would be calculated as:

$CE = w_{i2}x_i^* / w_{i2} x_i$ ---------- (6)

That is, the ratio of minimum cost of observed cost. One can then calculate the Allocative Efficiency residually as:

$AE = CE / TE$ ---------- (7)

Note that the product of Technical Efficiency and Allocative Efficiency provides the overall Economic Efficiency. All three measures are bound by zero and one.

RESULTS DISCUSSION

Area under Agroforestry

It could be seen from the Table 1 that the majority of tree growers had Eucalyptus (65 per cent) and followed by Casuarina (35 per cent). Total area of eucalyptus and casuarina in the sample farms was 90.51 hectares and 58.48 hectares, respectively in the Western zone of Tamil Nadu. Area under casuarina was in high (32.28 ha) in the sample farms of Namakkal district. Whereas area under Eucalyptus was high in Erode district followed by Karur.

Productivity of Eucalyptus and Casuarina

In table 2 productivity of eucalyptus and casuarina are presented. It is evident from table that the mean yield of eucalyptus and casuarina were 80.40 and 88.63 tonnes per hectare respectively. The standard deviation of eucalyptus (10.14) was less than casuarina (5.39) which indicates there is a less risk of yield in the study area. The yield of eucalyptus ranged between 65 to 100 tonnes per ha whereas the casuarina yield ranged between 80 to 110 tonnes per ha.

Costs and Returns

Costs and returns are estimated for eucalyptus and casuarina and the same is presented in Table 3. It could be seen from the table that the total cost of cultivation of Eucalyptus was Rs.1,35,920 and gross income was Rs.2,00,913 per hectare. The total cost of cultivation and gross return of casuarina were Rs.1,45,841 and Rs.2,16,891 per hectare respectively. The cost of production for eucalyptus was Rs.1,691 per tonne whereas cost of production for casuarina was Rs.1,646 per tonne.The benefit-cost ratio for both eucalyptus and casuarina was 1.19 and 1.28 respectively which indicates that these tree crops were financially viable enterprise over an four year period rotation in the sample farms.

Resource Use Efficiency in Eucalyptus Production

It could be seen from the table 4 that the coefficient of multiple determinants (R^2) for casuarina production was 0.61. These have indicated that about 61 per cent of the variation in eucalyptus yields is influenced by the explanatory variables included in the model. In log-log production function, the estimated coefficient values are the production elasticity of the resources used. The coefficient of machine hours was positive and significant at one per cent level with a value of 0.35, which indicated that an increase in the usage of machinery by one per cent from mean level, *ceteris paribus* would increase the yield of eucalyptus pulpwood by 35 per cent. The variable seedlings and inorganic fertilizers were positive and significant at ten per cent level with coefficient value of 0.17 and 0.09 which indicates that a one per cent change in the number of seedlings and quantity of fertilizers from the existing mean level, *ceteris paribus* would increase the yield of eucalyptus pulpwood by 17.00 and 9.00 per cent, respectively.

Resource Use Efficiency in Casuarina Production

It could be evident from the table 5 that the coefficient of multiple determinants (R^2) for casuarina production was 0.57. These have indicated that about 57 per cent of the variation in casuarina yields is influenced by the explanatory variables included in the model. The coefficients of seedlings at five per cent positively significant and human labour and machinery variables were at 10 per cent positively significant which indicated that an increase in the usage of seedlings by one per cent from the existing mean level, *ceteris paribus* would increase the yield of casuarina pulpwood by 37.00 per cent and increase in the usage of Human labours and machinery hours by one per cent from existing mean level, *ceteris paribus* could increase the yield of casuarina pulpwood by 59.00 and 58.00 per cent, respectively.

Economic Efficiency

It is evident from the Table 6 and 7 that the technical efficiency of Casuarina farms (0.92) was found to be higher than Eucalyptus farms (0.79). This result suggests that the farmers were not utilizing their production resources efficiently, indicating that they were not obtaining maximal output from their given quantum of inputs. The average pure technical efficiency score was found to be high in casuarina (0.96) and in eucalyptus (0.91) which indicates that there is a low potential to increase the output level by 9 per cent in Eucalyptus and 4 per cent in Casuarina. The mean scale efficiency of Casuarina farms was higher (0.95) than Eucalyptus farms (0.86). This result showed that 5 per cent and 14 per cent of scale efficiency could be increased in casuarina and eucalyptus farms by operating in optimal scale size, given the current state of technology. If the farmers operate in optimal scale size, farm productivity and income will increase. If the farmers operate in optimal scale size, farm productivity and income would increase. Sathya (2011) [5] reported the mean scale efficiency of only 0.53 in tomato production and in Nigerian farms it was 0.69. [1]. Allocative

efficiency of both farms had same efficiency (0.82). This implies that yield could be increased by 18 per cent in the area through proper utilization of resources in optimal proportions given their respective prices and the current state of technology. Benjamin *et al.* (2011)[1] reported that the mean Allocative efficiency was low with 0.14 in Nigerian farms.

The Economic efficiency of casuarina was higher (0.75) than eucalyptus farms (0.65). This result suggests that the 25 per cent and 35 per cent of production costs in casuarina and eucalyptus were wasted relative to the best practiced farms producing the same output and facing the same technology in the study area. This extra production costs can be reduced at the allocatively and technically efficient point with the given current state of technology.

Frequency level of Different Efficiency Measures for Eucalyptus and Casuarina Farmers

The result in Table 8 & 9 shows that 37.11 per cent of eucalyptus sample farmers operated in a technical efficiency range of 71 to 80 per cent whereas 66.05 per cent of casuarina farms were in the range of 91-100 per cent. The same trend was observed in both scale and allocative efficiencies of casuarina and eucalyptus farms. Nearly 48 per cent of eucalyptus farms had the economic efficiency of less than 60 per cent, whereas nearly 40 per cent of casuarina farms had the economic efficiency of 71-80. The reason for higher efficiencies scores for casuarina is due to the short rotation (3 years) and good management practices.

CONCLUSION

The resource use efficiency of Eucalyptus revealed that seedlings, inorganic fertilizer and machine hours were significant whereas in Casuarina, seedlings, human labour and machinery hours were significant. Casuarina has the highest yield range from 80 to 110 tonnes per hectare whereas eucalyptus yield range from 65 to 100 tonnes per hectare. The area under eucalyptus was higher than casuarina in the sample farms with 90.51 hectares and 58.48 hectares respectively. The gross income from eucalyptus cultivation was Rs.2,00,913 per hectare and casuarina was Rs.2,16,891 per hectare which was higher in the shorter rotation. The benefit-cost ratio for both eucalyptus and casuarina was greater than one which indicates financial viability of these tree crops.

The mean technical efficiency of Eucalyptus and Casuarina based agroforestry model was 0.79 and 0.92 respectively which indicates that technical efficiency of eucalyptus and casuarina based agroforestry models could be increased by 21 and 8 per cent respectively through better use of available production resources. The mean allocative efficiency of both eucalyptus and casuarina was 0.82 implies that yield of pulpwood could be increased by 18 per cent in the area through proper utilization of resources in optimal proportions given their respective prices. The average economic efficiency of eucalyptus and casuarina was 0.65 and 0.75 respectively which shows that remaining 35 per cent and 25 per cent of production costs could be saved as per the best practiced farms producing the same output to meet the current demand of pulpwood from paper industries. The results indicated that proper intervention, the efficiency of these tree crops could be increased.

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Table 1: Area under Agroforestry in the Sample Farms.

Sl.No	District	Eucalyptus		Casuarina	
		Number of farms	Area in hectares	Number of farms	Area in hectares
1	Erode	38	35.32	12	13.96
2	Karur	38	31.31	12	12.34
3	Namakkal	21	23.88	29	32.28
4	**Total**	**97**	**90.51**	**53**	**58.48**
5	**Percentage**	**65.00**		**35.00**	

Table 2: Descriptive Statistics for Yield of Eucalyptus and Casuarina.

Sl.No	Particulars	Eucalyptus	Casuarina
1	Mean	80.40	88.63
2	Standard Deviation	10.14	5.39
3	Minimum	65.72	81.74
4	Maximum	100.04	109.67
5	Coefficient of variation (C.V)	12.50	5.68

Table 3: Costs and Returns of Eucalyptus and Casuarina (Rs/ha).

Sl.No	Particulars	Eucalyptus	Casuarina
1	Total cost of cultivation	135920	145841
2	Gross Return	200913	216891
3	Cost of production (Rs/Tonne)	1691	1646
4	BCR	1.19	1.28

Table 4: Resource Use Efficiency in Eucalyptus Production.

Sl.No	Explanatory variables	Regression coefficient	Standard Error	t-ratio
1	Regression constant	4.8265	0.8634	5.5899
2	Human labour (Mandays/ha.)	-0.1137 NS	0.0715	-1.5890
3	Machinery (hrs/ha)	0.3587***	0.0613	5.8477
4	Seedlings (No. /ha.)	0.1754*	0.0910	1.9270
5	Inorganic fertilizer (Kg/ha.)	0.0918*	0.0480	1.9119
6	Irrigation (hrs /ha)	-0.0832 NS	0.0634	-1.3122
7	Plant protection (Litre /ha)	-0.0793NS	0.1311	-0.6046
8	R^2	0.6152		
9	Adjusted R^2	0.5895		
10	F	23.9855*		

N = 97 ***- Significant at one per cent level, *- Significant at ten per cent level,NS - Non Significant

Table 5: Resource Use Efficiency in Casuarina Production.

Sl.No	Explanatory variables	Regression coefficient	Standard Error	t-ratio
1	Regression constant	1.2394	1.0870	1.1402
2	Human labour (Mandays /ha.)	0.5970*	0.3329	1.7932
3	Machinery (hrs/ha)	0.5883*	0.3089	1.9043
4	Seedlings (No. /ha.)	0.3771**	0.1394	2.7038
5	Inorganic fertilizer (Kg /ha.)	0.0868 NS	0.1003	0.8650
6	Irrigation (hrs /ha)	0.0981 NS	0.1115	0.8804
7	Plant protection (Litre /ha)	0.0306NS	0.0830	0.3686
8	R^2	0.5706		
9	Adjusted R^2	0.5146		
10	F	10.1904**		

N = 97 **- Significant at five per cent level, *- Significant at ten per cent level,NS - Non Significant

Table 6: Estimated Efficiency Measures for Eucalyptus Production.

Sl.No	Parameters	Mean	Standard Deviation	Minimum	Maximum
1	Technical Efficiency	0.79	0.12	0.58	1.00
2	Pure Technical Efficiency	0.91	0.07	0.74	1.00
3	Scale Efficiency	0.86	0.09	0.63	1.00
4	Allocative Efficiency	0.82	0.09	0.55	0.98
5	Economic Efficiency	0.65	0.13	0.44	0.98

Table 7: Estimated Efficiency Measures for Casuarina Production.

Sl.No	Parameters	Mean	Standard Deviation	Minimum	Maximum
1	Technical Efficiency	0.92	0.08	0.65	1.00
2	Pure Technical Efficiency	0.96	0.05	0.83	1.00
3	Scale Efficiency	0.95	0.06	0.72	1.00
4	Allocative Efficiency	0.82	0.07	0.62	0.98
5	Economic Efficiency	0.75	0.09	0.55	0.98

Table 8: Frequency Distribution of the Eucalyptus Farmers by Different Efficiencies.

Frequency Levels	Technical Efficiency	Pure Technical Efficiency	Scale Efficiency	Allocative Efficiency	Economic Efficiency
< 60	3 (3.09)	0 (0.00)	0 (0.00)	2 (2.06)	47 (48.45)
61 – 70	20 (20.61)	0 (0.00)	3 (3.09)	7 (7.21)	20 (20.61)
71 – 80	36 (37.11)	6 (6.18)	20 (20.61)	28 (28.86)	14 (14.4)
81 - 90	21 (21.64)	36 (37.11)	43 (44.32)	38 (39.17)	11 (11.34)
91 – 100	17 (17.52)	55 (56.70)	31 (31.95)	22 (22.68)	5 (5.15)
No. of. Farmers	97 (100.00)	97 (100.00)	97 (100.00)	97 (100.00)	97 (100.00)

(Figures in the parentheses indicated percentage to total)

Table 9: Frequency Distribution of the Casuarina Farmers by Different Efficiencies.

Frequency Levels	Technical Efficiency	Pure Technical Efficiency	Scale Efficiency	Allocative Efficiency	Economic Efficiency
< 60	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	2 (3.77)
61 – 70	1 (1.88)	0 (0.00)	0 (0.00)	2 (3.77)	14 (26.41)
71 – 80	4 (7.54)	0 (0.00)	2 (3.77)	20 (37.73)	21 (39.62)
81 - 90	13 (24.52)	7 (13.20)	7 (13.20)	22 (41.50)	12 (22.64)
91 – 100	35 (66.05)	46 (86.79)	44 (83.01)	9 (16.98)	4 (7.54)
No. of. Farmers	53 (100.00)	53 (100.00)	53 (100.00)	53 (100.00)	53 (100.00)

(Figures in the parentheses indicated percentage to total)

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RESEARCH ARTICLE

Evaluation of Phytochemicals and Antioxidant Activity in *Ganoderma lucidum* (W. Curst. Fr.) P. Karst. Extracts.

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ABSTRACT

Mushrooms are seasonal fungi, which occupy diverse niches naturally. Mushrooms represent a major source of potent pharmaceutical products. *Ganoderma lucidum* (W. Curst. Fr.) P. Karst. is a well known medicinal mushroom due to its therapeutic value. The present study was undertaken to evaluate the phytochemical analysis and antioxidative potential of *G. lucidum* extracts (hexane, ethyl acetate, ethanol, chloroform and aqueous). The result revealed that, all extracts indicated the presence of terpenoids, while the chloroform extract showed the presence of reducing sugars. The antioxidant activities of solvent extracts such as ethyl acetate, ethanol and aqueous of *G. lucidum* was determined by assessing the phenolic content as well as the radical scavenging activity. High total phenolic content was detected in the ethanolic (70 µg/ml GAE) and ethyl acetate extracts (47µg/ml GAE). The ethanolic extract showed 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging potentials of 82% at 100 µg/ml. Our results indicate the antioxidative potentials of *Ganoderma* extracts.

Keywords: Medicinal mushroom, *Ganoderma lucidum*, Phytochemicals, Antioxidants, Total phenolics, Solvent extracts.

INTRODUCTION

The genus *Ganoderma* belongs to the order Polyporales of the Kingdom basidiomycota [1]. *Ganoderma* is a highly valuable medicinal mushroom and is well recognized as a "miracle herb" due to its effectiveness in treating broad range of disease and disorders [2].*Ganoderma lucidum* (W. Curst:Fr) P. Karst is a well-known medicinal and an oriental fungus, which grows on a wide variety of dead and dying trees. *G. lucidum* also has a long history of use for promoting health and longevity in China, Japan and other Asian countries [3]. It is a woody mushroom commonly called as "Linzhi in Chinese, "Reishi" by the Japanese, "Hangul" or "Yeongi" in Korea, is also called "Glossy Ganoderma" or "Shiny Polyporus" in English and "Leman kwado" or "Burtuntuna" in Hause [4].

G. lucidum is a polypore that is soft (when fresh) corky and flat with conspicuous red varnished kidney shaped cap [5] the caps are irregularly knobby or elongated, but by maturity more or less fan-shaped; with shiny, vanished surface often roughly arranged into lumpy zones, red to reddish brown when mature, when young often with zones of bright yellow and white towards the margin. The stem is sometimes absent, but more commonly present; 3-14 cm long, up to 3 cm thick, twisted, equal or irregular, varnished and colored like the cap, often distinctively angled away from one side of the cap. The underside is cream colored and porous [6].

G. lucidum contains a wide variety of biochemical substance, including more than 119 different types of polysaccharides and mushroom nutraceuticals [7]. It is very unique in its pharmaceutical value and many health-promoting and therapeutic effects include the control of blood sugar level, modulation of the immune system, coronary heart disease, hypertension etc. *G. lucidum* is the most appealing, shelf fungi, and has not been reported from the plantation areas so far. Therefore, the present study is aimed to evaluate the presence of phytochemicals and estimate the antioxidative potential of *G. lucidum* extracts.

MATERIALS AND METHODS

Collection of sample

The fruiting bodies of *G. lucidum* were collected from the fallen trunk of Dipterocarpaceae tree species in the coffee plantations of Nelaji village, Kodagu district, Karnataka, in October 2012. The sample was air dried under shade to remove the moisture content. A specimen has been preserved in formalin (4%) and maintained.

Morphological observation of *G. lucidum*

The under surface of the fruiting body of *G. lucidum* was observed under stereotrinocular microscope (Lawrence and Mayo, India Ltd, Bangalore) and photographed for pore shape and spore size. Spores were dusted onto clean white sheets and observed under microscope for shape at different magnifications (10X, 40X and 100X).

Microscopic observation of spores

Thin hand sections of sample was taken and placed over a clean glass slide. The sectioned materials were treated with KOH solution (10%) in order to loosen the hyphae. The sections were washed with eosin (10%), later the sections were washed with water and finally stained with cotton blue. Sections were mounted using lactoglycerine as a mounting medium and cover glass was placed on each section. The prepared slides were observed under compound microscope (40X magnification). Later the spores were observed under Olympus Bx-40 bright field microscope (100X) and photographs of sections were documented.

Phytochemical analysis

The fruiting bodies of *G. lucidum* were weighed (500 g) and cut into small pieces, dried under shade and powdered with the help of mixer. This powder was preserved in a zip lock polythene cover until required for further use.

Preparation of solvent extracts using soxhlet method

The dried powder of *G. lucidum* (50 g) was filled in a thimble and then placed into a soxhlet chamber. Approximately 300 ml of solvents in the order of polarity like hexane, chloroform, ethyl acetate and ethanol was added to it. The mixture was extracted for 48 h, until the solvent fraction was completely extracted. The extracted solvent fractions were allowed to evaporate to remove the remaining solvent in the extracted fraction. Similarly other solvents were extracted, the dried extracts were scraped and placed in a clean pre-weighed Eppendorf tubes until for further use.

Preparation of extracts for phytochemical analysis

Aqueous extract: One g of sample was mixed with 50 ml distilled water and boiled on water bath for about 20 min and filtered. The obtained filtrate was termed as the aqueous extract which was used for further phytochemical tests.
Solvent extracts: The residue obtained from the solvent extracts were weighed as one mg and dissolved in one ml of the respective solvents and used for phytochemical studies. Preliminary qualitative phytochemical screening was done for all the extracts according to standard procedure [8].

Evaluation of Antioxidant activity

The antioxidant activities of the extracts were evaluated by two methods:

Estimation of total phenolic content

Total phenolic content were determined by Folin Ciocalteau (FC) method employing Gallic acid as standard (1mg/ml) as per the procedure of Volluri *et al*. [9] with some modifications. Different concentrations of standard (5-25 µg/ml) and the extracts (50-250 µg/ml) were taken in test tubes and 1.0 ml of FC reagent (1:1 dilution) was added. 2.0 ml of sodium carbonate (20%, w/v) was added and the mixture was allowed to stand for 45 min under the dark condition. After the specified incubation period, the absorbance of standard and samples were read at 765 nm using spectrophotometer. The concentration of total phenolics was expressed in terms of Gallic acid equivalents (µg/ml GAE).

DPPH radical scavenging assay

Different aliquots of standard (5-25 µg/ml) and aqueous extracts of plant sources were taken and the total volume was up to 250 µl with water / methanol respectively. To this 1.0 ml of DPPH (4 mg/100 ml) was added and the tubes were kept in dark for incubation at room temperature for 20 min. The absorbance of 517 nm was calculated based on the extent of reduction in the color [10].

RESULTS

Microscopic observation of *G. lucidum*

G. lucidum was collected from the plantation area of Nelaji village, Kodagu district, Karnataka. This was located on dead and fallen tree species belonging to Dipterocarpaceae. The basidiocarp was laterally stipitate or eccentric, 4–7 × 3– 8 × 1 cm, laccate, brittle, stipe reddish brown, 5–7 cm long and 1cm diameter. The upper surface is lacccate, sulcate, semidull and dark reddish brown. The margin is 2 mm in thickness, sterile, yellowish to reddish brown (Fig. 1).Pore surface yellowish cream; pore 6 per mm, irregular; tube 3–5 mm long, unstratified, whitish brown, contex 2 mm thick, coffee colour, thickening towards the base of the stem. Basidiospore is 6.6–7.5 × 8.3–9.1 μm, rugose and obovate (Fig. 2).

Phytochemical analysis

Phytochemical analysis conducted for five solvent extracts of *G. lucidum viz*., hexane, chloroform, ethyl acetate, and ethanol, aqueous indicated the presence of different phytochemicals. Among the solvent extracts, all extracts showed the presence of terpenoids (Table 1).The chloroform extract of *G. lucidum* indicated positive results for reducing sugars. *G. lucidum* solvent extracts did not indicate the presence of other phytochemicals like tannins saponins, flavonoids, steroids, anthroquinones, phlobatannins, glycosides and alkaloids.

Determination of total phenolic contents of solvent extracts of *G. lucidum*

The total phenolic content of *G. lucidum* was examined for different solvent extracts by using the Folin-Ciocalteau reagent with Gallic acid as standard. The results revealed that, in the ethanolic extract, high phenolic content was detected (70 µg/ml) followed by ethyl acetate (47 µg/ml), aqueous (28 µg/ml) and hexane (25 µg/ml) extracts. The chloroform extract showed low amount of phenolics (10 µg/ml). Thus high phenolic content was detected in the ethanolic extracts only. The result of the phenolic content of extracts is shown in Fig. 3.

Evaluation of antioxidant activity

The antioxidant activity of the *G. lucidum* was determined by DPPH scavenging activity using ascorbic acid as standard.The results showed that the ethanolic extract showed radical scavenging activity of 82% at 100 µg/ml followed by aqueous extract (76%) and ethyl acetate extract (67%). Potent antioxidant activity was identified in the ethanolic extract among the different solvent extracts of *G. lucidum* (Fig. 4).

DISCUSSION

In the present study, the fruiting bodies of *G. lucidum* collected from the coffee plantation of Nelaji village, Kodagu district, Karnataka, was analyzed for the presence of phytochemicals and antioxidant activity in solvent extracts.The basidiomycetous fungus, *G. lucidum* is distributed worldwide. In India, the occurrence of *G. lucidum* on gulmohar trees (*Delonix regia* Raf.) of Kerala is reported [11,12]. Likewise, the fruiting bodies have been collected from Maharashtra, Pune University campus, Tamil Nadu and Uttarkhand [13,14,15]. In our study, *G. lucidum* was collected from the plantation area of Nelaji village, Kodagu district, Karnataka. The fungus was located on dead and fallen Dipterocarpaceae tree species. The occurrence of the fungus was reported from Shivamogga district, Karnataka state from an old *Eugenia jambolana* tree [16].

The fungus was identified by their morphological characters like red shining, kidney shaped irregular, knobby and fan like cap, caps were corky in texture and pores were round to irregular in shape. In India, only nine species of *Ganoderma* have been reported until now. In our study, the phytochemical analyses were conducted for solvent extracts of *G. lucidum,* indicated the presence of terpenoids and reducing sugars. These phytochemicals are implicated in various biological activities. Terpenoids are the largest group of natural compounds have many biological activities and used for the treatment of human diseases. The phytochemical screening revealed the presence of glycosides, saponins, flavonoids, alkaloids, steroids and reducing sugars in the aqueous extracts of *G. lucidum* [12].The aqueous and ethyl acetate extracts of *G. lucidum* revealed the presence of alkaloids, flavonoids and saponins. The phytochemical analysis of methanolic and aqueous extracts of *G. lucidum* conducted by Kamra and Bhatt [15], confirmed the presence of phenols, flavonoids and ascorbic acid. The phytochemical screening of *G. lucidum* powder in various organic solvents like methanol, ethyl acetate and n-butanol, revealed the presence of flavonoids, reducing sugars, tannins, cardiac glycosides, saponins and terpenoids [4].

In the present study, the antioxidant activities in the solvent extracts were tested by two methods. The total phenolic content was determined in five solvent extracts of *G. lucidum* and high concentrations of phenolics were detected in ethanolic and ethyl acetate extracts. The total phenolic content of five edible and medicinal mushrooms cultivated in Korea revealed that high concentration of phenolics were present in the medicinal mushrooms in comparison to the edible mushrooms [17]. Phenolics possess various biochemical activities such as antioxidant, anticarcinogenic and antimutagenic activity.In our study, the antioxidant activity of *G. lucidum* in three solvent extracts (ethyl acetate, ethanol, aqueous) was evaluated. High radical scavenging activities were identified in the ethanolic as well as aqueous extracts. Significant antiperoxidative activity in the ethanolic extracts and antioxidative activity of peptides as well as the chloroform extract of *G.lucidum* has been reported [12,18,19].Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease.

CONCLUSION

Ganoderma lucidum (W. Curst:Fr) P. Karst., the highly medicinal mushroom is effective in the treatment of various ailments. The present work was aimed to evaluate the phytochemical analysis of *G. lucidum* in five solvent extracts and results indicated the presence of terpenoids in all extracts and reducing sugars in chloroform extract. The presence of phytochemicals such as terpenoids, total phenolics and radical scavenging potentials indicate that these extracts could be beneficial for the isolation of newer phytochemicals. Since, the extract demonstrated the presence of terpenoids in all solvent extracts, it can be taken up for further evaluation in *in vivo* models. As terpenoids are demonstrated to have a number of pharmaceutical applications, further purification and testing of extracts may prove its beneficiary effects.

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Table 1: Phytochemical test for different solvent extracts of *G. lucidum.*

Test	Solvent extracts				
	Hexane	Chloroform	Ethyl acetate	Ethanol	Aqueous
Tannins	-	-	-	-	-
Saponins	-	-	-	-	-
Flavonoids	-	-	-	-	-
Terpenoids	+	+	+	+	+
Steroids	-	-	-	-	-
Anthraquinones	-	-	-	-	-
Phlobatannins	-	-	-	-	-
Glycosides	-	-	-	-	-
Alkaloids	-	-	-	-	-
Reducing sugars	-	+	-	-	-

"+" = Positive for test, "-" = Negative for test.

Fig. 1. Friuting bodies of *G. lucidum* (W. Curst. Fr.) P. Karst.
A. Habit on tree trunk **B.** Close up **C.** Emergence of stipitate basidiocarp **D.** Mature basidiocarp.

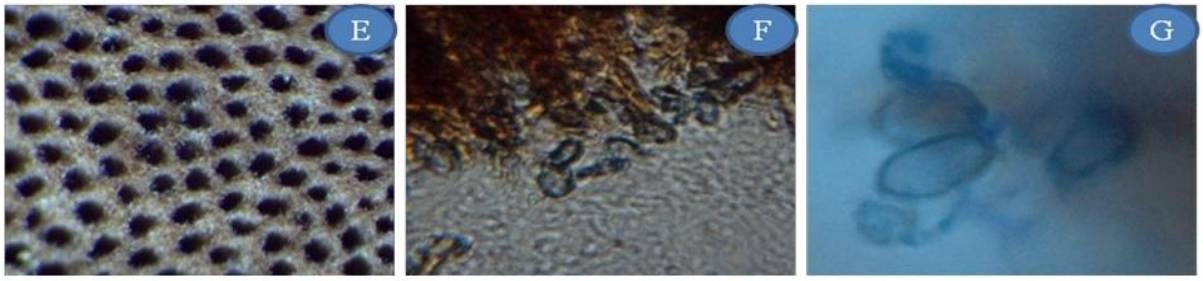

Fig. 2. Morphological characters of pore surface and spores of *G. lucidum*
A. Pore surface (20X) B**.** Spore morphology (40X) C**.** Spore structure (100X).

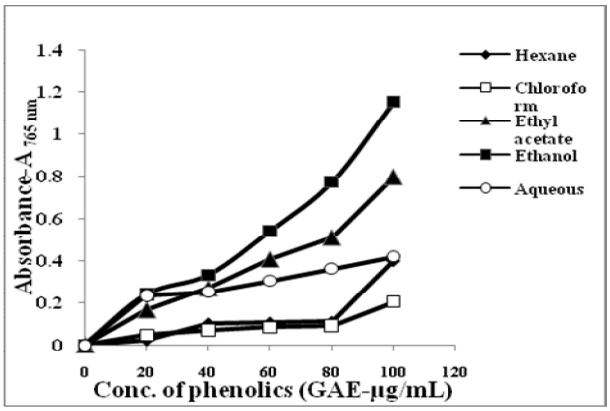

Fig. 3. Determination of total phenolic content in solvent extracts of *G. lucidum.*

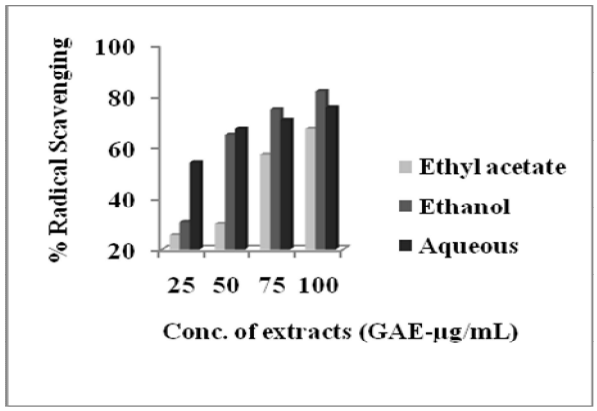

Fig. 4. Percent radical scavenging activity of solvent extracts from the fruiting bodies of G. *lucidum.*

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RESEARCH ARTICLE

Evaluation of Bore Well Water Quality for Crop Production in Karaikal Region, India.

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ABSTRACT

An experiment was undertaken to assess the quality and suitability of bore well water for commercial crop production at Karaikal district of Puduchery (Union Territory), India.Water samples from 20 bore wells (08 community wells and 12 farmers bore well) were taken to evaluate the suitability of irrigation water for crop production. Water samples were collected from individual wells during January 2011 and were analyzed for pH, EC and ionic composition to work out various quality indices. The results of the analysis had revealed that the pH of the well waters ranged from 6.5 to 7.6 and the electrical conductivity (EC) ranged from 0.60 to 5.94 dS m^{-1}. Among the cations, potassium was the lowest and its content ranged from 0.0 to 1.28 cmol L^{-1} while, Sodium recorded the highest and ranged between 4.04 – 50.56 cmol L^{-1}. Among the anionic composition, in general, chloride dominates in most of the bore wells. The depth of wells had a significant influence on majority of the parameters studied. It was observed that pH, RSC and Permeability Index increased with depth, whereas EC, Ca^{2+}, Mg^{2+}, Cl^{-}, SO_4^{2-} and Potential Salinity decreased. As per the USDA classification of irrigation waters for the suitability to agricultural use, six number of bore wells (30 per cent) were classified as C_3S_2, five number of bore wells (25 per cent) were of C_4S_2 class, four numbers (20 per cent) were of C_3S_1 class and three numbers (15 per cent) were of C_4S_1 class.

Keywords: crop production, electrical conductivity, Permeability Index, Potential Salinity.

INTRODUCTION

One of the most important inputs of intensive agriculture is water. The results of the various experiments had indicated that just giving the optimum irrigation could double the yield of a crop.Keeping this in mind, the Government of India had constructed various irrigation projects all over the country, which had brought about 45 per cent of the cultivable area under irrigation. In the U.T of Puducherry, an area of 25,600 and 13,300 ha of land entirely lies in the coastal regions of Puducherry and Karaikal, respectively.

The cropping pattern in Karaikal is Rice – Rice – Rice fallow pulse / cotton and is mainly based on the water availability from the river Cauvery and monsoon rains. In recent decades, due to the existence of monsoon uncertainty at catchment areas of river Cauvery, the supply of water from the reservoir is not only delayed but also drastically reduced to very low levels. In order to overcome these problems, the Government of Puducherry had drilled Community bore wells in Karaikal region. Apart from this community bore wells there are number of shallow bore wells available in the farmers' holdings. Due to non – availability of water in time and quantity, supplementary irrigation sources from ground water are exploited on community sharing basis. However, due to proximity to sea, rich fossiliferous marine beds of the Pleocene age (at 54 to 77m) and Cuddalore sand stones of Miocene age (at 194 to 371m), the suitability of the community bore wells are to be analyzed for sustainable cropping programme in this region. Hence, the underground water quality of Nedungadu region of Karaikal where most of the agricultural activity is undertaken is analyzed to test its suitability for irrigation.

MATERIALS AND METHODS

Water samples were collected from twenty bore wells during January, 2011. Based on the depth of bore well, the bore wells were divided into three categories *viz*. shallow (<100' depth), medium (100 – 250' depth) and deep (>250' depth) wells.Water samples were collected in clean polythene bottles from individual wells and were assigned with code numbers. Before sampling, the motor was allowed to run for about 15 minutes and the water was collected in the sample bottles after rinsing with the same bore well water. The sample bottles were tightly capped and brought to the laboratory for further analysis. The water samples were analyzed for pH, EC and for their ionic composition by employing standard methods [1,2]. The various quality indices were also worked out by employing the formulae given in Table 1.The intrusion of sea water into the ground water is determined based on the ratio of chloride to carbonate + bicarbonate as given by Todd (1995)[10]. This ratio of Cl^-/ CO_3^{2-}+ HCO_3^- is mentioned as Cl^-/HCO_3^- ratio in this study for the convenience of expression, though the carbonate content is also accounted for the calculation of this ratio.

RESULTS AND DISCUSSION

The pH, EC and the ionic composition of the water samples were provided in Table 2. The pH values of the bore wells ranged from 6.51 to 7.62 with a mean of 7.2 and co-efficient of variation of 4.0 per cent. The depth of the bore well had no significant influence on the pH of the water. All the bore well water is normal in pH and the decrease in pH might be due to increase in EC[8].The results had shown that EC values ranged from 0.6 dS m^{-1} to 5.94 dS m^{-1} with a mean of 2.2 and co-efficient of variation as 55.6 per cent. The EC is high to very high in all depth of bore wells as per USSL Staff (1954)[11]. The EC in deep bore wells was very high than shallow and medium bore wells. The high EC values were also confirmed by the dominance of Na^+ cation in association with Cl^- and SO_4^{2-} anion [3,5]. The effect of saline irrigation water may vary according to the soil type and crops chosen. The calcium content was found to be higher at deeper bore well compared to shallow depth. The dominance of calcium in ground water may indicate that they are recharged by surface waters as opined earlier by Handa (1989)[6]. In all the bore wells the Mg^{2+} dominates the Ca^{2+} concentration which also confirms the increase in EC of water. The domination of Mg^{2+} content over Ca^+

content in all depth of bore wells indicates the intrusion of sea water into the ground water [6]. The increased magnesium in irrigation water might increase the adsorption in soil which in turn lower the aggregate stability and deteriorate soil structure. The results of bore well water had shown that Na value ranged from 4.0 to 50.6 c mol L^{-1} with a mean of 20.5 and co-efficient of variation of 50.0 per cent. The Na^+ content recorded in deep bore wells was comparatively higher than shallow and medium bore wells. Although Na salts are harmful, the relative effect on soil and crop depend upon the associated anions. The K^+ content of the well water was ranged from 0.0 to1.28 cmol L^{-1}. The K content of deeper wells was higher than medium and shallow wells.

The results had shown that most of the bore well water registered a low value of Carbonate ion, which might be due to conversion of CO_3^{2-} into HCO_3^- in water sample. There is no significant variation in CO_3^{2-} content among the depth of bore wells. The HCO_3^- content in bore well water ranged from 3.0 to 9.0 c.mol L^{-1} with a mean of 6.3 and co-efficient of variation of 28.4 per cent. The HCO_3^- content recorded in shallow bore wells was comparatively higher when compared to medium and deep wells. The results revealed that Cl^- value ranged from 2.2 to 39.4 cmol L^{-1} and was higher than HCO_3^- concentration, which is indicated by the higher EC values. The chloride content increased irrespective of depth of bore wells which indicates the sea water intrusion especially in coastal areas. The adverse effect of salinity in presence of Cl^- can be caused to the crop growth especially specific ion toxicity to some crops [4]. The SO_4^{2-} concentration did not vary much with depth of bore wells. The predominance of SO_4^{2-} over HCO_3^- in some of the bore wells might indicate the process of sea water intrusion is in action [7,12]. The quality parameters were worked out and furnished in Table 3. The lowest SAR values were recorded in medium well and differ markedly from other two depths. Fifty per cent of bore wells recorded high sodicity hazard, which in due course if used for irrigation increase the Na^+ concentration in soil. Based on the SAR and EC values, 30 per cent of bore wells can be grouped under C_3S_2, 25 per cent under C_4S_2, 16 per cent under C_3S_1, 12 per cent under C_4S_1 and 5 per cent under C_2S_1.The salinity increases with depth and among 20 bore wells 11 were unsafe for irrigation. The results had shown that the RSC value ranged from -10.4 to10.2 cmol L^{-1}. The negative value of RSC indicates that Cl^- and SO_4^{2-} anion are dominating in the water. RSC decreases with depth indicating that salinity hazard is higher compared to sodicity hazard. Among 20 bore wells, 80 per cent of bore wells are unsafe for irrigation as per RSC criteria.It was observed that the Soluble Sodium Percentage was higher in all the bore wells and no correlation between SSP and depth. It was also observed that the Potential Salinity values recorded in deep wells were higher than the medium wells. Permeability Index values ranged from 0.3 to 3.3 and increased with depth. The Puri's Salt index value ranged from 20.5 to 1632.5 and all the bore wells except one showed positive values and hence unsafe for irrigation[9].The chloride to bicarbonate ratio had shown that the value ranged from 2.3 to 18.5 with a mean of 11.9 and co-efficient of variation of 38.1 per cent.The results had shown that the ratio recorded in deeper wells was higher than the medium wells. This ratio was taken as an index [10,11] to identify the sea water intrusion in irrigation water. As per the results, 65 per cent of bore wells was intruded with sea water.The intrusion has positive correlation with depth of bore wells.

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Table 1. Quality indices of irrigation water

Sl. No.	Parameters	Formula	Authors
1	Sodium Adsorption Ratio*	$Na^+/\sqrt{(Ca^{2+}+Mg^{2+})/2}$	USSL Staff (1954)
2	Soluble Sodium Percentage*	$[Na^+/(Ca^{2+}+Mg^{2+}+Na^++K^+)]X100$	Wilcox (1948)
3	Residual Sodium Carbonate*	$(CO_3^{2-}+HCO_3^-)-(Ca^{2+}+Mg^{2+})$	Eaton (1950)
4	Potential Salinity*	$Cl^-+½SO_4^{2-}$	Doneen (1963)
5	Permeability Index*	$(Na^+ + HCO_3^-/ Ca^{2+}+Mg^{2+}+Na^+)X100$	Doneen (1975)
6	Salt Index**	(Total Na^+-24.5)-[Total Ca^{2+}-(Ca^{2+}combined as Ca CO_3^{2-} +$Ca(HCO_3)_2$)X4.85]	Puri (1949)
7	Ratio of Cl^- to HCO_3^-*	$Cl^-/(CO_3^{2-}+HCO_3^-)$	Todd (1995)

* All ionic composition are expressed in c mol L^{-1} ** All ionic composition are expressed in ppm

Table 2. pH ,EC, Cations and Anions of bore well waters of Nedungadu Commune, Karaikal

Bore well No	Depth in feet	pH	EC	Ca	Mg	Na	K	CO_3	HCO_3	Cl	SO_4
			dS m^{-1}	----------(cmol L^{-1})----------							
1	85	7.39	1.55	0.8	2.0	20.16	0.108	4	9	7.8	1.79
2	110	7.32	2.51	0.8	2.6	24.07	0.279	4	4	16.6	2.93
3	110	7.33	1.71	0.8	2.8	19.07	0.318	4	7	7.2	1.89
4	120	7.17	3.00	1.0	5.8	29.28	0.654	6	6	18.4	6.07
5	120	7.38	1.36	0.8	2.4	14.35	0.387	4	8	4.2	0.72
6	120	7.19	2.59	1.8	4.8	32.50	0.449	4	8	8.8	17.50
7	150	7.5	1.81	1.2	2.2	18.76	0.290	6	6	7.4	2.09
8	150	6.9	2.36	2.2	4.8	22.30	0.508	6	9	11.6	2.19
9	160	7.26	2.52	2.2	5.2	23.04	0.105	4	8	14.4	4.27
10	160	7.53	1.88	1.4	3.6	12.76	0.408	8	3	5.0	0.47
11	180	6.9	0.60	2.4	4.0	4.04	0.562	0	5	2.2	0.15
12	170	6.91	4.42	5.2	15.4	31.02	0.641	4	7	34.8	6.07
13	185	7.12	1.95	2.2	4.8	14.83	0.300	4	6	9.0	3.27
14	143	7.36	1.63	1.4	2.0	18.91	0.295	4	7	6.8	1.99
15	179	7.42	1.37	1.6	2.0	13.35	0.621	4	5	7.2	0.80
16	182	7.21	1.15	2.6	2.2	10.87	0.226	4	4	5.4	1.15
17	205	6.51	0.86	1.6	3.2	7.24	0.000	4	4	2.6	0.39
18	1028	7.18	5.94	4.4	14.8	50.56	1.228	4	8	39.4	17.50
19	1081	7.62	2.26	3.8	4.6	19.50	0.703	6	5	12.8	3.88
20	1008	6.7	3.44	9.0	11.4	23.93	0.826	4	6	24	8.66
Min		*6.5*	*0.6*	*0.8*	*3.6*	*4.0*	*0.0*	*0.0*	*3.0*	*2.2*	*0.2*
Max		*7.6*	*5.9*	*9.0*	*15.4*	*50.6*	*1.2*	*8.0*	*9.0*	*39.4*	*17.5*
Mean		*7.2*	*2.2*	*2.4*	*4.9*	*20.5*	*0.4*	*4.4*	*6.3*	*12.3*	*4.2*
SEd		*0.3*	*1.2*	*2.0*	*4.2*	*10.3*	*0.3*	*1.5*	*1.8*	*10.1*	*5.1*
CV (%)		*4.0*	*55.6*	*84.1*	*86.6*	*50.0*	*64.1*	*34.9*	*28.4*	*82.5*	*120.7*

Table 3. Quality Indices of bore well waters of Nedungadu Commune, Karaikal

Bore well No	Depth in feet	SAR	RSC	SSP	PS	PI	Cl : HCO_3 Ratio	Salt Index	USDA class
1	85	17.04	10.2	87.39	8.70	127.00	0.60	944	C_3S_2
2	110	18.46	4.6	86.74	18.07	102.18	2.08	1033	C_4S_3
3	110	14.21	7.4	82.95	8.14	115.00	0.65	918	C_3S_2
4	120	15.88	5.2	79.71	21.43	97.78	1.53	1425	C_4S_2
5	120	11.34	8.8	80.00	4.56	127.35	0.35	810	C_3S_2
6	120	17.89	5.4	82.18	17.55	103.58	0.73	1130	C_4S_2
7	150	14.39	8.6	83.57	8.44	111.73	0.62	1163	C_3S_2
8	150	11.92	8	74.82	12.69	106.82	0.77	1148	C_4S_2
9	160	11.98	4.6	75.43	16.54	101.97	1.20	874	C_4S_2
10	160	15.25	7.2	75.20	5.24	111.30	0.45	1297	C_3S_2
11	180	2.26	-1.4	36.74	2.28	86.59	0.44	-164	C_2S_1
12	170	9.67	-9.6	59.36	37.83	73.65	3.16	767	C_4S_1
13	185	7.92	3	67.01	10.64	95.42	0.90	685	C_3S_1
14	143	14.50	7.6	83.65	7.79	116.14	0.62	857	C_3S_2
15	179	9.95	5.4	75.98	7.60	108.26	0.80	709	C_3S_1
16	182	7.02	3.2	68.38	5.97	94.89	0.68	555	C_3S_1
17	205	4.67	3.2	60.13	2.79	93.36	0.33	568	C_3S_1
18	1028	16.32	-7.2	71.22	48.15	83.94	3.28	1293	C_4S_2
19	1081	9.52	2.6	68.18	14.74	87.81	1.16	928	C_4S_1
20	1008	7.49	-10.4	53.00	28.33	67.52	2.40	235	C_4S_1
Min		*2.3*	*-10.4*	*36.7*	*2.3*	*67.5*	*0.3*	*-164*	-
Max		*18.5*	*10.2*	*87.4*	*48.2*	*127.4*	*3.3*	*1425*	
Mean		*11.9*	*3.3*	*72.6*	*14.4*	*100.6*	*1.1*	*859*	
SEd		*4.5*	*6.0*	*12.7*	*11.9*	*15.9*	*0.9*	*375*	
CV (%)		*38.1*	*180.4*	*17.5*	*83.0*	*15.8*	*78.9*	*43.7*	

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RESEARCH ARTICLE

The Influence of High Altitude on Economic Efficiency of Broiler Farming in Kerala, India.

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ABSTRACT

An investigation was undertaken to assess the economic efficiency of commercial broiler farming in high altitude area of Wayanad District of Kerala. The study was carried out under the Entrepreneurial Training program by the students of College of Veterinary and Animal Sciences, Pookode, Wayanad. A total of 314 day old commercial broiler chicks of Vencobb – 400 strain were reared under deep litter system for a period of 42 days. The standard management practices *viz*., vaccination, lighting, watering, feeding etc. were followed with necessary modifications taking in to account of the temperature and relative humidity of the region. Lighting was provided for first 10 days at the rate of 1.5 watt per chick. The chicks were fed with maize grit for first few hours. Commercial pellet crumbles as pre starter; starter and finisher were fed from 1st to 10th, 11th to 28th and 29th to 42nd days, respectively. *Ad lib* water was given with water soluble glucose in luke warm water for the first 3 days and later up to 10 days with commercial preparations of liver extract. The daily feed intake and weekly weight gain was recorded regularly. The study revealed a mortality rate of 1.27 per cent .The average market weight at 42nd day was 2.53 kg with an overall FCR of 1.66. The feed conversion ratios for the earlier first, second, third, fourth and fifth weeks were 1.04, 1.27, 1.37, 1.37 and 1.57, respectively. The Performance Index, Production Efficiency and Economical efficiency was studied. The cost of production for a kilogram broiler meat on live weight was Rs. 53.77 and the birds were sold at local market with an overall profit of Rs. 6.23 per kg live weight. Based on the results it was concluded that the broilers can be raised economically on commercial basis in the climatic conditions of Wayanad.

Keywords: Commercial broiler farming; Cost: Benefit analysis; FCR; High altitude.

INTRODUCTION

During 12th Five Year plan (2012 -2017), Indian poultry industry envisages growth rate of 11 per cent for commercial broilers, 7 per cent for layers and 3 per cent in rural poultry by indigenous advancements in genetic capabilities, veterinary health, poultry feed, poultry equipment, and poultry processing sectors. (Planning Commission of India, 2012). The production capacity has responded with increased integration and large scale implementation of contract poultry farming. Farmers in India have moved from rearing country birds in the past to rearing hybrids which ensure faster growth of chicks, higher eggs per bird, increased hatchability, low mortality rates, excellent feed conversion and consequently sustainable profits to the poultry farmers. Poultry industry contributes about Rs. 400 billion accounting for about 0.7 per cent of the national GDP and provides employment to over five million people in the Country (CARI, 2012).

Indian poultry meat production (broiler - carcass weight) increased from less than 1.0 million tons in 2000 to 3.4 million tons in 2012 with per capita consumption increasing from 0.8 kg to 2.8 kg per annum during same period. The healthy growth in poultry output over last decade makes India one of the fastest growing major world market in the segment with future growth potential remaining strong on back of wide gap against global per capital consumption norms and favorable socio economic factors.Despite such progress, the average per capital availability in India is still merely 52 eggs and 2.3kg of poultry meat and in Kerala is very low at 72 eggs/ year and that of poultry meat is at 0.9 Kg per year (Deepa G Menon, 2009) against the ICMR's recommended levels of 180 eggs and 11kg meat per annum. By Year 2030 in India, the demand for meat and eggs is likely to shoot up to 5.9 and 9.5 million metric T, respectively (CARI,2012).

According to Balram S. Yadav, MD, Godrej Agrovet, during 2013, the cost of production per bird at the farm level is about Rs 50-60, while the selling price is around Rs 60-70 in Maharashtra. A live bird costs about Rs 80-90 a kg in the market while a dressed bird costs Rs. 130- 135. National Egg Coordination Committee (NECC), explained during the same period that in Andhra Pradesh to keep the cost of production in control, poultry farmers reduced capacity by 20 per cent and farm gate price of Rs 75-83 a kg (live) where the break-even production cost was Rs 60 per kg live weight.Kerala consumes 2.4 lakh tonnes of poultry meat every year and makes for a market of Rs. 3,360 crore in size. Internal production is just 90,000 tonnes, explaining the dependence on outside sources. This is too big an opportunity for the local entrepreneurs to start broiler breeder/commercial poultry farms. The states like Andhra Pradesh, Tamil Nadu and Karnataka scale up their farm activities from 5000 to 50000 birds per week placement.

At present, In Kerala 10 -15 private hatcheries working as satellite hatcheries to the local production of chicks and chicken meat. Approximately 40 -50 thousand direct employment is generated through broiler production. (Deepa G Menon, 2009). Even though the commercial broiler production started by private parties in Kerala, no leading broiler integrators like Venkateshwara Group, Pune; Suguna Poultry Farms Ltd, Coimbatore; Pioneer Poultry Group, Coimbatore; Godrej Agrovet Ltd, Mumbai; Sky Lark group, North India; Jafa com feed had under no circumstance thought to start their integrating activities in Kerala.This may be due to lack of knowledge about climatic factors and economic viable management practices for broiler production. The ideal thermal zone for poultry production is between 24^{o}C and 26^{o}C. However, the environmental temperature in India during major period of the year is above the ideal temperature zone. The typical poultry houses in our country are open sided type, where the diurnal and seasonal variations in the environment directly impact the chicken (Usayran *et al*., 2001). In the prevailing climate, the birds are under heat/cold stress which affects the growth, egg production, egg shell quality, nutrient utilization (Usayran *et al*., 2001) and immunity (Mashaly *et al*., 2004) of birds. The mean ambient temperature is expected to rise by 1.4 – 5.8 ^{0}C by the end of 21st century (International Panel on Climate Change, 2011). Further it will worse the broiler industry and economic losses due to climate variations may amount to more than 10000 million per annum in India. In these circumstances, the requirement of Kerala broiler meat may be meeting out by promoting broiler farms in different districts of Kerala.

Among these, Wayanad district geographically located at 11. 27' to 15. 58' N latitude and 75. 47' to 70. 27' E longitude in Brahmagiri, western Ghat range of hills with altitudes of 700 meters to 2100 meters above mean sea level. The normal average annual rainfall in the district is 341.7 cm, out that 276 cm during the southwest monsoon (June – September), 34 cm in the northeast monsoon (October –November) and the rest in other months. It is recorded that number of rainy days during year 2004 was 128 with maximum temperature of 33.97^{o} C and minimum temperature of 13.87^{o} C (Vinaya Chandran, 2007). The minimum, maximum temperature, availability of water and land border connectivity with Tamilnadu and Karnataka is more suitable to start the commercial broiler farming activity in Wayanad district.

However, the economic efficiency of broiler farming is decided upon land availability, labor cost, climatic factors, availability of input materials like day old chicks, good quality raw materials for feed preparation/feed industry, vaccines, technical inputs etc. The feed efficiency is a major criterion for defining the performances to broiler chickens. In Europe, for calculation of feed efficiency is used ratio between feed intake and weight gain. In broilers highest proportion from feed ingested are used for growth because for maintenance function have low requirements. Therefore, feed efficiency is very good in broilers which induced decreased FCR value (Leeson *et al*., 1996) In this context, experimental trial was conducted undertaken to study the economic efficiency of commercial broiler farming in high altitude area of Wayanad District of Kerala. The study was carried out under the Entrepreneurial Training program by the students of College of Veterinary and Animal Sciences, Pookode, Wayanad, Kerala, India.

MATERIALS AND METHODS

A total of three hundred fourteen day old commercial broiler straight run chicks of Vencobb – 400 strains were reared in deep litter system with required floor space of 0.5 square ft per chick for first 14 days of age and chick drinker and waterer was placed, one each for 50 chicks with chick guards. After 14th days of age floor space was arranged with 1 sq ft per chick. The study was conducted at College of Veterinary and Animal Sciences, Pookode for a period of 42 days (12th June, 2012 to 27th July, 2012).The farming management recommended in Broiler Management Manual Vencobb-400, *viz*., vaccination, lighting, watering, feeding etc., were followed with necessary modifications taking in to account of the temperature, rainfall and relative humidity of the region. Commercially available RDVF (Lasota strain), IBD, and RDVF (Lasota booster) vaccines were given at 1st, 2nd and 3rd week of age respectively.The lighting schedule was different depending the chickens age (24 h light from 1 d to 7 d; 23 h light: 1 h dark from 8 d to 35 d- with dark periods on the night time and 24 h light from 36 d to 42 d) and provided 1.5 watt per chick for first 10 days.

The chicks were fed with maize grit for first few hours. Commercial pellet crumbles as pre starter; starter and finisher were fed from 1st to 10th, 11th to 28th and 29th to 42nd days, respectively. *Ad lib* water was given with water soluble glucose in luke warm water for the first 3 days and later up to 10 days with commercial preparations of liver extract. The daily feed intake, weekly weight gain, weekly feed conversion ratio was recorded regularly. Cost-benefit analysis also studied. The economic efficiency parameters *viz* Performance Index was calculated as mentioned by Jahan *et al*., 2006; Feed Conversion Ratio, European efficiency factor, European Broiler Index

$$\text{Performance Index} = \frac{\text{Live Weight in Kg}}{\text{Feed Conversion Ratio}} \times 100$$

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Feed consumed in kg}}{\text{Live weight gain in Kg}}$$

European Production Efficiency Factor (EPFF)

$$= \frac{\text{Live Weight (Kg) x Liveability (\%)}}{\text{Age at depleted (days) x Feed Conversion efficiency}} \times 100$$

$$\text{European Broiler Index} = \frac{\text{Daily weight gain (g) x survival (\%)}}{\text{10 x Feed Conversion Ratio}}$$

RESULTS

Economic Efficiency

The calculated European Production Efficiency Factor (EPEF) in this present study was 361.40 and European Broiler Index calculated as 355.0. The average body weight gain during 42 days was 60.11 gram with 99.79 survivability. Meanwhile, the live weight per bird at depletion age of 42nd age was 2.525 kg. The economic viability and performance of commercial broiler farms are decided upon by Survivability per cent, FCR at marketed age. Analysis of performance data *viz* weekly cumulative feed intake, weekly weight gain and Feed Conversion ratio (FCR), Survivability per cent, Performance Index were summarized in Table-1. Efficiency of feed measured by FCR (kg feed/kg gain) was graphically represented in Figure 1.

The feed conversion ratio recorded in present study was 1.07 during first week to 1.66 during seventh week and the survivability during this 42 growth period witnessed only 4 birds out of 314 chicks reared. The average day old broiler chick weigh was 39.69 gm. The FCR was calculated based on live weight gain during this experiment period. The data referring of Production Efficiency broiler chickens from this study are shown in Table 2. The Economic Efficiency of Broilers is summarized in Table -3.The cost: benefit analysis of the broiler farming is summarized in Table-4.

DISCUSSION

The feed conversion ratio reported in this study for Vencobb-400 strain was with in the reported value of 1.59 (Kumar *et al*., 2010) and 1.93 (Mehala *et al*., 2008) at 42nd days of age.Many broiler integrators follow measure of feed conversion ratio, European Production Efficiency Factor (EPEF), European Broiler Index (EBI) to asses the economic viability of the broiler farms. Out of these, in Europe EPEF and EBI are more widespread performance indicators used which assess environmental, climatic and management variables within and among broiler farms. Marcu *et al*., 2013 recorded EPEF values from 260.49 to 376.18 in his study with Ross 308 and Cobb 500 broiler strains for a depletion period of 42 days. The EBI values reported in the same study was 233.63 to 370.23. In this present study, the EPEF and EBI values are 361.40 and 355 respectively. The high values of EPEF and EBI in this study indicate suitable environmental, climatic and management variables in Wayanad. EPEF is used in many countries of the world as a tool for measuring growing performances to broiler chicken [Van *et al*, 2003; Broiler Management Manual Ross-308, 2009]. EBI values are always lower than EPEF, because in the ADG calculation was excluded the chicks weight to one day (Van, 2003).

The Performance Index (PI) reported by Osman *et al*., 2007 for third week to sixth week ranges between 39.61 to 93.23, where as in our study the Performance Index ranges from 59.49 to 154.22 during the same weeks of age.Probably the most important non-dietary factor influencing feed conversion is the ambient temperature of the poultry house.

Chickens are homeo-therms (warm-blooded) meaning they maintain a relatively constant body temperature regardless of the environmental temperature. Broilers perform best when there is minimal variation in house temperature over a 24 hour period of time. The meat, feed ratio was calculated to express the production efficiency; the calculated ratio in the present study was 1.456.

Apart from environmental or climatic factors, the main factors influencing the profit margin is cost of day old chicks (Rs.20/- to Rs.35/- in a year)**,** feed cost which occupies 76.60% of cost per kg live weight gain. The other factor which affect profit margin of broiler farming is market price of live bird at farm level.Osman *et al*., 2007 reported that the economical efficiency of the broiler was 3.02, where as 0.456 was reported in the present study. The difference may be due to the selling price of live bird weight and feed price in the market. In India, the commercial broiler rates at farm level are fixed by the Broiler Coordination Committee (BCC) at Palladam, Coimbatore, Tamil Nadu for southern states and the price fluctuates daily.

During this study period (on 27th July,2012), the BCC farm rate fixed at palladam was Rs.51/- per Kg live weight and broiler farm purchase rate at Wayanad would be + Rs.8 – Rs.10/- fixed by local purchaser who arrange vehicle, manpower to load the birds. The preferable market live weight at Wayanad by the local purchaser is 2.5 kg; where other parts of the Kerala they prefer live weight of 1.750 to 1.800 Kg. During the study period the live and dressed weight market was studied and Rs.90 and Rs.130 per Kg respectively. It could be concluded that, the economic efficiency performance indicators like EPEF and EBI are critically assess environmental, climatic and management variables in the commercial broiler farms indicates in Wayanad climate broilers could perform its genetic potential.

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Table 1:Weekly Cumulative feed intake, weight gain FCR, Performance Index and Survivability.

Week	Mean Cumulative Feed Intake (gms)	Mean Cumulative weight gain per bird (gms)	Feed Conversion Ratio (FCR)	Performance Index (Per cent)	Survivability (Per cent)
First Week	149.20	143.00	1.04	17.12	99.68
Second Week	514.56	405.00	1.27	34.65	99.68
Third Wk	1068.04	780.00	1.37	59.49	99.35
Fourth Week	1855.10	1350.00	1.37	101.09	100
Fifth Week	3000.17	1915.00	1.57	124.20	100
Sixth Week	4194.31	2525.00	1.66	154.22	100

Table 2:Production Efficiency of Broilers.

Parameter	Mean Value
Average Feed Intake (Kg/bird)	4.194
Live Weight per bird (Kg)	2.525
Feed Conversion Ratio	1.66
Value of Live Weight Rs/bird	151.50
Value of Feed (Rs/bird)	104.01
Meat –Feed Price Ratio	1.456

Table 3:Economic Efficiency of Broilers.

Parameter	Mean Value
Average Feed Intake (Kg/bird) = a	4.194
Price /Kg Feed = b	Rs.24.80
Total Feed Cost = a x b =c	Rs.104.01
Average Live Body weight gain (Kg/bird) = d	2.525
Price /Kg Live Weight = e	Rs.60
Total revenue = d x e =f	Rs.151.5
Net revenue = f - c = g	Rs.47.49
Economic Efficiency = g/c	0.456

Table 4: Cost Benefit Analysis of the Broiler Farming.

Parameter	Cost (Rs)	Remarks
A. Expenditure		
Cost of Chicks	9000	(Rs.30 per chick x 300 chicks)
Cost of Commercial Broiler Feed	32,240	Includes Maize Grit, Broiler pre starter, starter and finisher feed (Rs.24.80 Kg of Feed)
Miscellaneous Expenses	846	Litter material, glucose, medicines, vaccines
Total cost	42,086	Cost of Production Rs.53.77 per Kg
B. Income		
Sale of Birds	46965	(782.75 Kg *Rs 60 per kg)
Net Profit	Rs.6.23 per Kg Live Weight	Rs.46965 – Rs.42086 = (Rs.4879/782.75 Kg)

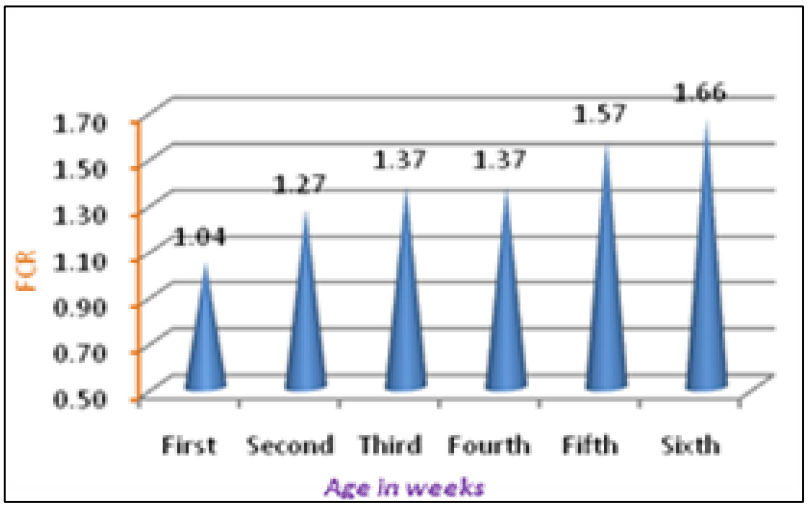

Figure 1: Feed Conversion Efficiency Ratio.

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RESEARCH ARTICLE

Influence of plant based Molluscicide on the Hepatopancreas of terrestrial slug *Laevicaulis alte* (Ferrrusac) (Mollusca : Gastropoda).

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ABSTRACT

Laevicaulis alte is one of the pulmonate pest infesting vegetables crops. Mostly they eat the vegetation available in their habitat and also devour cultivated vegetables and tubers like Tomato, Carrot , Potato and Hibiscus flowers. According to malacologists the slug *L. alte* is a notorious pest and not inferior to any other insect pests in damaging the agricultural crops.Therefore, controlling of this particular slug pests is important to minimize the infestation on agriculture land. Since two decades, malacologists recommend the organic and inorganic synthetic compounds such as calcium arsenate copper sulphate and calcium cyanide etc. to control the mollusc species. But these compounds are highly toxic and also target non-specific organisms. Malacologists recommend phytochemicals from plants as it can be conveniently used as molluscicides so that only selective organism is targeted with less detrimental effect on beneficial organisms. Thus the present investigation has been elicited to study the efficacy of plant based phyto-chemicals in regulating the mollusc in an experimental design.

Keywords: *L.alte,Agricultural* crops, ,Molluscicides,*M.azedarach, S.brevistigma.* Malacologist , Phytochemicals*, S. amaranthoids*.

INTRODUCTION

The phylum mollusca which include a substantial number of species, spread over a variety of ecological niches, have been considered a fairly successful group among the invertebrates. Next to Arthropoda, phylum mollusca have more than 100,000 living species apart from nearly 35,000 fossil forms identified so far (Hickman *et al*., 1982). Pulmonata

which forms one of the subclasses of Gastropoda contributes largely towards the abundance of molluscan species. The snail and slugs are among the most troublesome pests in many gardens and landscapes. Among the slugs the most notorious are the grey garden slug (*Deroceras reticulatum*), the banded slug (*Lehmannia poirieri*) and the three banded slug (*L.valentiana*). The slugs infest on a variety of commercially important plants such as banana, peanut, carrot etc. The slug *L. alte* has attained pest status in Cauvery Delta region and this evokes an alarming call for immediate control to save the agricultural crops and vegetables in this particular infected area. During the last two decades, several attempts have been made to control this terrestrial slug by using several organic and inorganic synthetic compounds, not only in India but also in different countries. But the toxic and persistent nature of chemical pesticides has instigated the researchers to call on their research towards the use plant products as alternative environmental safe compounds to control the molluscan species with less hazardous substances but ,more effective and readily available one (Upathayay and Sing, 2011; Sing *et al*., 2012).

Recently the researchers have attempted different formulations of molluscicides in combination with different amino acids as an attractants and one such combination is found to be very effective against *L. acuminate* (Kumar *et al*., 2012). The plant *Melia azedarach* has long been recognised as an insecticidal and medicinal plant all over the world (Kahn *et al*., 2001; Chistokhodova *et al*., 2002).The ethanol extract of *Sphaeranthus amaranthoides* has been reported for treatment including that of blood disorders, filariasis, fever and piles (Kirtikar and Basu, 1971; Swarnalatha *et al*., 2009). *Sarcostemma brevistigma* Wight (Asclepiadaceae) commonly known as soma (Sanskrit) is a leafless, trailing shrub. A fraction of this plant extract has been reported to have anti-allergic and anti-inflammatory activities (Oberai, 1985; Saraf, 1988). So there is an impetus on research towards the use of plant based products as alternative environmental friendly compounds with minimal side effects, to control the molluscs and which are more effective and readily available (Upadhyay and Sing, 2011; Sing *et al*., 2012). Hence, the present study was designed to investigate the impact of *M. azedarach, S. amaranthoids* and *S.brevistigma* extracts on the biochemistry of reproductive organs of *L. alte* and also to assess molluscicidal properties if any.

MATERIALS AND METHODS

Collection of slug

The specimens of *L. alte* were collected particularly from shady moist places and green lawns of Annamalai University campus, Chidambaram. The collected slugs were temporarily stocked in perforated polythene bags and were brought to the laboratory, Department of Zoology, Raja Doraisingam Government Arts College, Sivagangai.

Rearing of slug

The collected slug were then housed in wooden cages (40x22) layered with wet humus at the base as medium for creeping. The top of the cages were covered with glass plates to facilitate easy observation. The animals in cages were maintained at low temperature and placed at a corner to avoid the radiation effects of sun light. In each cage only ten slugs were maintained in order to avoid overcrowding.

Experimental Design

All the experimental cages were cleaned every day to prevent any infection due to deposition of excreta and leftover food stuff. The slugs showing signs of disease and fungal attack were removed periodically during pre-experimental stage. The plant leaves of *M. azedarach, S. amaranthoids* and *S. brevistigma* were collected from the backyard of houses in and around Sivagangai. Only mature slugs were chosen for experimental studies. The mature slugs were divided into seven groups with ten slugs in each group. In these seven groups one group is designated as control, which is not subjected to any treatment. The remaining six groups are considered as treatment groups. The six treatment

groups were split into three groups comprising of doublets, and every doublets receive sublethal I and II formulated from the plant extracts of *M. azedarach, S. amaranthoids* and *S. brevistigma* respectively. The treatment regimens were divided into two, the sub-lethal dose- I and the sub-lethal dose -II. For the sub-lethal dose-I the extracts were formulated differently for the three plants, for *M. azedarach* the extracts were 10ppm at concentration*, S. amaranthoides* at 1ppm concentration and *S. brevistigma* at 30ppm concentration. In the same manner for the sub-lethal dose- II, *M. azedarach* at 5ppm concentration*, S. amaranthoides* at 0.5ppm concentration and *S. brevistigma* at 15ppm concentration. The six experimental and the control groups were housed in separate cages. The hepatopancreas of the control and the treated animals were dissected out for enzyme biochemical analysis. The experiments were conducted for a period of 30 days and the animals sacrificed for enzyme biochemical analysis on 1st, 3rd, 7th, 15th and 30th day. The leaf extract was subjected to soxhlation with methanol solvent as described by Fang *et al*. (1999).

RESULTS

The slug *L. alte* were injected with sub lethal dose- I a concoction of extract constituting 3% of *M. azedarach,* 2.26% of *S. amaranthoides* and 1.58 % of *S. brevistigma* leaf extracts for five alternative days and the hepatopancreas were analysed for their alkaline phosphatase activities at different time intervals such as 1st , 3rd ,7th ,15th and 30th day of experiment. The mean values calculated from the AKP activities obtained from both control and treated slugs at 1st, 3rd, 7th, 15th and 30th day of experiments were reported in Table 1 and in graph (Figure 1). The mean AKP activities in the hepatopancreas of control slug were noted as 0.58±0.052 µmol/min^{-1}/mg protein. This level declined to 0.24 ±0.011 µmol/min^{-1}/mg protein and 0.18 ±0.010 µmol/min^{-1}/mg protein in *M. azedarach* treated slug on the 1st and 2nd day of observation. In the 3rd day and 4th day of experiments, the AKP activity fluctuated by increasing and decreasing intermittently with a mean value of 0.35± 0.038 µmol/min^{-1}/mg protein and 0.30 ± 0.025 µmol/min^{-1}/mg protein respectively. Eventually at 30th day of experiment the AKP activities showed a sharp rise recording 0.39 ± 0.037 µ µmol/min^{-1}/mg proteins. Throughout the entire period of study in treatment groups the AKP activities showed little fluctuations, but it never reached the control values. The mean percentage reduction over control were observed as 58.62, 68.96, 39.65 and 48.27 µmol/min^{-1}/mg protein respectively for 1st, 3rd, 7th, 15th and 30th day of treatments.

In the hepatopancreas of plant extract *S. amaranthoides* treated slugs, the AKP activities shows the declining trends from the 1st day to 15th day of experiment compared to the control slugs. They were noted as 0.52 ± 0.028 µmol/min^{-1}/mg proteins, 0.37 ± 0.011 µmol/min^{-1}/mg proteins, 0.31 ± 0.016 µmol/min^{-1}/mg proteins and 0.15 ± 0.007 µmol/min^{-1}/mg proteins for 1st, 3rd, 7th and 15th respectively. The mean percentage reduction over control were calculated as 13.33 µmol/min^{-1}/mg proteins, 38.34 µmol/min^{-1}/mg proteins and 48.33µmol/min^{-1}/mg proteins respectively for 1st, 3rd, 7th and 15th day of treatments. Thereafter, in the 30th day of experiment, the AKP values exhibited a abrupt increase in their activities which were noted as 0.72± 0.059 µmol/min^{-1}/mg proteins. Here the percentage elevation over control observed was 21.67 µmol/min^{-1}/mg.

In *S. brevistigma* treated hepatopancreas, the AKP activities expressed deceasing trends during the 1st, 3rd and 7th days of treatment and the values recorded were 0.40 ± 0.024, 0.35 ± 0.019 and 0.22 ± 0.012 µmol/min^{-1}/mg proteins respectively. Thereafter, the AKP activities showed a gradual increment till the 15th and same trend followed till the end of the experiment. But the increase in value during the 15th day of treatment never exceeded the values recorded with control slugs 0.42 ± 0.031 µmol/min^{-1}/mg proteins. Ironically at the 30th day of treatment the AKP activities surged enormously accounting to 0.67± 0.046 µmol/min^{-1}/mg proteins. Their percentage over control values were noted as 23.63 and -21.81.In the sub-lethal dose - II treatment, the slug *L. alte* were treated with 5ppm of *M. azedarach*, 0.5ppm of *S. amaranthoides* and 15ppm of *S. brevistigma* for five alternate days and the alkaline phosphatase activities were estimated from the both control and treated slug's hepatopancreas on the 1st, 3rd, 7th, 15th and 30th day of experiment. The mean values calculated for both control and treated slugs were reported in table 2 and it is also plotted in Figure 2. The mean AKP of hepatopancreas in control slugs were within the range of 0.56 ± 0.048 to 0.59 ± 0.047 µmol/min^{-1}/mg proteins. In *Melia azedarach* treated slugs the AKP level showed irregular fluctuations from the 1st day to 30th day of treatment. The sequence of fluctuations were noted as C >1st day > 3rd day <

5th < 30th day. In 15ppm extracts of *S. brevistigma*, the AKP level in slugs were 0.50 ±0.04, 0.43 ± 0.033, 0.36 ± 0.027 , 0.23 ± 0.015 and 0.17± 0.012 µmol/min-1/mg proteins on the 1st, 3rd, 7th , 15th and 30th day respectively. From the first day of experiment to that of the 30th day AKP levels exhibited a declining effect. The mean percentage decline in AKP of control on the 1st, 3rd, 7th , 15th and 30th of experiment were noted as 10.71%, 23.21%, 35.71%, 58.92% and 69.64%.

In 0.5ppm extracts of *S. amaranthoides*, the AKP level declined to 0.47 ± 0.035µmol/min-1/mg proteins on the 1st day but, the control maintained at 0.58 ±0.051µmol/min-1/mg proteins. A further decline recorded in the experimental animals to 0.41 ±0.037 on 3rd day, followed by 0.28± 0.018, 0.22 ±0.020 and 0.20±0.019 µmol/min-1/mg proteins respectively on 5th, 7th and 15th day. The AKP level declined gradually in all observed days, which are from the first day of experiment to 30th day. The percentage over control of increasing of AKP decline from 1st day to 30th day of experiment is noted as 18.96%, 29.31%, 57.72%, 62.06% and 65.56%.

DISCUSSION

Worldwide, from the last two decades, various synthetic molluscicides are used to control the intermediate host mollusc for Schistosomiasis disease. The shell less terrestrial slug *L. alte* has been reported to cause great economic loss in agriculture worldwide; however, there is no specific compound available to control these pests without being harmful to the non-target organisms. Due to the hazardous environmental effects of niclosamide, its toxicities to non-target organisms and even man the search for alternative safe molluscicides is still ongoing (Abdelrazek *et al.,* 2007). More than 1000 plant species and their extracts have been screened for molluscicidal activities (El-Bolkiny *et al.*, 1997).Probably suggests that many plants with molluscicidal properties remain to be discovered. Several promising plant molluscicides have been identified. Previous studies have shown that potency levels of plant samples vary significantly according to season and geographical location of the plants, such unpredictable trends in the potency of plant molluscicides militate, against their selection of control program (Brackenbury, *et al*.,1997). Various phytochemicals, viz., oils from Neem, Garlic, Gin-ger, Cedar etc individually or in combination, polyphe-nolic compounds, proanthrocyanins, flavonoid, azadirachtin, quinoline alkaloids, dinocophylline, epidinocophiline are isolated and successfully tested for potent molluscicidal activity on *Lymnea* and *Indoplanorbis* species (Singh and Singh, 1997; Muraleedharan *et al*., 1997; Hmumouchi et al., 1998;Nelymar *et al*., 1999; Bilia *et al.*, 2000).

Al-Sharkawy *et al* (1996) reported that the alkaline phosphatase of *B. alexandrina* digestive gland decreased after exposure to *Ammimajus.* Atlam (2000) stated that *B. alexandrina* haemocytes after acute exposure to *E. peplus* showed lower activity of both acid and alkaline phosphatase. Finally, ElMehaEawy and Rizk (2000) showed a decrease in alkaline phosphatase activity and increase in acid phosphatase after long-term exposure of *B. alexandrina* to diazinon. In the present investigations the plant extract *M. azedarach, S. amaranthoids* and *S.brevistigma* treated hepatopancrease the AKP activities were observed as deceasing trends for 1st, 3rd and 7th days of treatment. Thereafter, the AKP activities showed a gradual increment both in 15th and 30th days of treatments. Even though, there was elevation of AKP in the 15th day of treatment it never exceeded the AKP level observed in control slugs. From the above results, it is evidenced that the phyto-compounds present in all three plants such as*, M. azedarach, S. amaranthoids* and *S.brevistigma* have some effective molluscicidal properties. This may be due to the presence of some important molluscicidal compounds such as satriterpenoids, saponins, steroidal glycosides and alkaloids in all three plant extracts. Brackenbury (1999) has reported that the toxic effect is due to the plant secondary metabolites like triterpenoids, saponins, ste-roidal glycosides and alkaloids.

Various phytochemicals, viz., oils from Neem, Garlic, Gin-ger, Cedar etc individually or in combination, polyphe-nolic compounds, proanthrocyanins, flavonoid, azadirachtin, quinoline alkaloids, dinocophylline, epidinocophiline are isolated and successfully tested for potent molluscicidal activity on *Lymnea* and *Indoplanorbis* species (Hmumouchi *et al.,* 1998; Bilia *et al.*, 2000). The purification and analysis for chemical nature, confirmed the *C. nocturnum* compound as steroidal saponin. Marston and Hostettmann (1987); Naqvi *et al*. (1996) and Abdel *et al*. (1997) have reported that, the saponin are best molluscicidal compound. In many plants the molluscicidal activity is

due to the presence of saponin contents (Osman *et al*., 2007; Singh and Singh 2010) and alkaloid components (Melendez and Capriles, 2002; Ahmed and Rifaat 2005; Silva *et al*., 2005 and Singh *etal*., 2010). *Euphorbia aphylla* (Euphorbiaceae), *Ziziphus spina-christi* (Rhamnaceae) and *Enterolobium contortisiliquum* (Fabaceae) have been described as plants rich in saponin and or alkaloids (Mimaki *et al*., 2004; Anthony, 2005; Osman *et al*., 2007 and Siddiqui *et al*., 2009).

The phytocompounds present in all there three plants reduce the defence mechanism of the slug by reducing the alkaline phosphatase, acid phosphatase as well as reserve food materials glycogen from the slug. Hence, in the present study *Melia azedarach, Sphaeranthus amaranthoids* and *Sarcostemma brevistigma* acts as a powerful molluscide against the slug *Laevicaulis alte.* Further studies on these plants are needed to verify, whether the extracts of these plants are toxic to other invertebrates sharing the same habitat with *Laevicaulis alte.* The outcome will certainly give an idea that above plants can be used in terrestrial environment with negative ecological consequences. More studies on the mode of action of active molluscicidal components in snail body are also required to explore its full potential as molluscicide.

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Table 1. Alkaline phosphatase activities (µmol/min^{-1}/mg protein) in the hepatopancreas of sub lethal dose-I concentrations of *Melia azedarach, Sphaeranthus amaranthoides* and *Sarcostemma brevistigma* extracts *treated* slug *Laevicaulis alte.*

Days of exposure	Name of the plants		
	Melia azedarach	***Sphaeranthus amaranthoides***	***Sarcostemma brevistigma***
Control	0.58±0.052	0.60 ±0.043	0.55 ±0.035
1st day	0.24 ±0.011	0.52 ±0.028	0.40 ±0.024
3rd day	0.18 ±0.010	0.37 ±0.011	0.35 ±0.019
7th day	0.35± 0.038	0.31 ±0.016	0.22± 0.012
15th day	0.30 ±0.025	0.15 ±0.007	0.42 ±0.031
30th day	0.39 ±0.037	0.72± 0.059	0.67±0.046

Table 2. Alkaline phosphatase activities (µmol/min^{-1}/mg protein) in the hepatopancreas of sub lethal dose-II concentrations of *Melia azedarach, Sphaeranthus amaranthoides* and *Sarcostemma brevistigma* extracts *treated* slug *Laevicaulis alte.*

Days of exposure	Name of the plants		
	Melia azedarach	***Sphaeranthus amaranthoides***	***Sarcostemma brevistigma***
Control	0.59±0.047	0.56 ±0.048	0.58 ±0.051
1st day	0.22 ±0.010	0.50 ±0.041	0.47 ±0.035
3rd day	0.15 ±0.011	0.43±0.033	0.41 ±0.037
7th day	0.27 ± 0.019	0.36±0.027	0.28± 0.018
15th day	0.25 ±0.015	0.23±0.015	0.22 ±0.020
30th day	0.20 ±0.013	0.17±0.012	0.20±0.019

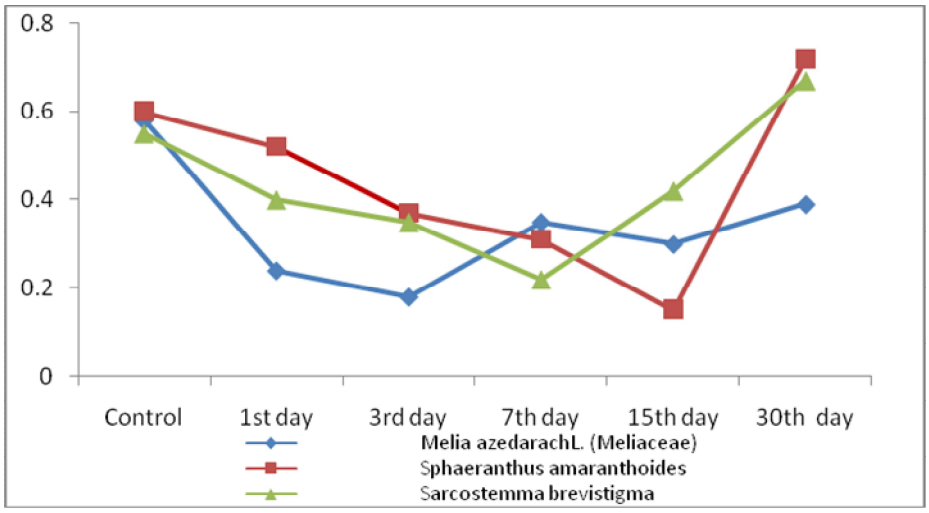

Fig.1.Alkaline phosphatase activities (µmol/min^{-1}/mg protein) in the hepatopancreas of sub lethal dose-I concentrations of *Melia azedarach, Sphaeranthus amaranthoides* and *Sarcostemma brevistigma* extracts *treated* slug *Laevicaulis alte.*

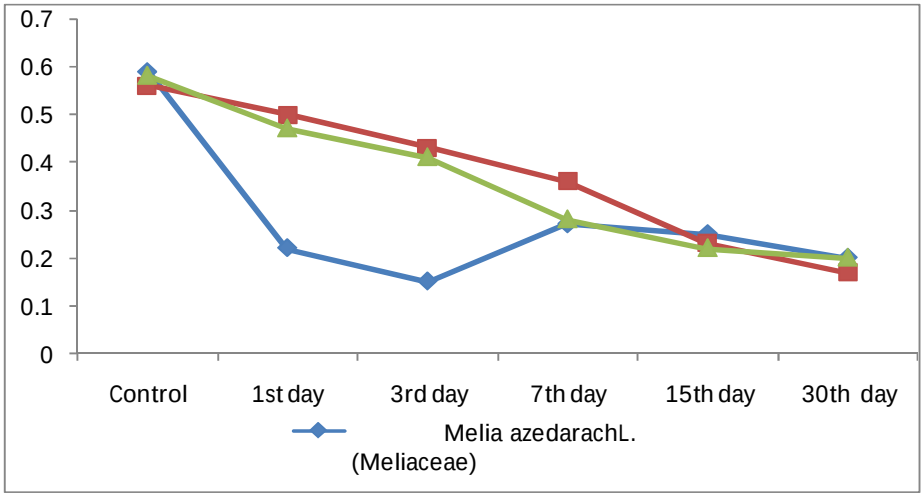

Fig.2.Alkaline phosphatase activities (µmol/min^{-1}/mg protein) in the hepatopancreas of sub lethal dose-II concentrations of *Melia azedarach, Sphaeranthus amaranthoides* and *Sarcostemma brevistigma* extracts *treated* slug *Laevicaulis alte.*

INSTRUCTION TO AUTHOR (S)

Manuscripts should be concisely written and conform to the following general requirements: Manuscripts should be type written in double-space in A4 sized sheets, only on one side, with a 2 cm margin on both sides. Research Papers should have more than 15 pages, Review Articles in the range of 15-30 pages and Short Communications up to 15 pages, inclusive of illustrations. Pages should be numbered consecutively, starting with the title page and the matter arranged in the following order: Title page, Abstract, Introduction, Materials and Methods, Results, Discussion or Results and Discussion, Acknowledgements, References, Illustrations (Tables and figures including chemistry schemes along with titles and legends) and figure and Table titles and legends. Abstract should start on a separate page and each table or figure should be on separate sheets. The titles "Abstract" and "Introduction" need not be mentioned. All other section titles should be in capital letters while subtitles in each section shall be in bold face lower case followed by a colon.

Title Page - Title page should contain title of the paper in bold face, title case (font size 14), names of the authors in normal face, upper case (font size 12) followed by the address(es) in normal face lower case. The author to whom all correspondence be addressed should be denoted by an asterisk mark. The title should be as short as possible and precisely indicate the nature of the work in the communication. Names of the authors should appear as initials followed by surnames for men and one given-name followed by surname for women. Full names may be given in some instances to avoid confusion. Names should not be prefixed or suffixed by titles or degrees. Names should be followed by the complete postal address or addresses with pin code numbers of the place(s), where the research work has been carried out. At the bottom left corner of the title page, please mention "*Address For correspondence" and provide a functional e-mail address. Address of the corresponding author to whom all correspondence may be sent should be given only if it is different from the address already given under authors' names. Trivial sub-titles such as 'Title', 'Author', 'Address' or 'Place of Investigation' shall not be included in the title page. Title page should be aligned centre except for "* Address For correspondence". Provide a running title or short title of not more than 50 characters.

Abstract - Should start on a new page after the title page and should be typed in single-space to distinguish it from the Introduction. Abstracts should briefly reflect all aspects of the study, as most databases list mainly abstracts. Short Communications as well as Review Articles should have an Abstract.

Key-words - Provide four to ten appropriate key words after abstract.

Introduction - Shall start immediately after the Abstract, as the next paragraph, but should be typed in double-space. The Introduction should lead the reader to the importance of the study; tie-up published literature with the aims of the study and clearly states the rationale behind the investigation.

Materials and Methods - Shall start as a continuation to introduction on the same page. All important materials used along with their source shall be mentioned. The main methods used shall be briefly described, citing references. Trivial details may be avoided. New methods or substantially modified methods may be described in sufficient detail. The statistical method and the level of significance chosen shall be clearly stated.

Results - All findings presented in tabular or graphical form shall be described in this section. The data should be statistically analyzed and the level of significance stated. Data that is not statistically significant need only to be mentioned in the text - no illustration is necessary. All Tables and figures must have a title or caption and a legend to make them self-explanatory. Results section shall start after materials and methods section on the same page.

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Discussion - This section should follow results, deal with the interpretation of results, convey how they help increase current understanding of the problem and should be logical. Unsupported hypothesis should be avoided. The Discussion should state the possibilities the results uncover, that need to be further explored. There is no need to include another title such as "Conclusions" at the end of Discussion. Results and discussion of results can also be combined under one section, Results and Discussion.

Acknowledgements - Should be given after the text and not in the form of foot-notes.

References - Should be numbered consecutively in the order in which they are first mentioned in the text (not in alphabetic order). Identify references in text, tables, and legends by Arabic numerals in superscript in square brackets. References cited only in tables or figure legends should be numbered in accordance with the sequence established by the first identification in the text of the particular table or figure. Use the style of the examples below, which are based on the formats used by the international journals. The titles of journals should be abbreviated according to the style used in international journals. Use complete name of the journal for non-indexed journals. Avoid using abstracts as references. Information from manuscripts submitted but not accepted should be cited in the text as "unpublished observations" with written permission from the source. Avoid citing a "personal communication" unless it provides essential information not available from a public source, in which case the name of the person and date of communication should be cited in parentheses in the text. For scientific articles, contributors should obtain written permission and confirmation of accuracy from the source of a personal communication. The commonly cited types of references are shown here, for other types of references such as electronic media; newspaper items, etc. please refer to ICMJE Guidelines (http://www.icmje.org).

Articles in Journals

1. Devi KV, Pai RS. Antiretrovirals: Need for an Effective Drug Delivery. Indian J Pharm Sci 2006;68:1-6.
   List the first six contributors followed by *et al*.
2. Volume with supplement: Shen HM, Zhang QF. Risk assessment of nickel carcinogenicity and occupational lung cancer. Environ Health Perspect 1994; 102 Suppl 1:275-82.
3. Issue with supplement: Payne DK, Sullivan MD, Massie MJ. Women's psychological reactions to breast cancer. Semin Oncol 1996;23(1, Suppl 2):89-97.

Books and other Monographs

4. Personal author(s): Ringsven MK, Bond D. Gerontology and leadership skills for nurses. 2nd ed. Albany (NY): Delmar Publishers; 1996.
5. Editor(s), compiler(s) as author: Norman IJ, Redfern SJ, editors. Mental health care for elderly people. New York: Churchill Livingstone; 1996.
6. Chapter in a book: Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Brenner BM, editors. Hypertension: pathophysiology, diagnosis, and management. 2nd ed. New York: Raven Press; 1995. p. 465-78.

Illustrations: Tables - Should be typed on separate sheets of paper and should not preferably contain any molecular structures. Only MS word table format should be used for preparing tables. Tables should show lines separating columns but not those separating rows except for the top row that shows column captions. Tables should be numbered consecutively in Arabic numerals and bear a brief title in capital letters normal face. Units of

measurement should be abbreviated and placed below the column headings. Column headings or captions shall be in bold face. It is essential that all tables have legends, which explain the contents of the table. Tables should not be very large that they run more than one A4 sized page. Tables should not be prepared in the landscape format, i. e. tables that are prepared width wise on the paper.

Figures - Should be on separate pages but not inserted with in the text. Figures should be numbered consecutively in Arabic numerals and bear a brief title in lower case bold face letters below the figure. Graphs and bar graphs should preferably be prepared using Microsoft Excel and submitted as Excel graph pasted in Word. These graphs and illustrations should be drawn to approximately twice the printed size to obtain satisfactory reproduction. As far as possible, please avoid diagrams made with India ink on white drawing paper, cellophane sheet or tracing paper with hand written captions or titles. Photographs should be on glossy paper. Photographs should bear the names of the authors and the title of the paper on the back, lightly in pencil. Alternatively photographs and photomicrographs can be submitted as jpeg images. Figure and Table titles and legends should be typed on a separate page with numerals corresponding to the illustrations. Keys to symbols, abbreviations, arrows, numbers or letters used in the illustrations should not be written on the illustration itself but should be clearly explained in the legend. Avoid inserting a box with key to symbols, in the figure or below the figure. In case of photomicrographs, magnification should be mentioned either directly on them or in the legend. Symbols, arrows or letters used in photomicrographs should contrast with the background. Method of staining should also be mentioned in the legend.

Chemical terminology - The chemical nomenclature used must be in accordance with that used in the Chemical Abstracts.

Symbols and abbreviations - Unless specified otherwise, all temperatures are understood to be in degrees centigrade and need not be followed by the letter 'C'. Abbreviations should be those well known in scientific literature. *In vitro, in vivo, in situ, ex vivo, ad libitum*, *et al*. and so on are two words each and should be written in italics. None of the above is a hyphenated word. All foreign language (other than English) names and words shall be in italics as a general rule. Words such as carrageenan-induced inflammation, paracetamol-induced hepatotoxicity, isoproterenol-induced myocardial necrosis, dose-dependent manner are all hyphenated.

Biological nomenclature - Names of plants, animals and bacteria should be in italics.

Enzyme nomenclature - The trivial names recommended by the IUPAC-IUB Commission should be used. When the enzyme is the main subject of a paper, its code number and systematic name should be stated at its first citation in the paper.

Spelling - These should be as in the Concise Oxford Dictionary of Current English.

SHORT COMMUNICATIONS

The journal publishes exciting findings, preliminary data or studies that did not yield enough information to make a full paper as short communications. These have the same format requirements as full papers but are only up to 15 pages in length in total. Short Communications should not have subtitles such as Introduction, Materials and Methods, Results and Discussion - all these have to be merged into the running text. Short Communications preferably should have only 3-4 illustrations.

REVIEW ARTICLES

Should be about 15-30 pages long, contain up-to-date information, comprehensively cover relevant literature and preferably be written by scientists who have in-depth knowledge on the topic. All format requirements are same as

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those applicable to full papers. Review articles need not be divided into sections such as materials and Methods and Results and Discussion, but should definitely have an Abstract and Introduction, if necessary.

PUBLICATION FEE

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