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CONTENTS

SUPPRESSING EFFECT OF *ASPERGILLUS FUMIGATUS* AND *BACILLUS SUBTILIS* ON *MELOIDOGYNE INCOGNITA*

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ABSTRACT

Study was conducted on the possible potential mechanism of root-knot nematode management by using two of the most promising bio-control agents (*Aspergillus fumigatus* and *Bacillus subtilis*) at different concentrations of culture filtrate (CF) *viz.* 0% (control), 25, 50, 75 and 100%. Single egg-mass was kept in petri plate containing 5 ml suspension of each concentration. Plate was incubated for 5 days in BOD at 28±2 °C for juveniles hatching. After 5 days of incubation counting of total hatched and mortile juveniles was done. Results revealed that the culture filtrates of *B. subtilis* and *A. fumigatus* caused high mortality and great suppression in egg hatching at all the concentrations. The CF of *B. subtilis* and *A. fumigatus* at 100% concentration was found to be highly effective in both mortality and in suppression of egg hatching and followed by the low concentrations. This study indicated that the metabolites released by the bacteria and fungi having toxic effects against root-knot nematode, *M. incognita*.

Key words: *Bacillus subtilis, Aspergillus fumigatus, Meloidogyne incognita, culture filtrate, egg mass, hatching, mortality.*

Indian economy still is based on agriculture but most of agriculture products are damaged by one or another various kind of pests. Among which root-knot nematodes is the serious in terms of economic loss in a variety of crops. To control such nematode pests, tons of nematicides are being used by the farmers. Due to the environmental and health concern, various bio-control agents have replaced the chemical nematicides. Besides natural and cultural techniques to control the nematodes, several biotic and abiotic factors, including chemical and physical properties of the soil and soil micro biota, affect the ability of applied beneficial organisms to colonize, disperse and produce necessary compounds inhibitory to plant pathogens (Duffy and Défago, 1999; Dickson *et al.*, 1994). Nonpathogenic rhizobacteria and fungi have been shown to be promising microorganisms for controlling the plant-parasitic nematodes (Siddiqui and Mahmood, 1999: Anke, *et al.*, 1995). Initial investigations on antagonistic rhizobacteria against *Meloidogyne* spp. include works of Zavaleta-Mejia and van Gundy (1982), Becker *et al.* (1988) and Kloepper *et al.* (1992). In a screening programme, isolates from the rhizosphere of wild and cultivated plant species caused marked reduction in *Meloidogyne javanica* penetration of mungbean roots (Siddiqui *et al.*, 2001). The aim of the present investigation was to determine the potentiality of fungi (*Aspergillus fumigatus*) and (*Bacillus subtilis*) culture filtrate *in vitro* hatching and mortality of hatched juveniles of *Meloidogyne incognita*.

MATERIALS AND METHODS

During a survey of various vegetable cultivated fields of district Meerut, some species of soil borne fungi and rhizobacteria were isolated from the rhizospheric roots of the host plants. The strains of bacteria and fungi were cultured on nutrient agar medium and Potato agar medium respectively and sub cultured for the purification. The identified strains were the rhizobacteria (*B. subtilis*) and four species of fungi *viz. Aspergillus fumigatus, A. flavus, A. niger* and *A. terreus*, of which *A. fumigatus* was chosen for

the experimental trial as it produces toxicogenic metabolites but not aflatoxicogenic (Wilson *et al.*, 1968) and is a promising antagonist to nematodes (Tripathi *et al.*, 2006; Siddiqui *et al.*, 2004).

Culture filtrate was obtained from the pure culture of *A. fumigatus* grown for a week in BOD at 28±2°C in 250 ml capacity Erlenmeyer flasks containing 100 ml potato dextrose broth (PDB) medium. Whatman filter paper (No. 1) was used to filter the broth. To obtain CF of *B. subtilis*, pure culture was grown in King's broth liquid medium (King *et al.*, 1954) at 28±2°C in 250 ml capacity Erlenmeyer flasks for 5 days in BOD. The liquid medium was centrifuged at 2800 g for 20 minutes. The supernatant was discarded and the pellet re-suspended in KB liquid medium and for the preparation of culture filtrate, the remaining half of the bacterial culture was centrifuged again at 2800 g for 20 minutes. The pellet was discarded and the supernatant collected in a beaker. The supernatant was passed through two layers of Whatman No. 1 filter paper and the filtrate was collected in the beaker.

Further low concentration of CF were prepared as 100% (10 ml CF alone), 75 % (7.5 ml CF+2.5 ml distill water), 50% (5 ml CF+5 ml distilled water), 25% (2.5 ml CF+7.5 ml distilled water) and 0% (10 ml distilled water alone) for the determination of ovicidal and larvicidal activities. *In vitro* the surface sterilized one egg mass of *M. incognita* was kept in 5 ml suspension of each concentration in glass petri plates. Egg mass in distilled water alone served as control. Each treatment was replicated thrice and petri plates were kept in BOD at 28±2°C for 5 days. After 5 days of incubation, the number of total hatched and dead juveniles was counted and percentage mortality as well as inhibition in hatching was calculated.

RESULTS AND DISCUSSION

Ovicidal Activities of Culture Filtrates of both the bio-agents

Culture filtrate of *B. subtilis* was found significant than the CF of *A. fumigatus* at all the concentrations and the highest inhibition in hatching was occurred at 100% concentration of both *B. subtilis* and *A. fumigatus*, 85.71% and 70.14%, respectively and followed by the low concentrations in comparison to control (Table 1).

Larvicidal activity of culture Ffiltrates of both the bio-agents

The observations on larvicidal activities showed the highest mortality of J_2 in CF of *B. subtilis* in comparison to *A. fumigatus* over total hatching at all the concentrations and followed by the low concentrations. At lowest concentration (25%), the CF of *A. fumigatus* and *B. subtilis* showed 4.71% and 3.85% mortality, respectively (Table 1).

The observations of the present study revealed that the toxins released by fungus may suppressed the egg hatching and caused mortality of J_2 while the enzymes of the bacteria may hydrolyzed the cuticle of the J_2 and degrade the gelatinous matrix of the egg-mass and inhibit the egg hatching. Most of the soil-inhabiting species of *Bacillus* secretes collagenolytic/proteolytic enzymes that can hydrolyze collagens in their natural forms. The entire surface of plant parasitic nematodes is covered by a multilayered cuticle (Bird and Bird, 1991) and the major structural components of these cuticles are collagens (Kingston, 1991). Hence in the author's opinion, cuticle degradation could be an effective way of controlling parasitic forms of root-knot nematodes. Sigal *et al.* (1998) isolated collagenolytic/proteolytic enzyme from the *Bacillus cereus* and purified and characterized against *Meloidogyne javanica*. Galper *et al.* (1989) reported that the application of collagen to the soil led to the enrichment of collagenolytic microorganisms which drastically reduced the number of galls caused by *M. javanica* on tomato roots. Bin-Li *et al.* (2005) reported that *B. subtilis* (B7) exhibited strong nematicidal activity by killing the second stage larvae of *M. javanica and* toxic principles of bacterium B7 showed the highest juvenile mortality and enhanced the growth of mungbean plants. Active compounds that affect egg hatch and second-stage juvenile mobility

Table 1: Effect of *B. subtilis* and *A. fumigatus* culture filtrates on egg hatching and mortality of *M. incognita* juveniles after 5 days of incubation.

Treatment	*B. subtilis*				*A. fumigatus*			
	Hatching (J2)	Inhibition over control (%)	Mortality (J2)	Mortality (%)	Hatching (J2)	Inhibition over control (%)	Mortality (J_2)	Mortality (%)
CF @ 100%	28.00±1.53 * (26.00-31.00)	85.71	13.33±0.88 (12.00-15.00)	47.60	60.00±2.89* (55.00-65.00)	70.14	18.00±1.15 (16.00-20.00)	30.00
CF @75%	28.67±0.88 * (27.00-30.00)	85.38	12.67±0.33 (12.00-13.00)	44.17	99.00±1.73* (96.00-102.00)	50.90	13.33±0.88 (12.00-15.00)	13.46
CF @ 50%	39.33±1.20 * (37.00-41.00)	79.93	4.67±0.33 (4.00-5.00)	11.87	136.67±4.41 * (130.00-145.00)	32.00	10.67±0.88 (9.00-12.00)	7.80
CF @ 25%	95.00±1.73 * (92.00-98.00)	51.53	3.67±0.88 (2.00-5.00)	3.85	183.67±0.88 * (182.00-185.00)	8.62	8.67±0.67 (8.00-10.00)	4.71
CF @ 0%	196.00±4.93 (188.00-205.00)	0.00	0.00	0.00	201.67±4.41 (195.00-210.00)	0.00	0.00	0.00

Data are mean of three replicates; ± are √-transformed values.
* Significant different as compared to control at 0.05 levels.
CF = Culture filtrate; J_2 = Second stage juvenile

of *M. incognita* and *Heterodera glycines* were identified from nematode-associated fungi and from rhizosphere-inhabiting bacteria and fungi (Nitao *et al.*, 1999; Meyer *et al.*, 2000). Earlier workers reported that several other microbial bio-agents such as *Arthrobotrys*, *Bacillus*, *Burkholderia*, *Paecilomyces*, *Pseudomonas*, and *Verticillium* might be used to enhance systems for the management of plant-parasitic nematodes (Kerry, 1998; Sikora and Hoffmann-Hergarten, 1993; Siddiqui and Mahmood, 1996; Stirling, 1991). Nemec *et al.* (1996) suggest that bio-agents are very efficacious to control root diseases of vegetables and citrus. The studies indicated that the bacterium and fungal filtrates are promising antagonists to *M. incognita* and have potential for application as novel nematicide, and as live bio-control agents.

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REFERENCES

Anke, H., Stadler, M., Mayer, M.A. and Sterner, O. 1995. Secondary metabolites with nematicidal and antimicrobial activity from nematophagous fungi and Ascomycetes. *Canadian J. Bot.*, **73**: S932-S939.

Becker, J.O., Zavaleta-Mejia, E., Colbert, S.F., Schroth, M.N., Weinhold, A.R., Hancock, J.G. and vanGundy, S.D. 1988. Effects of rhizobacteria on root-knot nematodes and gall formation. *Phytopathol.*, **78**: 1466–1469.

Bin-Li, G-L. X., Soad, A. and Cooseman, J. 2005. Suppression of *Meloidogyne javanica* by antagonistic and plant growth-promoting rhizobacteria. *J. Zhejiang Univ. Sci.*, **6B**: 496-501.

Bird, A.F. and Bird, J. 1991. The exoskeleton. In: *The structure of Nematodes*. Academic Press Inc., p. 55-68.

Dickson, D.W.; Oostendorp, M.; Giblin-Davis, R.M. and Mitchell, D.J. 1994. Control of plant-parasitic nematodes by biological antagonists. In: *Pest Management in the Tropics Biological Control-A Florida Perspective* (D. Rosen, F.D. Bennett and J.L. Capinera, eds.). Intercept Ltd., U.K. pp 575-601.

Duffy, B.K. and Défago, D. 1999. Environmental factors modulating antibiotic and siderophore biosynthesis by *Pseudomonas fluorescens* biocontrol strains. *Appl. Environ. Microbiol.*, **65**: 2429–2438.

Galper, S.; Cohn, E.; Spiegel, Y. and Chet, I. 1989. Nematicidal effect of collagen-amended soil and the influence of protease and collagenase. *Revue de Nematologie*, **13**: 67-71.

Kerry, B.R. 1998. Progress towards biological control strategies for plant-parasitic nematodes. *The 198 Brighton Crop Protection Conference: Pests and Diseases* 3: 739-746.

King, E.O.; Ward, M.K. and Raney, D.E. 1954. Two simple media for the demonstration of pyocyanin and fluorescein. *J. Lab. Clin. Med.*, **44**: 301–307.

Kingston, I.B. 1991. Collagen genes in *Ascaris*. Parasitic Nematodes- Antigens, Membranes and Genes. In: *Cevage Of Structural Proteins During Assembly Of The Head Bacteriophage T4* (M.W. Kenedy, ed.). Laemmli UK (1970). Nature, **227**: 680-685. pp. 66-83.

Kloepper, J.W.; Rodriguez-Kábana, R.; McInroy, J.A. and Young, R.W. 1992. Rhizosphere bacteria antagonistic to soybean cyst (*Heterodera glycines*) and root-knot (*Meloidogyne incognita*) nematodes identification by fatty acid analysis and frequency of biological control activity. *Plant & Soil*, **139**: 75–84.

Meyer, S.L.F.; Massoud, S.I.; Chitwood, D.J. and Roberts, D.P. 2000. Evaluation of *Trichoderma virens* and *Burkholderia cepacia* for antagonistic activity against root-knot nematode, *Meloidogyne incognita*. *Nematol.*, **2**:871-879.

Nemec, S.; Datnoff, L.E. and Strandberg, J. 1996. Efficacy of biocontrol agents in planting mixes to colonize plant roots and control root diseases of vegetables and citrus. *Crop Protection*, **15**: 735-742.

Nitao, J.K.; Meyer, S.L.F. and Chitwood, D.J. 1999. *In vitro* assay of *Meloidogyne incognita* and *Heterodera glycines* for detection of nematode-antagonistic fungal compounds. *J. Nematol.*, **31**: 172-183.

Siddiqui, I.A.; Ehteshamul-Haque, S. and Shaukat, S. 2001. Use of rhizobacteria in the control of root rot-root knot disease complex of mungbean. *J. Phytopathol.*, **149**: 337–346.

Siddiqui, I.A.; Shaukat, S.S. and Khan, A. 2004. Differential impact of some *Aspergillus* species on *Meloidogyne javanica* biocontrol by *Pseudomonas fluorescens* strain CHA0. *Lett. Appl. Microbiol.*, **39**: 74-83.

Siddiqui, Z.A. and Mahmood, I. 1996. Biological control of plant-parasitic nematodes by fungi: A review. *Bioresource Technology*, **58**: 229-239.

Siddiqui, Z.A. and Mahmood, I. 1999. Role of bacteria in the management of plant-parasitic nematodes: A review. *Bioresource Technology*, **69**: 167-179.

Sigal, S.; Hedva, S.; Ilan, C. and Yitzhak, S. 1998. Purification and characterization of a *Bacillus cereus* collagenolytic/proteolytic enzyme and its effect on *Meloidogyne javanica* cuticular proteins. *European J. Plant Pathol.*, **104**: 59-67.

Sikora, R.A. and Hoffmann-Hergarten, S. 1993. Biological control of plant-parasitic nematodes with plant-health promoting rhizobacteria. In: *Pest management: Biologically Based Technologies*. (R.D. Lumsden and J. L. Vaughn, eds.). Proceedings of Beltsville Symposium XVIII. American Chemical Society. Washington, D.C. pp. 166-172.

Stirling, G.R. 1991. *Biological Control of Plant-Parasitic Nematodes*. CAB International, Wallingford, UK.

Tripathi, P.K.; Singh, C.S.; Prasad, D. and Singh, O.P. 2006. Use of fungal Bio-agents for the management of *Meloidogyne incognita* infecting tomato. *Ann. Pl. Protec. Sci.* **14**: 194-196.

Wilson, B.J.; Campbell, T.C.; Hayes, A.W. and Richard, T.H. 1968. Investigation of reported aflatoxin production by fungi outside the *Aspergillus flavus* group. *Appl. Microbiol.*, **16**: 819-821.

Zavaleta-Mejia, E. and van Gundy, S.D. 1982. Effects of rhizobacteria on *Meloidogyne* infection. *J. Nematol.*, **14**: 475–476.

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STUDY IN CHANGES OF BIOCHEMICAL COMPOSITIONS IN *LABEO ROHITA* (HAM). WITH AGE GROWN IN ALKALINE SOIL POND

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ABSTRACT

Biochemical compositions viz. moisture content, fat, protein, tryptophan, lysine, cholesterol, carbohydrate and total ash were investigated of Indians major carp Labeo rohita (Ham.) under alkaline soil pond of pH 9.5 of six, eighteen, thirty six, and sixty months age of experimental fishes. Value of moisture content was decreasing with age attaining maximum value 78.4% in test fishes of soil pH 9.5 as compare to 79.8 % in normal soil pond in six month old fish. Moisture content varied inversely proportional with fat content with increasing age in both type of fishes with maximum value of fat content 2.89% in sixty month old fish .Protein and amino acids (tryptophan and lysine content) varied with age. The maximum value of protein 18.10%, tryptophan 68.92mg/g N and lysine 560mg/Gn were reported in different age group of fishes. Similarly maximum value of ash content (1.76%) and carbohydrate (4.89%) was recorded in eighteen and sixty month's old fishes respectively in alkaline soil pond. Overall, it was reported that there was no significant change in biochemical composition of muscle with age, however decrease in protein and increase in fat content was reported with increase of age, but corresponding values were lower in alkaline soil pond as compare to normal soil pond fishes.

***Key words** : Biochemical compositions, Alkaline soil pond, Protein, Trytophan, Lysine, Cholestrol and Corbohydrate etc.*

Fish production in India shares 4.81% of total world annual fish production and contribute 5.3% agricultural GDP of India. In the global fish market, India trades only 2.4% , may be apart from contributing nutritional security .Quantitatively fish production in India is increasing at much faster rate compared to food grains but consumption of fish among fish eating populations is only 56% @9 Kg per capita per annum. Therefore, as to ascertain the nutritional security some changes in Biochemical Compositions under rearing environment with growth is necessarily require, because growth is a harmonic development of important tissues such as bones, connective tissues, muscles and adipose tissues etc which comprises major fish products. It results from simulations and retention of chemical components as protein, lipids, carbohydrates, and minerals with normal and altered development of specific tissue resulting changes in composition (Benqit et al, 1995).Among the essential factors rearing environment including pH are some factors which affect the chemical compositions and protein requirement in fishes. But very little information is available on study of variability in biochemical changes in relations to age in alkaline pond as compared to normal soil ponds to assess the quality attributes in test fishes.

MATERIALS AND METHODS

The details of the Experimental materials and methods used in the present investigation have been reported else where Prakriti and Rao,A.P.(2010)

Experimental site:

Samples of fishes of different age groups were collected from fish ponds of the college of fisheries N.D.University of Agriculture and Technology Kumarganj Faizabad U.P,from newly constructed ponds of soil pH 9.5 and another lime activated 5 years old pond of pH 7.0. Biochemical analysis of muscles and body traits were conducted in laboratories of Department of fisheries and Agricultural Biochemistry of the University. Details of experimental set up may be seen elsewhere Prakriti and Rao,A.P (2010)

Biochemical analysis:

The representative sample of flesh was taken from different parts of the body with the help of sharp knife and scissor. Efforts were made to remove the intramuscular spines as much as possible with the help of scissor, knife and forceps .Samples were weighed for wet weight and then kept in oven at 70c temperature for 6-8 hours for drying. Samples were turned and temperature was maintained approximately 45c for 15-16 hours for complete drying. After drying, all the samples were removed from the oven and placed into desiccators in order to avoid contamination from atmosphere .Before the start of biochemical analysis of samples were grinded to fine powder with the help of pestel and mortar for sake of analysis and powder was stored in air tight bottles.

1. Moisture Content:

Moisture content was determined by drying the known amount of sample in a oven at 60c -70c for 8 -10 hours .Moisture content obtained by subtracting dry weight from fresh weight and it was expressed as percent fresh weight.

2. Protein Content:

The protein content was determined by Lowry's (1951) method depends on the development of colour due to reaction of alkaline copper reagent with protein.

Ten percent homogenized samples was prepared in water and centrifuged at 5000 rpm for 20 minutes. Supernatant was collected and taken 1ml of it in another test tube .Added 1ml of 10 percent TCA in it and left tube for 30 minutes and then it was centrifuged .Supernatant was discarded and residue was collected. This residue was dissolved in 0.1 N NaoH solutions. 0.5 ml of this solution was taken and made the volume up to 1 ml by distilled water.5ml alkaline copper reagent was added. Left the test tube for 5-10 minutes and then 5ml Folin's reagent was added. All the test tubes were shaked properly. The intensity of colour was recorded on spectronic -20 against bland solution at 650 nm.

3. Fat Content:

Fat content was estimated by Soxhlet method as described in A.O.A.C (1970). The powdered sample was placed in the extractor of the Soxhlet apparatus .The pre weighed flask, containing a solvent (petroleum ether 40C to 60C BP) was heated on a water bath. Thus, the glass assembly with sample was refluxed on a water bath continuously for 8-10 hours for extraction of fat. Finally, the solvent in the receiving flask (capacity 100ml) was distilled off leaving behind the pure fat in the flask. The flask was weighed with fat and the net weight of fat was collected and expressed in percent.

4. Carbohydrate Content:

Carbohydrate content was determined by the method described by Yam and Willis (1954). One gram dried sample was transferred to 100 ml stoppered measuring cylinder. Ten ml distilled water was added and stirred with the help of long glass rod to disperse the sample thoroughly .Thereafter ,13 ml of 52 percent perchloric acid was added and frequently stirred for 20 minutes. The volume was made up to 100 ml by addition of distilled water. It was then mixed properly and filtered by using what man's filter paper no 42 into a 250 ml volumetric flask. .It was diluted up to mark with distilled water and mixed properly.

Three test tubes were taken and added reagent in the following manners-

1. Took one ml sample in 1st test tube.
2. One ml water for blank sample in 2nd test tube.
3. For standard, 1.0 ml glucose (100ml /mg) was pipetted in to separated test tube.

Now, 5ml anthrone was added in each test tube and was kept in water bath for 12 minutes than cooled down at room temperature .Finally, the intensity of colour was recorded at 620 nm against blank reading on spec tonic -20.

5. Tryptophan Content:

Tryptophan content was estimated by the method of Spies and Chamber, (1949). 0.2 g homogenous fish sample was transferred in to a 100ml conical flask ten ml 19 N NH_2SO_4 was also added. The content of conical flask was kept for 12 hours in dark place. After expiry of period ,1.0 ml distilled water ,1.0ml dimethyl amino benzaldehyde (30 mg dissolved in 100ml 2N H_2SO_4) and 0.1 ml of sodium nitrite solution (0.045 percent in water)were added .This was kept for 30 minutes for co lour was measured in photoelectric colorimeter (Spectronic -20) at 620 nm.

6. Lysine Content:

Lysine content was estimated by the method described by Felker *et al.*, (1978).Fifty mg very fine fish sample was taken in 250 ml volumetric flask. Fifty ml of buffer solution (0.055N tera sodium phosphate pH- 9.4) was added gentle shaking and kept on platform of shaker for two hours at room temperature. Then it was centrifuged at 10,000 rpm for 10 minutes. The supernatant was collected and absorbance was taken at 420 nm. Sulphuric acid (50mg /ml) aqueous solution was added and again the solution was kept for shaking gentle. The absorbance was recorded at 420nm on spectronic -20. The difference in two readings was the lysine content in the sample.

7. Cholesterol Content:

Cholesterol content was estimated by the method Cheyne (1964) and expressed in unit as mg/ 100g. The stock standard solution of cholesterol was prepared by dissolving 50mg of cholesterol in 25ml of glacial acetic acid .Thus 1.0 ml of this solution containing 2ml or 2000 micrograms of cholesterol was taken.

For standard:

0.5ml of cholesterol stock standard solution was taken in a test tube and 4-5 ml of glacial acetic acid was added to it. Thus there was 1000 micrograms of cholesterol in this solution .Four test tubes were taken and numbered as 1,2,3and 4 and thereafter ,0.25, 0.50 , 0.75 and 1.0ml of cholesterol working solution was transferred to the test tube number 1,2,3 and 4, respectively. The quantity of all the tube was equalized by adding 0.75ml, 0.50ml and 0.25ml glacial acetic acid to make 1.0 ml volume in test tube. Test tube 4 already having 1.0 ml solution.

Ferric chloride working solution was prepared and 6ml of this solution was taken in each test tube and then added 4 ml of sulphuric acid and mixed the content by slow inversion and allowed to cool for half an hour. A blank sample was also prepared by adding 1.0 ml glacial acetic acid in one test tube. Added 6ml of working solution of ferric chloride and 4ml of sulphuric acid in it. , mixed by slow inversion and allowed to cool for half an hour along other 4 test tubes .The co lour intensity of standard samples was recorded on spectronic -20 at 540nm.

For sample:

15 ml of 3:1 alcohol ether mixture was taken in a 2.5 ml volumetric flask and 0.1 ml of homogenized fish sample was added to it with the help of micro pipette .The content were shaken thoroughly and allowed to stand for 30 minutes with occasional mixing. The volume was made up to 25 ml by addition of 3:1 alcohol ether mixture and filtered by whatman's no 42 filter paper in to 25ml test tube. A 2ml aliquot was transferred into another test tube and the moisture was evaporated on water bath and dried residue was allowed to cool and 6ml ferric chloride working solution was added. The tube was placed on boiling water bath. During this interval 0.05 ml of standard cholesterol solution was taken in a test tube and 6ml of ferric chloride was added in a test tube containing standard solution .Another tube containing 6ml of ferric chloride working solution was taken for preparation of blank solution too.

The tube placed in boiling water bath was removed and allowed to cool down at room temperature and 4ml sulphuric acid was added in each

of the test tube. The standard and blank solution containing tube, 4ml of sulphuric acid was also added in it. All the test tubes were tightly stoppered and mixing was carried by slow inversion. The content were allowed to stand for 30 minutes for optimum colour development .The intensity of colour was measured on spectronic -20 at 540 nm and reading for blank and standard were also recorded and the cholesterol content was calculated.

8. Total Ash Content:

Total ash content was estimated by the method as described by Hart and Fisher (1971). Two gram sample of fish was dried at 70 c temperature and transferred to ash less filter paper. The ignition of sample was carried out to non luminous flame in pre weighed silica crucible. The crucible was placed in muffle furnace. The temperature was maintained at 525- 560 (+25c) for about 5-6 hours to burn organic matters. After expiry of period, the crucible was transferred to desiccator for cooling to avoid absorption for moisture by ash. The cold ash along with silica crucible was weightened and the result was calculated and reported on moisture free basis.

RESULTS AND DISCUSSION

Biochemical composition

1. Moisture content

Average moisture content recorded in different age group of fishes of soil ponds pH 7.0 and 9.5 has been shown in table 1

The maximum moisture content in alkaline pond fishes was 78.41 percent in six months old fishes and minimum 76.73 percent in sixty months old fishes as compare to 79.81 and 77.60 % in 7.0 pH soil pond respectively.

The moisture content was not significantly differed over the effect of age in Rohu of both types of ponds. It ranged from 78-76 percent with maximum 78.4 percent in six months old fish. Minimum 76.63 percent moisture was reported in sixty months old fish of saline soil pond. With the increase of age in the fishes, a decrease in moisture content was recorded in both types of test fishes. It may be due to the increase in fat content .Similar results were also observed by Pandey *et al.* (1976) in various freshwater fishes.

2. Fat Content

Fat content in different age group in soil pond pH 7.0 and 9.5 has been presented in table-2. The fat content (pH9.5) was ranged between 1.60 to 2.89 percent in sixty months old fishes followed by 2.65, 1.78and 1.60 in thirty six, eighteen and six months old fishes, respectively.

Fat content was significantly affected by age in the experimental fishes. In the alkaline soil pond fishes it ranged from 1.60 to 2.89 percent .Fat content of whole body and flesh increased regularly with age .This increase might be due to the decrease of moisture content in the fishes with the increase of age .Increase in fat percentage may be due to the increase in food

Table -1: Variation in moisture percent in experimental fish Rohu at different age groups.

S.No.	Months	Moisture content (%)	
		pH 9.5	pH 7.0
1.	6months	78.41	79.81
2.	18 months	78.02	79.22
3.	36 months	77.20	78.30
4.	60 months	76.63	77.60
	C.D at 5%	NS	NS

Table -2: Variation in fat content percent in experimental fish Rohu at different age groups.

S.No	Months	Fat content (%)	
		pH 9.5	pH 7.0
1.	6 months	1.60	1.60
2.	18 months	1.78	1.88
3.	36 months	2.65	2.73
4.	60 months	2.89	2.98
	C.D.at 5%	68.73	71.65

intake by the fish in later stage .Similar types of results were found in Benoit *et al.* (1995) in carps.

3. Protein Content

The protein content in the experimental fish Rohu is presented in table 3. The protein content of fishes grown in alkaline pond was lower than normal soil pond and ranged between 18.40 to 16:60 percent during the observations .The maximum 18.40 percentage of protein was in the six month old fish followed by 17.87, 17.19 and 16.60 percent in eighteen , thirty six and sixty months old fish ,respectively . Minimum 16.60 percent was recorded in sixty months old fish in alkaline soil pond.Protein content was decreasing with age in both type of test fishes and were nonsignificant among themselves

Protein content of test fish Rohu grown in alkaline pond was not significantly affected with the age. Protein content in the experimental fishes of normal soil pond ranged from 18.10 to 15.82 percent in age of six to sixty months and was non- significantly higher from normal soil pond fishes. In the observation protein content decreased with increasing age but posses' higher value in small age (6 months) i.e. 18.10 percent and low in higher age group (sixty months).i.e. 15.82 percent in both groups of test fishes. These values were in agreement with those reported earlier by Gopakumar (1997). In the present experiment no correlation was found between protein and ash content. Gross protein fraction is very stable with aging .However, 70-72% of the protein of flesh is composed

Table -3: Variation of protein content percent in experimental fish Rohu at different age groups.

S.No.	Age	Protein content	
		pH 9.5	pH 7.0
1.	6 months	18.40	18.10
2.	18 months	17.87	17.31
3.	36 months	17.19	16.56
4.	60 months	16.60	15.82
	C.D. at 5%	NS	NS

of myofibrillar protein from muscle (Suzuki, 1981) and marked qualitative change occurs in myofibillar protein during the development. (Benoit *et al.*, 1995).

4. Tryptophan Content

The table 4 showed that tryptophan content in the experimental fish Rohu of different age groups in saline soil pond within the range of 49.30 to 68.92mg/g N as compare to 50.30 to 70.18 mg/g N in neutral soil pond fishes .The maximum tryptophan 68.92mg/gN was also found in sixty months of fish followed by 64.83, 61.01 and 49.30 mg/gN in thirty six, eighteen and six months old fish, respectively. Minimum tryptophan content 49.30 mg/g N was recorded in six months of fish .Tryptophan content

was varied significantly among themselves in both groups of fishes.

Tryptophan content was significantly differ with age. Tryptophan content in the experimental fish of alkaline pond ranged from 49.30 to 68.92mg/g N in six to sixty months age .maximum tryptophan was recorded in sixty months of fish where as minimum was in six months old fish of both soil ponds. Tryptophan increased with age this data is well supported by Sankar and Ram Chandra (2001). However, Love (1980) found a decrease in tryptophan content with age .The essential amino acids contributed to 41.51% of the total amino acid present in the fishes. Many workers have determined the amino acid in various fishes. However no marked differences in the amino acid content were observed in various age groups. In the present experiment increasing pattern in tryptophan content was observed with age .However, no relation was established with protein in any of test group of fishes.

5. Lysine Content

The lysine content in experimental fish Rohu at different age groups grown in alkaline soil pond was reported in Table -5. And compared with neutral soil pond fishes. The lysine content in saline soil pond fishes was in the range of 470to 560 mg/g N during the observation .maximum lysine content 560mg/ N was observed in sixty months old fish followed by 512, 482and 470mg/g N in thirty six, eighteen and six months of fishes respectively. Lysine content of fishes grown in higher pH recorded non-significantly lower value as compared to lower pH pond fishes.

Table -4: variation in tryptophan content (mg/gN) in experimental fish Rohu at different age groups.

S.No.	Age	Tryptophan content (mg/g N)	
		pH 9.5	pH 7.0
1.	6 months	49.30	50.30
2.	18 months	61.01	62.30
3.	36 months	64.83	66.00
4.	60 months	68.92	70.18
	C.D. at 5%	7.25	8.33

Lysine content was not significantly differ with age. Lysine content in the experimental fish of alkaline pond ranged from 470to 560mg/g N in six to sixty months age .Maximum 560mg/gN lysine content was in sixty months old fish where and minimum 470mg/gN in six months old fish . Similar trend of variation in lysine content were reported in neutral soil pond fish. The results were also in agreement with the observation made by Love (1980) in the maturity fish where lysine content was increasing. The data is also well supported by Sankar and Ram Chandran (2001)

6. Cholesterol Content

The cholesterol content in Indian major carp *Labeo rohita* shown in Table-6 revealed that maximum cholesterol content 88.98 in thirty six months old fish followed by 87.12 ,85.21and 85.61 mg/100g were available in eighteen ,sixty , and six months of fishes respectively. All the treatment varied significantly among them. Cholesterol content in fishes grown in higher pH posses lower as compared to neutral soil pond fishes.

The cholesterol constant was significantly affected by the age in experimental fishes of alkaline soil pond .It was ranged from 86.40 to 91.15 mg/100g in six to sixty months of age .Maximum cholesterol content was recorded in thirty six month old fish where as minimum in six month of old fish . No definite trend of variability in cholesterol was noticed in Rohu with age .In the experiment initial increase in cholesterol level

Table- 5: variation in Lysine content (mg/g N) in experimental fish Rohu at different age groups.

S.No.	Age	Lysine content (mg/g N)	
		pH 9.5	pH 7.0
1.	6 months	470	480
2.	18 months	482	494
3.	36 months	512	524
4.	60 months	560	572
	C.D. at 5%	NS	NS

was noticed followed by decreasing pattern. Similar results were noticed by Geri *et al* (1995).in *cyperus cario*. However, Love (1980) stated that cholesterol have no relation with the age of fish.

7. Total Ash Content

The total ash content in the experimental fish of Labeo rohita of saline soil pond and normal soil pond have been reported in Table-7. The total ahs content in alkaline soil pond fishes varied from 1.68 to 1.12 percent .Maximum 1.68% was observed in eighteen months old fish followed by 1.32,1.21and 1.12 percent in thirty-six , sixty and six months of fish ,respectively lower than normal pH soil pond fishes.

The ash content was not significantly different with the age. In the experiment it ranged from 1.12to 1.68 percent in six to sixty months old fishes grown in alkaline soil pond .Maximum ash content was recorded in eighteen months old and minimum in six months old fish. Ash content was increased from six to eighteen months old fishes and decreased continuously .Present observation is supported by Geri *et al* .(1995) in *cyperus carpio* .Sankar and Ram Chandra (2001) have not get any clear trend of ash content in Indian major carps with the size.

Table -6: variation in cholestrolcontent (mg/100g) in experimental fish Rohu at different age groups.

S.No.	Age	Cholesterol content (mg/100g)	
		pH 9.5	pH 7.0
1.	6 months	85.61	86.40
2.	18 months	87.12	89.10
3.	36 months	88.98	81.15
4.	60 months	85.21	87.60
	C.D. at 5%	2.91	3.07

Table -7: variation in Total Ash content percent in experimental fish Rohu at different age groups.

S.No.	Age	Total Ash content (%)	
		pH 9.5	pH 7.0
1.	6 months	1.12	1.13
2.	18 months	1.68	1.76
3.	36 months	1.32	1.37
4.	60 months	1.21	1.25
	C.D. at 5%	NS	NS

Table -8: variation in carbohydrate content percent in experimental fish Rohu at different age groups.

S.No.	Age	Carbohydrate content (%)	
		pH 9.5	pH 7.0
1.	6 months	3.87	4.20
2.	18 months	4.43	4.90
3.	36 months	4.76	5.25
4.	60 months	4.89	5.45
	C.D. at 5%	NS	NS

8. Carbohydrate Content

Carbohydrate content in experimental fish Rohu (*Labeo rohita*) grown in soil pond of pH 9.5 and 7.0 were presented in Table -8 .The carbohydrate content of fishes of soil pond 9.5 was in the range of 3.87to 4.89 percent as compared to normal pH range 4.2 to 5.45. The maximum carbohydrate was 4.89 percent in the sixty months of fish followed by 4.76, 4.43and 3.87 percent in thirty six, eighteen and six month's age of fish respectively. Higher pH non – significantly reduced the carbohydrates % in the fishes.

The carbohydrate content was not significantly affected by the age. In the experiment it ranged from 3.87to 4.89 percent in six to sixty months old fishes grown in alkaline pond as compared to 4.20 and 5.45% in test fishes of normal soil pond. Minimum carbohydrates content was reported in six months old fishes where as maximum in sixty month old fishes. An increasing trend was observed in carbohydrate content with age. This may due to the increase calorific value of fish in later age and it may also be related to the increase in fat content with age. This is in agreement with the results obtained by Sanker and Ramachandran (2001) in Indian major carps

REFERENCES

A.O.A.C. 1970. Official method of Analysis. Association of official Agril. Chemistry, Washington D.C. 434.

Benoit, F.; Durante, H.A.; Laroche, M.; J. And Vallot, D. 1995. Growth and meat quality relations in carp. *Aquaculture*, 129:265-297.

Cheyne, G.A. 1964. Estimation of cholesterol techniques in clinical pathology. *Blackwell Scientific Publication* (Oxford), 201.

Felker,C.;Libonaukas,C.K. and Wainer,G.1978.*Crop Sciences*,18(3):489-490.

Geri ,G.; Poli, B.M .; Gualtieri ,M .; Lupi,P.and Parisi,g.1995.Body traits and chemical composition of muscles in the common carp(*Cyprinus carpio* L.) As influenced by age and rearing environment .*Aquaculture*, 129:329-333.

Gopakumar, K. 1997. Annal report- Biochemical composition of Indian Food Fish. *Central Institute of Fisheries Technology*, Cochin.

Hart, F.L. and Fisher, H.S. 1971. Modern food analysis. Springer Verlog, New York.

Love, R.M. 1980. The chemical biology of fishes. *Academic Press*, London and New York.

Lowery's O.H.; Roserbrough, N.J.; Farm, A.L. and Randall, R.J. 1951. Protein measurement with folinPhenol reagent. *J. Biol. Chem.*, 193:265-275.

Pandey, B.N.; Datta Munshi, J.S.; Choubey, B.J. and Pandey, P.K. 1976. Seasonal variation in body composition in relation to breeding cycle of on air breathing fish, Heteropneustes fossilis (Bloch). Inland Fish. Soc. India, 8:91- 102.

Prakriti and Rao,A.P.2010. Morphometric and meristic characters in Indian Major carps with age grown in alkaline soil *pond Asian journal of envoirnmental science (communicated).*

Sankar T.V. And Ramachandran, A.2001 . Changes in biochemical composition in Indian major carps in relation to size .*Fishery technology*, 38(1):22-27.

Spies, J.T. and Chamber, D.C. 1949. Chemical determination of tryptophan protein. Analysis Chem., 21:1249.

Suzuki, T. 1981. Fish and Krill protein processing technology. Applied Science Publishers, London.

Yam, E.W. and Willis, A.J. 1954. The estimate of carbohydrate in the plant extract by anthrone. *Biochem. J.*, 87:508-514.

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SEASONAL ALTERATIONS IN THE MACROPHYTIC FLORA OF CHANDO LAKE, BASTI (U.P.)

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ABSTRACT

Present study deals the seasonal alterations in the macrophytic fauna of a large ox-bow lake of Manorma river, Chando Lake, Basti (U.P.) The study was made during June 2009 to May 2010 and total 18 species of macrophytes were recorded from Chando Lake covering an area of about 650 hectares. Five species belong to floating type, four to marginal type, and nine to submerged type. Marophytic vegetation was dominated by Myriyophyllum spathulatum, Ceretotphyllum demersum, Hydrilla verticillata, Potamogeton crispus, Najas minor and Typha latifolia. Various macrophytes of Chando Lake, their prevelance and respective families have been noticed.

Key Words : *Macrophytes, Chando Lake, Seasonal Alterations, Submerged Macrophytes, Emergent Macrophytes, Marginal Macrophytes, Floating Macrophytes.*

Littoral zones can play a role as buffer zones located at the interphase of the terrestrial and aquatic environment and are valued for their capacity to remove nitrate, phosphate or potassium from the lake ecosystems (Van Donk *et al.*, 1993), particularly from sediments as well as from agricultural run off (Jansson *et, al,* 1998). Littoral zones are described as nutrient rich systems with very high productivity and rapid nutrient cycle (Gopal and Masing, 1990; Coopes *et. al.*, 1999), strongly affected by water level fluctuation, their duration, frequency, rate of filling and drying, the timing or wave exposure (Scheffer, 1998, Casanova and Brock, 2000). In eutrophic ecosystems, this zone is mostly created by emergent macrophytes which can uptake large quantities of phosphorus and nitrogen as well as potassium from the sediments via, their root system (Carignan and Kalff, 1980). Shallow lake ecosystem are most senstive to hydrological changes since, even small alterations may significantly influence nutrient cycle and macrophytic distribution and bio-diversity (Casanova and Brock, 2000; Seabloom and Vander valk, 2003; Egentson *et. al.* 2004;. Kallner *et. al.*, 2007; Choinski, 2006; Lawniczak, *et. al.*, 2008, 2010).

Long term data are essential to understand the trajectory of alterations in macrophyte communities and their ability to take up nutrients, particularly in water bodies exposed to human pressure (Hellsten et. al., 1996; Egertson *et. al.*, 2004). Macrophytes are excellent indicators of the ecological status of aquatic bodies, mainly because they integrate environmental changes over periods of few years, and reflect the cumulative effects of successive disturbances (Tremp and Kohler, 1995).

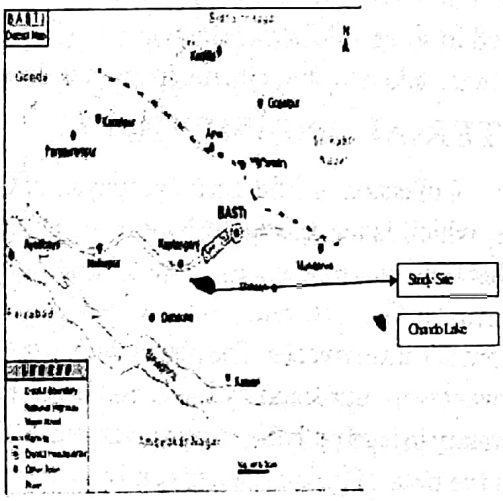

Fig1: Locational Map of Chando Tal

Besides, the role of aquatic macrophytes exercising an important role in the cycling of nutrient, they also provide shelter and protection to the aquatic fauna as well as attract other organisms upon which majority of the aquatic fauna feed. Abundance of the macrophytes is considered to be an indicator of the trophic status of any aquatic body. Aquatic macrophytes are essential if healthy fish stocks are to be mentained in a natural aquatic body. Opuszynski and Shireman (1995) reported that the water bodies with some plants or combination of plant species seems to be better fish habitat than other. Carpenter and Loudge (1986) pointed out that submerged macrophytes have major effect on productivity and cycle in freshwater because they occupy key interphase in streams and lake ecosystems. Alternations in the aquatic macrophytes may have major consequences for productive potential of commercially important fish fauna. Keeping this in view, the specific objectives of present study were to record the prevelance of various catagories of macrophytes viz, floating, submerged, marginal, dominant, subdominant, rare etc. with their respective families. Knowledge about responses of individual species to hydrological conditions may enable more effecient restoration or promote ecologicaly senstive hydrological management. Therefore, macrophytes of a vast chando, an oxbow shallow lake of river Manorma, covering an area of about 650 hectare, located in south of district Basti (U.P.) (Fig. 1) has been undertaken to study its macrophytes diversity.

MATERIAL AND METHODS

Collection of different macrophytes of Chando Lake, which is an oxbow of Manorma river and a shallow one was made using a metal quadrant of 50 cm. in size. The quadrant was lowered into water at random at various places. The plants with in the area of quadrant were uprooted by hand and brought to the laboratory in hard pollythein bags and were indentified with the help of guide lines given by Arber (1920); Biswas and Calder (1955); Edmondson (1959); Neetham (1962); Spence (1964) and Haslam (1978).

To study the distribution of macrophytes in Chando Lake, Basti (U.P.) in relation to seasonal alteration, water samples were collected at monthly intervals of an year for analysis of various physicochemical parameters. The standard suggested procedures of Adoni, (1985) and APHA, (2005); were followed and has been published by Shukla *et al.*, 2011. The macrophytes were arranged in their respective families.

RESULTS AND DISCUSSION

A precise list of macrophytes recorded at different sites of Chando Lake has been shown in Table 1. Total 18 species of macrophytes were observed from Chando lake during the study for an year of which four species belong to marginal type, five species floating type and rest nine submerged type as shown in table 1. The dominant, subdominant and rare catagries of the macrophytes present in the lake have been tabulated in Table 2. Observations of macrophytes were grouped into four catagries namely, abundant, Incidental, Present and Rare. Also the dominant, subdominant and rare macrophytes are listed. The citation of the macrophytes was made on three bases as shown in Table 1, 2 and 3 respectively.

1. On the basis of occurance during different months of study period (June to May)

2. On the basis of pattern of prevelance.

3. On the basis of the respective families.

The macrophytes observed in our study belong to different familes viz., *Azolla filiculoidies* of family Azollaceae; *Chara vulgaris* of family Characeae; *Ceratophyllum demersum* of family Ceratophyllaceae; *Eichhorhia crassipes* of family Pontedcriaccae; *Equisetum fleviatible* of family Equisetaceae; *Hydrilla verticellata* of family Hydrocharitaceae; *Ipomea aquatica* of family Convolvulaceae; *Marsilea* of family Marsilaceae; *Myriophyllum spathulatum* of family Haloragaceae; *Najas minor* of family Najadaceae; *Nelumbo mucifers* of family Nymphaceae; *Nymphaea stallata* of family Nymphaceae; *Pistia stratiotes* of family

Table – 1: Occurrence of Macrophytes in Chando Lake during the study period.

Taxa	Months (June 2009- May 2010)											
	June	July	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
Floating Plants												
Azolla filiculoides	R	R	I	I	I	I	A	A	A	A	A	R
Eichhornia	R	I	I	A	A	A	A	A	A	A	P	P
crassipes	P	P	P	I	I	I	P	I	I	I	P	P
Pistia stratiotes	R	I	I	I	I	P	A	R	R	R	R	
Nymphaea stellata	I	I	I	A	A	A	P	R	R	I	I	I
Nelumbo nucifera												I
Submeraged	I	I	A	A	A	A	A	P	P	P	I	
plants	I	I	I	A	A	A	R	A	A	A	A	I
Chara vulgaris	P	P	P	P	P	P	A	R	R	R	I	I
Ceratophyllum	I	I	A	A	A	A	P	A	A	A	A	
demersum	I	I	I	I	I	P	A	P	A	A	A	I
Equisetum	I	I	A	A	A	A	P	A	A	A	A	A
fluviatiles	I	I	I	I	I	P	A	P	P	P	P	A
Hydrilla verticillata	I	I	P	P	P	A	P	A	A	A	P	I
Myriophyllum	I	I	I	P	P	P	A	A	A	A	A	P
spathulatum												R
Potamogeton	R	R	I	I	I	I	P	P	P	P	P	
crispus	P	P	A	A	A	P	P	P	I	I	I	P
Utricularia stellaris	P	P	P	P	P	P	P	P	P	P	P	I
Vallisneria spiralis	P	P	P	A	A	A	A	A	A	A	A	P
Najas minor												A
Marginal Plants												
Ipomea aquatica												
Marsilea sp.												
Polygonum												
glabrum												
Typha latifolia												

A= Abundant, I= Incidental, P= Present, R= Rare

Table – 2 – On the basis of pattern of prevelance.

Dominant	Sub-dominant	Rare
Myriophyllum spathulatum	*Nelumbo nucifera*	*Equisetum fluviatile*
Ceratophyllum demersum	*Azolla filiculoides*	*Nymphaea stellata*
Hydrilla verticillata	*Vallisneria spiralis*	
Eichhorinia crassipes	*Utricularia stellaris*	
Potamogeton crispus	*Pistia stratiotes*	
Najas minor	*Impomea aquatica*	
Typha latifolia	*Marsilea*	
	Polygonum glabrun	
	Chara vulgaris	

Table. 3 – List of Macrophytes recorded from Chando Lake and their families.

Taxa	Family
Azolla filiculoidies	Azollaceae
Chara vulgaris	CharaceaeCeratophyllaceae
Ceratophyllum demersum	Pontederiaceae
Eichhornia crassipes	Equisetaceae
Equisetum fluviatile	Hydrocharitaceae
Hydrilla verticellata	Convolvulaceae
Impomea aquatica	Marsileaceae
Marsilea	Haloragaceae
Myriophyllum spathulatum	Najadaceae
Najas minor	nymphaeaceae
Nymphaea stellata	Nymphaeaceae
Pistia stratiotes	Araceae
Polygonum glabrum	Ploygonaceae
Potomogeton crispus	Potamogetonaceae
Potamogeton pectinatus	Potamogetonaceae
Typha latifolia	Typhaceae
Utricularia stellaris	Lentibulariaceae
Vallisneria spiralis	Hydrocharitaceae

Araceae; *Polygonum globrum* of family Polygonaceae; *Potamogeton crispus* of family Potamogetanaceae; *Typha latifalia* of family Typhaceae; *Utriculia stellaris* of family Lentibulariaceae and *Fallisneria spiralis* of family Hydrocharitaceae.

Some of these macrophytes were abundant in different months as shown in Table 1. The dominant species were seven in number, sub dominant species were nine in number and two were spcies rare as shown in Table 2, with their corresponding families as shown in Table 3.

Aquatic macrophytes are amongst those factors which a fishery manager may try to understand and include in his strategies for optimizing capture fishery in inland waters. Plant species composition, distribution and percentage cover may determine the fish species composition, individual fish species production, Since, Chando lake is a natural, unpolluted and physico-chemically balanced lake hence, the presence of the macrophytes may be regarded as an index for productive potential of the fishes for this locality as also reported by various workers in the aquatic bodies of different countries (Vander wygeart *et al.*, 2003; Solli and Varhoeven, 2007; Kumar *et. al.* 2006; Roem and Berendse, 2000; Geusewel and Koerselman; 2002; Lawniczak *et. al.*, 2010)

Aquatic macrophytes can also be efficient indicators of water quality and their presence may inhance water quality due to their ability to absorb excessive load of nutrients (Coops *et.al.*, 2004; Deegan, 2007; Egertson *et.al.* 2004; Engloner, 2009; Hoosbeck *et.al.*, 2002; Jonsson *et.al.*, 1998; Kallner *et.al.*, 2007, Lawniczak *et al.*, 2008. These properties have been used in waste water treatment as well as in biomanipulation of water bodies for enhancing fish production.

Aquatic macrophytes provide an enormously large surface area for the growth of microflara and fauna and aid in anchoring the fish spawn. However, due to their high growth rates, they have been considered to be an indicator of trophic status as cited by Welch, (1952) and Kohler, (1957). The macrophytes recorded total 18 members in number and most of the macrophytes were found to be abundant during winter months. It has also been observed that the area where macrophytes are thickly covered, the fish fauna were densily found there. These observation support the findings Borawa *et. al.*, (1979); Shireman *et.al.*, (1981); Killgore *et.al.*, (1989); Randall *et.al.*, (1996); and Kumar *et al.*, (2006). Macrophytic vegetation in our study is limited to the areas where anthropogenic activities exist much. Such findings are in support of the observation of Srivastava, (1998); in Kerwans reservoir Bhopal, (M.P.) and Kumar *et.al.*, (2006) in Sikandarpur reservoir, Basti (U.P.). Aquatic macrophytes constitute one of the major link in the interrelated environment and some worker believe that macrophytes are the central link which determine the stability of the freshwater ecosystem. They occupy a key postition in the net work of ecological relation between nutrients, zoo, phytoplankton and macroinvertebrate fauna and they determine the carrying capacity for the population of some fishes like Cyprinids, Eels, Pikes etc. (Din, Nie, 1987). Submerged macrophytes have major effects on productivity potential and biogeo-chemical cycle of various micro and macro nutrients in freshwater because macrophytes occupy key place in aquatic ecosystems (Carpenter and Lodge, 1986). It is also true that alterations in the aquatic macrophytes may have major consequences for commercially important fish fanna. Different water levels also exercise a profound impact on the prevelance of macrophytes. This indicates differential senstivity of macrophytes to alterations in the hydrological regime.

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REFERENCES

A.P.H.A. 2005: Standard methods for the examination of water and waste water. Amarican Public health association and *waste pollution control federation, New York* (19th ed.)

Adoni, A.D. 1985 : Work Book on Limnology, Pratibha Publisher, Sagar.

Arben 1920: Water plants, Cambridge University Press, London.

Berawa, J.C.; Kerby, J.H.; Huifh, T. and Mullis, A.W. 1979: Curriuck sound fish population before and after infestation by Eurasian water-milfoil. *Procs. Ann. Conf. SE Assoc. Fish and Wild Life Agencies*, 32: 520-528.

Biswas, K. and Calder, C.C. 1995: Handbook of common water and marsh plants of India and Burma; *Hlth Bull., (24) Malaria Bureau No. 11, Govt. of India Press,* Calcutta; pp. 1-216.

Consanova, M.T. and M.A. Brock 2000: How do depth, duration and frequency of flooding influence the establishment of wetland plant communities? *Plant Ecology* 147: 237-250.

Carpenter, S.R. and Lodge, D.M. 1986: Effect of submersed macrophytes on ecosystem. *Aquat. Bot.*, 26:341-360.

Choinski, A. 2006 : Atlas of Polish lakes (in Polish). Adam Mickiewicz Uni. press, Poznan, Poland.

Coops, H., J. Hanganu, M. Tudor and W. Oesterberg 1999 : Classfication of Danube Delta lakes based on aquatic vegetation and Trubidity. *Hydrobiologia* : 415 : 187 – 191.

Coops, H., J.T. Vulink and E.H. Van Nes 2004: Managed water levels and the expansion of emergent vegetation along a Lakeshore *Limnologica*: 34:57-64

Deegan, B.M.; S.D. White and G.G. Ganf 2007: The influence of water level fluctuation on the growth of four emergent macrophyte species. *Aquatic Bot.*; 86: 309-315.

De Nie, H.W. 1987: The decrease in aquatic vegetation in Europe and its consequences for fish populations. EIFAC/CE CPI Occassional paper No. 19. FAO, Rome, pp 52.

Edmondson, W.T. 1959: Freshwater Biology.2nd. New. York, London John viley and sons, pp 1-1248.

Egertson, Ch., J.A. Kopaska and J.A. Downing 2004: A century of change in macrophyte abundance and composition in response to agricultural eutrophication. *Hydrobiologia*, 524. 145-146.

Englonar, A.T. 2009; Structure, Grouth, dyamics and biomeass of reed (Phragmites australis) a review Flora 2004: 331-346.

Gopal, B. and V. Mising 1990: Biology and ecology. In Pattern. B.C. (ed.), Wetland and shallow continental water bodies. SPB Academic Publishing. The Hague : 91-239.

Gusewell, S. and W. Koreselman 2002: Variation in nitrogen and phosphorus conectration of wetland plants. Perspectives in Ecology; *Evolution and systimatics*: 5. 37-61.

Haslam, S.M. 1978: Riner plants the macrophytic vegetation of water courses. *Combridge Univ. Press, London,* pp 1- 396.

Hellsten, S.M. Marttunen, R. Palomaki, J. Richimaki and E. Alasaarela 1996: Towards an ecologically based regulation practice in Finnish Hydroelectric lakes. Regulated Rivers: *Research and Management;* 12: 535-545.

Hoosbeek, M.R.N.; Breemen, Van.; Vasander, H.; Buttlr, A. and Berendse, F. 2002: Potassium limits potential growth of bog vegetation under elevated atmospheric Co_2 and N. deposition. *Global Change Biology;* 8: 1130-1138.

Jonsson, A.; Floke, C. and Langaas, S. 1998: Quantifying the nitrogen retention capacity of natural wetlands in the large-scale drainage basin of the Baltic Sea. *Landscape Ecology.* 13: 249-262.

Kohler, A. 1957: Submerge Macrophyton and the gaseltschaften also Indikatoren der Gewssebelustung. Beitr. *Natur. Forsch. Sudw. DEL.*, 34: 149-159.

Kallner Bostviken, S.; Eriksson, P.G.; Ekstrom, A. and Konderski, K. 2007: Seasonal denitrification potential in wetland sediments with organic matter from different plant species. *Water, Air and Soil Pollution.* 183: 25-35

Kumar. V., T.A. Qureshi and J.P. Shukla 2006: Seasonal Changes in macrophytic vegetation of Sikandarpur reservoir, Basti (U.P.); *Nat. Journal of life Sci.*, 3 (Supp.) ; 509-512.

Lawniczak A.E., J. Zbierska and J. Kupiec 2008: Changes of nutrients concentrations in water sensitive to nitrate pollution from Agricultural sources in the samica stesze-wska River catchment. *Annals of Warsaw University of live sciences, Land Reclamation* 40: 15-25.

Meedham, J.L. and Needham, P.R. 1962: *A guide to the study of freshwater biology.* Holden Day *Inc. San. Franchsco,* P 108.

Nauniczak, A.E., J. Zbicrska, A. Choinski and W.S. Zczepaniok. 2010: Response of emergent macrophytes to hydrological changes in a shallow lake, with special refrence to nutrient cycling; *Hydrobiologia:* 656: 243-254.

Opuszynski, K. and Shireman, J.V. 1995: *Herbivorous. Fishes culture and use for weed management.* CRS Press, Boca. Raton, Florida, pp 223.

Randall, R.G., Minns, C.K., Cairns, V.W. and Moore, J.E. 1996: The relationship between and index of fish production and submerged macrophytes and other habital features at three littoral areas in the lakes. *Can. J. Aquat. Sci.*, 53 (Suppli.1): 35-44.

Roem, W.J. and F. Berendse 2001: Soil acidity and nutrient supply ratio as possible factor determining changes in plant speceis deversity in grassland and healthland communities. *Biological Conservation;* 92: 151-161.

Scheffer, M., 1998: Ecology of shallow lakes. Chapman and Hall, London. U.K.

Seabloom, E.W. and J. Vander Valk 2003: Plant diversity, Composition and invasion of restored and natural prairie pothole wetlands: Implications for restorations. Wetlands. 23: 1-12.

Shukla, Anuradha; Shukla, J.P. and Mishra, M. 2011. Plankton diversity in fresh water Chando lake. Basti (U.P.). *Proceeding challenges for Bio-Science in 21st Century.* 76-86.

Shireman, T.V., Colle, D.E. and Du Rand, D.F. 1981: Efficiency of rotenone sampling with large and small black nets in vegetated and open water habitats. *Trans. Amer, Fish Soc.* 110:77-80.

Sollie, S. and J.T.A. Verhoeven 2007: Nutrient cycling and retention along a littoral gradient in a Dutch shollow lake in relation to water level regime. In sollie, S. (ed.), Littoral Zoner in shallow lakes. Contribution to water quality in relation to water level regime. Ph.D. thesis, Utrecht University, Faculty of Science, Utrecht : 53-68.

Spence, D.H.N. 1982: The zonation of plant in freshwater lakes. *Advanced Ecological Reaserch;* 12: 37-125.

Tremp, Hand A. Kohler. 1995: The use fulness of macrophytes monitoring – systems, examplified on euturophication and acidification of running water; *Acta Botania Gallica;* 142: 541-550.

Vander Wygaert, I.J.J.L.D. Wienk, S. Sollie, R. Bobbink and J.T.A. Verhoeven. 2003: Long terms effects of yearly grazing by moulting *Greylag gees (Anser anser)* on reed *(Phragmites australis)* growth and nutrients dynamics, *Aquatic Bot.*; 75: 229-248.

Van Donk, E., R.D. Gulati, A. Iedema and J.T. Meulemans. 1993: Macrophyte related shifts in the nitrogen and phosphorus contents of the different trophic levels in a biomanipulated shollow lake. *Hydrobiologia;* 251:19-26.

Welch, P.S. 1952 : Limnology. 2nd ed. Mc. Grow Hill Book Co., New York, pp . 1-538.

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DEVELOPMENT OF ORANGE, CARROT, SESAME SEEDS CANDY THROUGH VALUE ADDITION WITH BASIL.

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ABSTRACT

The king of herbs, basil herb is one of the oldest and popular herbal plant rich in many notable health benefiting phyto-nutrients. Basil herb contains exceptionally high levels of beta-carotene, vitamin A. Orange, carrot are very good source of vitamin A, vitamin B, vitamin C and iron. Gingelly seed was one of the first oil seed known to mankind with its nutritive, preventive and curative properties. The present research work was conducted with the objective for development of orange, carrot, sesame seeds candy through value addition with basil to assess its sensory properties, to determine the nutritive value and to calculate the cost of the prepared product. Nutritional herbal candy was prepared by using three different treatments T_1 (carrot 70percent, orange 20 percent, gingelly seed 10 percent, basil 5 percent). T_2 (carrot 60percent, orange 25percent, gingelly seed 15 percent, basil 5 percent) and T_3 (carrot 50percent, orange 30percent, gingelly seed 20 percent, basil 5 percent). Sensory evaluation of the prepared product was carried out using the nine point hedonic scale. The experiment was replicated four times and the data obtained during investigation were statistically analysed by using analysis of variance (ANOVA) and critical difference (C.D) techniques. The cost of the candy was found on the basis of raw ingredients only, at prevailing prices candy was obtained in T_1 as compared to other treatment. The calculated nutritive value of product showed that as the percentage of carrot increased the carotene content increases, energy carbohydrate increase with increase of gingelly seed and orange. Over all it was concluded T_3 of orange, carrot, gingelly seeds candy through value addition with basil was found to be most acceptable.

Keywords: *Gingelly seed, hedonic scale, basil, carotene.*

The king of herbs, basil herb is one of the oldest and popular herbal plant rich in many notable health benefiting phyto-nutrients. This highly prized plant is revered as "holy herb" in many traditions all over the world. Basil leaves contain many notable plant derived chemical compounds that are known to have disease preventing and health promoting properties. Basil herb contains exceptionally high levels of *beta-carotene*, *vitamin A*, *cryptoxanthin, lutein* and *zea-xanthin*. These compounds help act as protective scavengers against oxygen-derived free radicals and reactive oxygen species (ROS) that play a role in aging and various disease process. Herbs, fruits and vegetables rich in zeaxanthin help to protect from *age related macular disease*(AMRD) especially in the elderly. Consumption of natural foods rich in vitamin-A known to helps body protect from lung and oral cavity cancers. Vitamin K in basil is essential for many coagulant factors in the blood and plays vital role in the bone strengthening function by helping mineralization process in the bones. Basil herb contains good amount of minerals like potassium, manganese, copper, and magnesium. Potassium is an important component of cell and body fluids which

helps control heart rate and blood pressure. Basil leaves are an excellent source of iron. Carrots are flooded with nutrients. No other vegetable or fruits contains as much carotene as carrots, which the body converts to vitamin A. This is truly versatile vegetable, rich in mineral salts and vitamins (B, C, D, and E). Oranges, like other citrus fruits, is an excellent source of vitamin C ; vitamin C is a powerful natural antioxidant. Consumption of foods rich in vitamin C helps body develop resistance against infectious agents and also, scavenge harmful, pro-inflammatory free radicals from the blood.Oranges also contain very good levels of vitamin A, and other flavonoid antioxidants such as alpha

Preparation of candy

In large, heavy bottomed iron saucepan, carrot and ½ cup of water was added

⇩

This was cooked until become soft for 30 minutes.

⇩

With the help of potato masher, carrots was mashed.

⇩

After this, sugar orange pulp and half of gingelly seed was added.

⇩

This was cook for 50-60 minutes.

⇩

Fresh basil leaves was finely chopped and added in the mixture.

⇩

After 10 minutes, the iron pan was removed from the fire and kept until it cools.

⇩

The mixture was spread on to the board and rolled into a 1/2inch rectangle.

⇩

Gingelly seeds was sprinkled on it.

⇩

1 diameter square pieces was cut for packaging.

⇩

Prepared candies were stored in airtight container

Experimental design:

Table 1. The trail of experimental design is as follows:

SNo.	Treatments	Specification
1.	T_1	Carrot (70%), Orange (20%), gingelly seeds (10%) + Basil (5%)
2.	T_2	Carrot (60%), Orange (25%), gingelly seeds (15%) + Basil (5%)
3.	T_3	Carrot (50%), Orange (30%), gingelly seeds (20%) + Basil (5%)

and beta carotenes. One of the first oil seeds known to mankind, sesame seeds are used in culinary as well as in traditional medicines for their nutritive, preventive and curative properties.

MATERIALS AND METHODS

Experimental site:

The experimental work for preparation of candy was done in, Nutrition Research Laboratory of Halina School of Home Science, Sam Higginbottom Institute of Agriculture, Technology & Sciences (Deemed-to-be-University), Allahabad U.P.

Procurement of raw materials:

Best quality of carrots, oranges, gingelly seed/sesame seed, sugar were purchased from the local market of Allahabad.

Organoleptic evaluation:

The Organoleptic evaluation of freshly prepared candy was done by a panel of 5 judges to assess the acceptability of the product based on the various attributes like colour, appearance, texture, flavor and taste. The evaluation will be done on the 9-Point Hedonic Scale based score card. **(Sri Lakshmi 2007)**

Calculation of nutritive value of prepared candy:

The nutritive value of prepared candy was calculated using food composition table from the book of Nutritive Value of Indian Foods by **Gopalan *et al.* (2004)**.Nutrient calculated was energy, protein, carbohydrate, fat, fiber, calcium, phosphorous, iron, carotene.

Calculation of cost of the product:

The cost of the product was calculated by basis of price of raw ingredient at rupees per kg.

Statistical analysis:

The data was statistically analyzed by using analysis of variance (two way classifications) and critical difference. **(Fisher 1995)**

RESULTS AND DISCUSSION

The experiment trail was replicated four times and in each replication of candy prepared from orange , carrot, gingelly seed through value addition of basil was evaluated by a panel of five judges by using 9 point hedonic scale for organoleptic properties.

The data obtained were statistically analyzed using analysis of variance and critical difference techniques the highest score obtained from flavour and taste of herbal candy from carrot, orange, gingelly seed with basil was recorded (8.80) in T_3 followed by (8.37) in T_1,(8.02) in T_2.The highest score obtained for texture of herbal candy from carrot, orange, gingelly seed with basil was recorded (8.70) in T_1 followed by (8.40) in T_3,(8.12) in T_2.The highest score obtained for the colour of herbal candy from carrot, orange, gingelly seed with basil was recorded (8.57) in

Table 2. Average sensory scores of different parameters for candy prepared from orange, carrot, gingelly seed with basil.

Parameters	Treatments			F 'Cal'	F 'tab'	Result
	T_1	T_2	T_3			
Flavour and taste	8.37	8.02	8.80	6.5	4.76	S
Texture	8.70	8.12	8.40	.91	4.67	NS
Colour	8.57	7.85	8.50	4.8	4.76	S
Over all acceptability	8.50	8.42	8.70	4.96	4.76	S

Table 3. Comparison of nutritive value of different treatments of candy from orange, carrot, gingelly seed with fresh basil leaves. (per 100 g)

Nutrients	Treatments		
	T_1	T_2	T_3
Energy (Kcal)	160.2	471	498
Protein (g)	3.5	5.8	6.7
Carbohydrate (g)	24.4	95	96
Fat (g)	5.4	7.5	9.7
Fiber (g)	3.8	3.7	3.8
Calcium (mg)	226	300	358
Phosphorous (mg)	492	469	525
Iron (mg)	5.4	5.7	7.4
Carotene (µg)	1548	1417	1286

T_1,followed by (8.50) in T_3,(7.85) in T_2.The highest score obtained for the overall acceptability of herbal candy from carrot, orange, gingelly seed with basil is recorded (8.70) in T_3,(8.50) in T_1,(8.42) in T_2. There was significant difference in flavour and taste of herbal candy from orange, carrot, gingelly seed with basil. Highest score for the texture of herbal candy from orange, carrot, gingelly seed with basil was recorded in T_1 (8.7).There was significant difference in colour of herbal candy from orange, carrot and gingelly seed with basil. There was significant difference in overall acceptability of herbal candy from orange, carrot and gingelly seed with basil.

The nutritive value of herbal candy prepared from orange, carrot and gingelly seed with basil T_3 was highest in energy, protein, carbohydrate. Carotene content of T_1 was highest followed by T_2 and then T_3

The highest average cost of the experimental candy from orange, carrot, gingelly seed with basil was recorded (47 Rs) in T_3 followed by T_2 (43 Rs) T_1 (38.5 Rs). The cost of T_3 treatment found was highest as the amount of gingelly seed, orange increases. Therefore, concluded treatment T_1 has lowest cost, and T_3 has highest cost. Calculated as basis of raw material, only at prevailing prices herbal candy was obtained in T_1 with all three herbs as compared to other treatment.

Results obtained from the statistical analysis revealed that the basil can be satisfactorily mixed with orange, carrot, gingelly seed to prepare nutritional candy.T_3 was scored highest in terms of flavour and taste, texture, colour and overall acceptability of candy prepared from carrot, orange, gingelly seed with basil. The calculated nutritive value of product showed that as the percentage of carrot increased the carotene content increases. Energy and carbohydrate increase with increase of gingelly seed and orange.

REFERENCES

Gopalan,C.; Rama Sashtri, B.V. and Balasubramanian, S.C. (2004). "Food and their nutrient content. Nutritive value of Indian foods". National Institute of Nutrition, Indian Council of Medical Research, Hyderabad: 29.

Fisher, S. (1995) "Analysis of variance- two way classification". B. R. S., 142(6); 56-58.

Srilakshmi,B (2007) "Food Science" "Sensory Evaluation" fourth edition. New age International (P) Limited Publishers, New Delhi: 286-287.

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CHROMIUM INDUCED ALTERATIONS IN SOME BIOCHEMICAL PROFILES DURING TESTICULAR CYCLE OF *COLISA FASCIATUS* (Bl. & Schn.)

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ABSTRACT

An attempt has been made in the present investigation to determine the toxicity of sub-lethal concentration of hexavalent chromium after long term exposure (30 days) on some biochemical profiles (total glycogen and lipid content) during different phases (preparatory, spawning and post-spawning) of testicular cycle of a tropical freshwater perch, *Colisa fasciatus* . Appreciable declining in the biochemical profiles in all phases of testicular cycle has been noticed and more pronounced during spawning phase. This study reflects the extent of toxic effects of hexavalent chromium on testis during its various phases.

Key Words : *Colisa fasciatus, Hexavalent chromium, Testicular cycle ,Total Glycogen, Total lipid.*

Freshwaters are highly vulnerable to pollution since they act as immediate sinks for the consequences of human activity always associated with the danger of accidental discharges or criminal negligence. Heavy metals constitute a core group of aquatic pollutants and additional concentrations of these metals accumulate in the aquatic ecosystems as a result of land based activities (Vutukuru *et al* , 2003). Chromium has been proved to be highly toxic to freshwater fishes and the deleterious effects caused by chromium to the fishes include depression in the metabolic rate, histopathological and hematological alterations (Vutukuru and Rao, 1996, 1999, Vutukuru, 2003). Chromium continues to be in widespread use in industry, paints , metal plating as corrosion inhibitor and its particulates enter the aquatic ecosystem through effluents discharged from textiles, tanneries, mining ,electroplating, dyeing and printing industries, photographic and pharmaceutical industries. In the natural environment, chromium exists primarily in the trivalent and hexavalent forms, however, the hexavalent forms predominant the trivalent form in natural waters. Hexavalent chromium potentially possesses toxicological, carcinogenic and mutagenic properties (Langard and Norseth, 1979). Heavy metals including chromium concentrated in the tissues of fishes enter human beings through food chain and because of cumulative action, cause potential health hazards , some times even lethal (Ui, 1972). The deleterious effects may result from the bioconcentration of metallic pollutants and their consequent binding with the biologically active constituents of the body such as aminoacids, enzymes, proteins and lipids. In this context, an attempt was made to investigate the long term effects of sublethal concentration of hexavalent chromium on glycogen and lipid profiles of *Colisa fasciatus*, a tropical freshwater perch during its preparatory, spawning and post spawning phases of testicular cycle.

MATERIALS AND METHODS

Fish, *Colisa fasciatus* of an average length 6.9 cm and weight 7.2 gm were procured from local lake for experimental purpose. These were acclimatized for 12 days in laboratory dechlorinated tap water having the following physicochemical

properties, analyzed by the procedures outlined by APHA (2005).

Temperature-	7.32 ± 1.4°C
pH-	7.32 ± 0.04
Hardness as $CaCO_3$-	128.30 ±4.12 mg/l
Electrical conductivity-	1296.62 µmho/cm

During acclimatization, fishes were fed on alternate days with dried shrimp powder at the rate of 5 % body weight. *Colisa fasciatus*, though is an air breather, even then aeration facilities were provided for 3-4 hours daily in controlled and experimental media. The SLC of hexavalent chromium as Potasium Chromate (Mark,India) was 4.8 mg/l (one tenth of LC_{50} 96 hours.)

Colisa fasciatus is an annual breeder. Its reproductive cycle though has been divided into 6 phases by Pandey and Mishra (1981), however, only three principal phases viz., preparatory, spawning and post-spawning have been choosen to observe alterations, if any, in the biochemical profiles (glycogen and lipid) under hexavalent chromium stress. Phases of testicular cycle , their duration and period of experiment run were as follows:

Phase	Experiment Run
Preparatory (March-April)	IInd week March- IInd week of April
Spawning (June-August)	IInd week of June-IInd week of July
Post Spawning (Oct.-Dec.)	IInd week of Nov.-IInd week of Dec.

Experiment in different phases was performed at natural photoperiod and ambient water temperature.

30 fishes in 30 l of glass aquarium were kept each in control and experimental media.

In each phase testis from 20 fishes from control and experimental were isolated. They were dried for 24 hours in hot air ovan at 50°C.

The total glycogen and lipid were estimated by adopting universally accepted standard procedures of Kemp and Kits, 1954 and Pandey *et al.* 1963 respectively and were expressed in mg/gm dry weight of the testis and only the average mean values (n=5) were presented to express the results. The values of the control and chromium treated fishes were compared with students "t" test (Baily, 1959).

RESULTS AND DISCUSSION

The total glycogen and lipid concentration in all the phases of testicular cycle of *Colisa fasciatus* exposed to sublethal concentration of hexavalent chromium are presented in Table-1. It becomes clear from the results that there is an appreciable decline in the aforesaid constituents under SLC of hexavalent chromium stress. Further to mention that level of glycogen and lipid in testis during its spawning phase was maximum in comparison to preparatory and post spawning which clearly points out towards the possible supply of carbohydrate content in the form of glucose for active maturation of male gametes (sperms). The increasing order decrease in the glycogen concentration during preparatory and spawning phases of testicular cycle may be due to its enhanced utilization as an immediate source to meet energy demands for sperm maturation under hexavalent chromium stress. It could also be due to the prevalence of hypoxic or anoxic condition which generally enhances glycogen

Table-1. Effect of sublethal concentration of hexavalent chromium on total glycogen and lipid content in mg/gm dry weight of testis during testicular cycle of *Colisa fasciatus* (n=5)
*= P<0.01 **= P<0.001

Phases	Glycogen in control ±S.D.	Glycogen in chromium stress ±S.D.	Percent change	Lipid in control ± S.D.	Lipid in chromium Stress ± S.D.	Percent change
Preparatory	18.64 ± 0.36	15.84 ± 0.28*	15.02	64.66 ±1.22	59.38 ± 1.42**	8.16
Spawning	24.04±0.22	16.86±0.28**	29.86	82.46±1.04	71.42±1.28**	13.38
Post-spawning	14.08±0.18	12.12±0.14*	13.92	38.12±1.02	35.26±1.08*	7.50

utilization in one way or other (Dezwaan and Zandee, 1973, Geetha *et al*, 1991,Shukla *et al* 2005). Present finding may well be correlated with the observations noticed by Chandravathi and Reddy, 1995, Shukla *et. al.*, 2005 and 2011.

The decrease in the lipid content during different phases of testicular cycle of *Colisa fasciatus* might be partly due to its utilization in cell repair and tissue organization with the formation of lipoprotein which is important constituent of cell membrane and cell organelles (Harper, 1983). Our Finding may well be correlated with the observations made by Ambrose *et. al.* 1994 , Vutuauru, 2003 and 2005 and confirms that the sublethal concentration of hexavalent chromium produces adverse impact on the lipid metabolism too.

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REFERENCES

Ambrose, T.; Cyril, A.K. L. and Vincent, S.R.L. 1994. Biochemical responses of *Cyprinus carpio communis* to toxicity of tannery effluent . *J. Ecobiol.* 6(3) 213-216

APHA 2005. Standard methods for the examination of water and waste water. 21st edition, Washington D.C.

Bailey, N.T.J. 1959. Statistical methods in Biology. The English Language Book Society, Great Britain. Chandravathi, M and Reddy, S.L.N.: 1995. In vivo effects of lead acetate on dehydrogenase activities and metabolites in the fresh water fish, *Anabas scandens* . *J. Ecotoxicol. Environ. Monit.* 5(2), 107-111.

Dezwaan, A. and Zandee, D.I. 1973. Body distribution and seasonal changes in glycogen content of the common sea mussel, *Mytilus edulis* . Comp. *Biochem. Physiol.* 43,53-55

Geetha, S.; Suryanarayanan, H. and Nair, N.B. 1991. On the nature of variations of biochemical constituents during the breeding cycle in *Heteropneustes fossilis* (Bloch). *Proc.Nat. Acad. Sci. India.* 61(B) 111,311-315

Harper, A.H.1983. Review of Biochemistry. 20th edition. Lange Medical Publication Co. California. pp1012.

Kemp, A.J.M. and Kits.V.H. 1994. A calorimetric micro method for the determination of glycogen. *J. Biochemical.* 56, 640-648

Langard,S. and Norseth, T. 1979. Chromium: In *Hand Book on the Toxicology of Metals*. Holland Biochemical Press, Netherlands. pp.383-394.

Pandey, S.V.; Khan, A.P. and Subhramanayam, T.A.V. 1963. Micro determination of lipids and serum fatty acids. *Analyst. Biochem.* 6(5), 120-125

Shukla, J.P.; Tripathi,S.; Chandel, B.S. and Dwivedi N.D. 2005. Deleterious effects of a metalloid (arsenic) on blood pyruvate level in a tropical fresh water fish, *Colisa fasciatus. Nat. J.Life Sci.* (1and2) 125 -126

Shukla, J.P.; Shukla Anuradha; Shukla, A.K. and Mishra, M. 2011. Deleterious effects of hexavalent chromium on the blood pyruvate level in the fingerlings of *Channa puncatatus J.Appl. Biosci.* (Accepted on January 7, 2011)

U.I.J. 1972. The changing chemistry of the Oceans. Almquist and Wiksells, Stockholm.

Vutukuru, S.S. and Rao, B.M.1996. Acute toxicity of chromium to the fresh water teleost fish, *Labeo rohita.* IInd. 14(2), 30-32

Vutukuru, S.S. and Rao, B.M.1999. Chromium induced histological alteration in the gill of fresh water teleost fish, *Labeo rohita.* (Ham.). *J. of Comp. Anim. Physiol..* 17(1),31-33.

Vutukuru, S.S. 2003. Chromium induced alterations in some biochemical profiles in the Indian major carp, *Labeo rohita.* (Ham.) Bull *Environ. Contam. Toxicol.* 70, 118-123.

Vutukuru, S.S. 2005. Acute effects of hexavalent chromium on survival, oxygen consumption, hematological parameters and some biochemical profiles of the Indian major carp, *Labeo rohita.* (Ham.). *Int.J.Env.Public Health.* 2(3) 456-462.

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IMMUNOPATHOLOGICAL ALTERATIONS IN SPLEEN OF *ASCARADIA GALLI* INFECTED AND CADMIUM EXPOSED WHITE LEG-HORN CHICKS

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ABSTRACT

White leg-horn chicks were given low (150 eggs) and high (1500) doses of pure eggs of *Ascaradia galli* and subsequently treated with cadmium acetate. After 16 and 32 days post-treatment, spleen showed various immunopathological changes. With low dose of infection, the capsular wall ruptured at various places and white pulp showed inflammatory edema and presence of secondary nodules. The high dose of infection caused separation of inner capsular wall with inflammatory and non-inflammatory edema. Sinus venosus showed lymphoid hyperplasia. Cadmium acetate caused increased production of lymphocytes and edema. Combined effect of *A. galli* infection and cadmium acetate treatment induced disorganization of capsular wall and depletion of lymphocytes.

***Key words**: Chicks,* Ascaradia galli *eggs, cadmium, immunopathology, spleen.*

In recent years the field of immunology has attracted wide attention in view of growing awareness regarding the need to modulate the host's immune system to achieve desirable effect for preventing any infection or diseased condition rather than treating it by chemotherapy in advanced stage after appearance of the symptoms. Advanced stage of infection results into various immunopathological, haematological and biochemical changes in the host. Therefore, an attempt has been made to record the immunopatholoical changes occurring in spleen of white leg-horn (WLH) chicks under *Ascaradia galli* infection followed by cadmium acetate treatment.

MATERIALS AND METHODS

Newly hatched white leg-horn chicks (WLH) were procured from Salim Hatchery, Meerut. Before initiation of the experiment, the chicks were housed in clean wood and steel cages in Animal Houses and acclimatized to laboratory conditions (temperature 36±2°C, photoperiod 18L, D. 6D) for 14 days. They were fed *ad libitum* on formulated chick feed (Hindustan Poultry Feed Ltd., India) during evening and morning and clean water was provided. The feeding was stopped 24 h before commencement of experiments to avoid dietary metabolic variations.

Collection of parasites: The adult parasitic *Ascaradia galli* were collected from intestine of freshly slaughtered fowl. The adult parasites were washed in double distilled water and kept in Lock-Lewis solution for egg laying. The pure embryonated 250 eggs were administered to laboratory maintained chicks. After 30 days, the parasites were collected from infected chicks maintained in laboratory whenever infective eggs were required. The chicks were autopcised and mature male and females parasites were obtained from the above chicks. All the experiments were done from the pure eggs obtained from experimentally infected chicks.

Toxicant used: An inorganic salt of the heavy metal cadmium of 99% purity namely cadmium acetate (BDH India Ltd, Mumbai) was selected.

Chronic exposure: Thirty six healthy chicks weighing (240±10 gm) were selected and divided into six equal groups. They were subjected to the treatments as -

(i) control, (ii) low dose of infection (150 embryonated eggs), (iii) high dose of infection (1,500 embryonated eggs), (iv) cadmium acetate 6.8 mg, (v) cadmium acetate 6.8 mg + low dose of infection (150 embryonated eggs) and (vi) cadmium acetate 6.8 mg + high dose of infection (1500 embryonated eggs). The chicks were regularly checked for activity, behaviour and weight as well as other related conditions during the entire course of investigation.

Chicks were anesthesized, decapitated and autopsy was performed. Spleen were removed and placed in 10% neutral buffer formalin. Organ sample were processed by standard histopathological techniques (Pearse, 1968). Sections were cut at 6 µ and stained with haematoxylin and eosin (H&E) for light microscopic examination.

RESULTS AND DISCUSSION

Immunopathological changes in spleen of WLH chicks on day 16 of treatment

Spleen is considered as secondary lymphoid organ in WLH chick. It is found close to right side of the junction between proventriculus and gizzard. Spleen of control WLH chick on day 16 appeared radish brown and rounded structure. The substance of spleen contained spleenic trebeculae which divided the sub-scapular tissues. Trabeculae were less or early seen in spleen of WLH chicks.

Spleen of the control WLH chicks comprised capsule and sub-capsule. The capsule was made up of an outer and inner layer of collagen and elastic fibers and externally surrounded by thin peritoneal layer. Outer layer is composed of collagen fibers and few elastic fibers forming a thin layer. Inner layer of capsule was made up of elastic fibers and fine collagen fibers forming a thick layer (Fig. 1).

Sub-capsule consisted of spleenic pulp broadly divided into two parts- white pulp and red pulp. White pulp consisted predominantly of area around branches of spleenic artery. The white pulp consisted of diffused network of reticular cells. White pulp contained number of scattered medium-sized and small lymphocytes. The red pulp was observed around the white pulp and consisted of loose spongy tissue made up of cellular cords surrounded by venous sinosis. The venous sinosis was irregularly shaped spaces between reticular cells of white pulp and red pulp. Red pulp also contained large number of lymphocytes and macrophages. The plasma cells and eosinophil cells were readily seen in the red pulp (Fig. 1).

On day 16 of the low dose of *Ascaradia galli* infection, spleen revealed the following changes. Capsules wall was found to be normal but separated at certain places. The white pulp revealed presence of secondary nodules and the number of lymphocytes decreased within white pulp area. The wall of blood arteries was found to be thickened. Well marked lymphoid hyperplasia was observed. The red pulp tissue revealed congested venous sinuses. The lumen of trabeculae was observed to be dilated (Fig. 3).

On day 16 of the high dose of *Ascaradia galli* infection, the capsular wall of spleen was found thickened at some places showing wavy and irregular shape. Marked changes were found in capsular wall with large number of red blood corpuscles and lymphocytes infiltration. Outer capsular wall was found separated from inner capsular wall at certain places. The inner capsular wall also showed separation from reticular tissue of the spleen at certain places. White pulp revealed dilated splenic sheathed blood vessels and some of them were sclerosed. It also revealed depletion of lymphocytes. The red pulp tissue revealed mild depletion of lymphocytes. Congestion was observed in large and small patches with huge number of RBCs and plasma cells. The venous sinuses revealed dilation parallel type of non-inflammatory edema (Fig. 4).

On day 16 of sublethal dose of cadmium acetate treatment (PT), capsular wall of the spleen was found to be thickened at some places with irregular number of vessels and some of them were sclerosed. White pulp tissue revealed mild depletion of lymphocytes. The secondary nodule was very distinct with clear space and infiltration of inflammatory cells. In red pulp area, parallel non-inflammatory edemas were evident and there was also present irregularly passage of venous sinuses. The reticular tissues revealed prominent cloudy swelling. The red and white pulp areas showed depletion of lymphocytes and the venous sinuses were highly dilated (Fig. 5).

Spleen of the chicks infected with low dose *A. galli* eggs and treatment of cadmium acetate (LPIV PT) on day 16 exhibited prominent vacuolization and inflammatory edema in outer and inner capsule walls. Outer capsular wall was slightly thickened and showed necrosis at various places. The white pulp tissue revealed severe depletion of lymphocytes. Few eosinophiles, plasma cells and the transformed lymphocytes were also recorded. The red pulp tissues were observed as loosely packed spongy tissue beneath the capsular wall. The depletion of lymphocytes was found to be prominent with congested sinuses. Inflammatory and non inflammatory edema were also observed (Fig. 6, 7).

Spleen of the chicks infected with high dose *A. galli* eggs and treatment with cadmium acetate (HPI/PT)on day 16 revealed thick and heavily regular non-inflammatory edema in outer layer capsular wall. The white pulp tissue revealed mild depletion of lymphocytes. Hyperplasia of sheated arteries were distinctly observed in white pulp area. Depletion on lymphocyte was found to be prominent. Hyperplasia with highly dilated venous sinuses was also revealed in the red pulp area. Both red and white pulps revealed non-inflammatory edema. The reticular tissue revealed cloudy swelling. The secondary lymphoid nodule showed prominent non-inflammatory edema and blood vessels present around it revealed thickened wall (Fig. 8).

Immunopathological changes in spleen of WLH chicks on day 32 of treatment

Structure of spleen of the control group of white leghorn chicks on day 32 exhibited the structure almost similar to those described for day 16 control chicks (Fig. 2).

Immunopathological changes in the spleen of the chicks infected with low dose (150 eggs) of embryonated *A. galli* eggs (LPI) on day 32 showed normal capsular wall. The white pulp tissue revealed the depletion of lymphocytes and congestion of venous sinuses was prominent. In red pulp area, inflammatory and non-inflammatory edema was observed at certain places and various secondary nodules were present. Depletion of lymphocytes and cloudy swelling in red pulp area were noticed. The reticular tissue of red and white pulp areas showed irregular and highly dilated venous sinuses (Fig. 9, 10).

The spleen of WLH chicks infected with high dose (1500 eggs) of embryonated *A. galli* eggs (HPI) on day 32 revealed the elasto-collagenous capsular wall as wavy structure and infiltrated with inflammatory cells. At some places, the capsular wall became quite thick, vacuolated with distinct non-inflammatory edema. The white pulp tissue revealed dilation of spleenic artery with very thick walls and infiltration of blood as well as inflammatory cells. The lumen of the artery also showed inflammatory cells. It also revealed depletion of lymphocytes and cloudy swelling was observed in the white pulp area. The red pulp exhibited abundant number of lymphocytes. The reticular tissue showed enlarged secondary nodules. Infiltration with blood cells and inflammatory cells around nodule inflammatory edema was observed. The venous sinuses were dilated and secondary nodule also showed vacuolization (Fig. 11).

Immunopathological changes in spleen of WLH chicks treated with sublethal dose of cadmium acetate (PT) on day 32 depicted ruptured outer capsular wall while inner capsular wall was thickened and separated. White pulp and red pulp areas appeared as loosely packed spongy tissue. There were present irregularly passage of venous sinuses and number of lymphocytes increased. Two secondary nodules were present showing mild edema and impregnation with lymphocytes (Fig. 12, 13).

Spleen of the WLH chicks infected with low dose (150 eggs) of embryonated *A. galli* eggs and treated with cadmium acetate (LPI/PT) revealed the capsular wall to be thin and irregular at certain places. Non-inflammatory edema was observed in inner wall of

...capsule. The thick walled sheathed artery was found ...section area. Lumen of the artery revealed infiltration ...cells and inflammatory cells. The endothelial ...displayed infiltration of inflammatory cells. Area ...the thick artery exhibited vacuolization. The venous ...revealed irregular dilation and white pulp cloudy swelling (Fig. 14).

Immunopathological changes in the spleen infected chicks with high dose (1500 eggs) of embryonated *A. galli* eggs and treated with cadmium acetate (HPI/PT) on day 32 showed marked changes in lymphoid tissue. The capsules wall was thickened at some places. The architecture of reticular tissue was quite disturbed and at most place reticular it was absent. Thick walled arteries were separated, depletion of lymphocytes observed. The capsular wall and reticular tissues revealed disorganized structure with vacuolization. Venous sinuses were highly dilated. The demarcation between red and white pulp was lost and depletion of lymphocytes in white pulp observed. Arteries were also present in white and red pulp (Fig. 15, 16)..

The present investigation revealed that the experimental *A. galli* infection and cadmium treatment induced marked immunopathological changes in spleen of WLH chicks due to low and high dose of embryonated eggs. The capsular wall of spleen was found to be quite thick and ruptured whereas red and white pulp revealed inflammatory edema with eosinophils and macrophages. With the high dose of embryonated eggs infected chicks, marked immunopathological changes like hyperplasia of follicles were observed during resent investigation owing to formation of secondary lymphoid nodule. The appearance of secondary lymphoid nodule in spleen is associated with the infective process as it was always found in the spleen of injected and treated chicks but absent in control chicks. Spleenic congestion and inflammatory as well as non-inflammatory edema were observed in infected and treated group of chicks which may be due to presence of scattered erythrocytes and lymphocytes. Depletion of lymphoid cells has also been observed in spleen of broilar chicks (Tanigueri *et al*., 1977; Bagurt *et al*., 1979; Holovzka and Jurajda, 1992; Stoev *et al*., 2000). Wedderburn (1974) observed hypertrophy of spleen in infected mice. Macrophages living in the spleen sinuses would impair normal blood flow that may effect in experimental parasitic disease. Similar

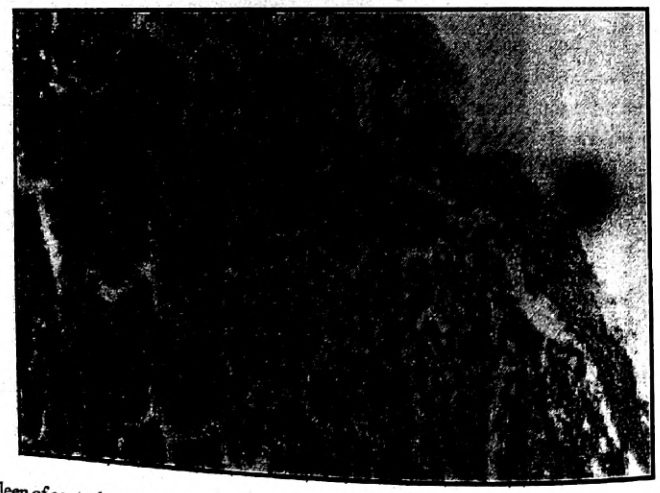

Fig. 1. Spleen of control chick on day 16 showing capsular wall, blood vessels and trabeculae. x 200.

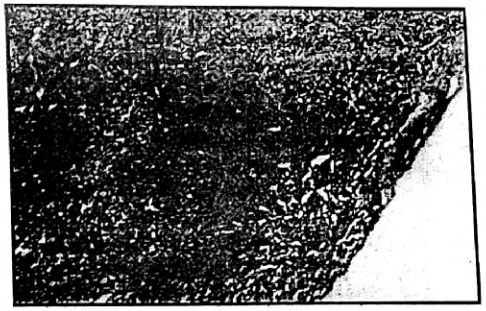

Fig. 2. Spleen of control chick on day 32 showing red and white pulp areas. x 200.

Fig.3. Spleen of chick with low dose of infection showing degenerative changes in capsular wall, white and red pulp area on day 16 of LPI. x 200

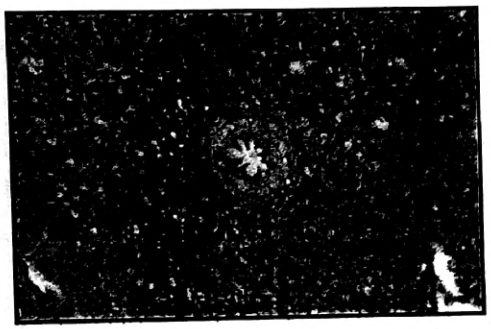

Fig. 4. Spleen of chick showing mild degenerative changes in white pulp area having small artery and red pulp area on day 16 of HPI. x 200

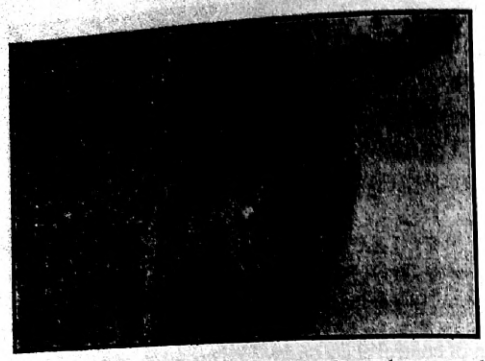

Fig. 5. Spleen exhibiting sinusoidal congestion due to huge patches of red blood cells and red pulp on day 16 of PT. x 200

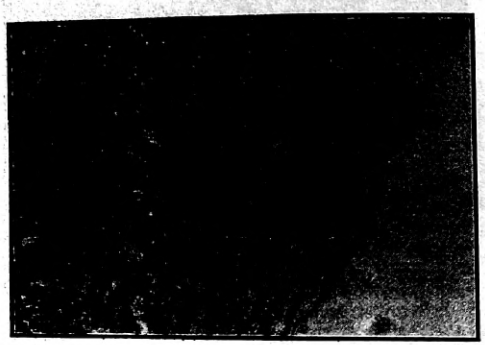

Fig. 6. Spleen depicting red pulp area and thick capsular wall on day 16 of LPI/PT. x 200.

Fig.7. Spleen showing dilated artery on day 16 of LPI/PT. x 200.

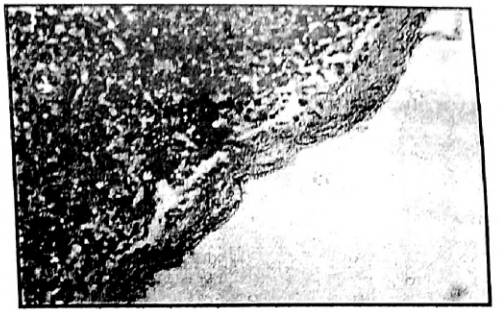

Fig. 8. Spleen exhibiting non-inflammatory edema in capsular wall, depletion of lymphocytes in red pulp area and small artery in white pulp area on day 16 of HPI/PT. x 200.

Fig. 9. Spleen showing thick capsular wall, inflammatory edema on day 32 of LPI. x 200.

Fig. 10. Spleen depicting hyperplasia of sheathed artery and abundant lymphocytes in white and red pulp area on day 32 of LPI. x 200.

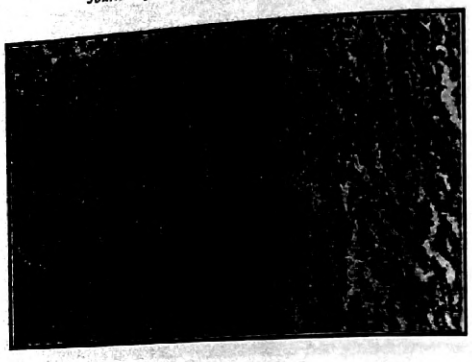

Fig. 11. Spleen showing parallel non-inflammatory edema in red pulp area on day 32 of HPI. x 200.

Fig. 12. Spleen exhibiting thick capsular wall, dilated venous sinuses and complete loss of lymphocytes on day 32 of PT. x 200.

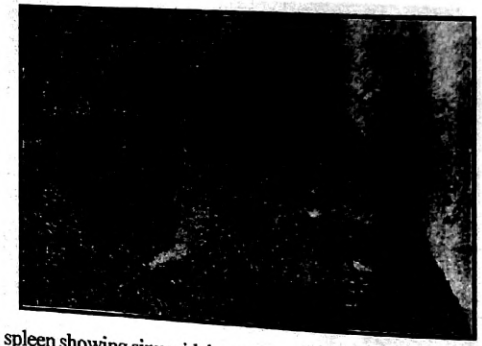

Fig.13. spleen showing sinusoidal congestion and depletion of lymphocyets cells in white pulp area on day of LPI/PT. x 200.

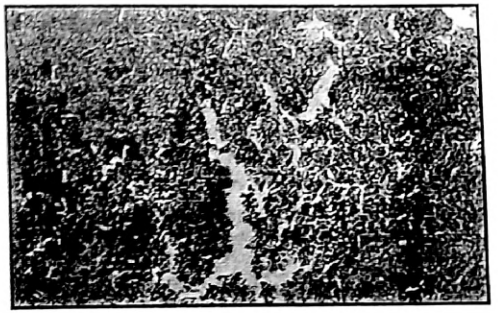

Fig. 14. Spleen showing irregular passage of venous sinuses on day 32 of LPI/PT. x 200.

Fig. 15. Spleen depicting non-inflammatory edema in capsular wall on day 32 of HPI/PT. x 200.

Fig. 16: Spleen showing scattered lymphocytes in red pulp tissue, dilated venous sinuses and secondary nodule on day 31 of HPI/PT. x 200.

changes were also observed in spleen having parasitic infections (Rogers *et al.*, 1975). Vincent and Ash (1978) reported changes of spleen during the course of infection with *B. malayi, B. pahangi* and *B. pateri.* The histology associated with *Longicallum alemmiscus* in spleen revealed variable amount of cellular infilteration (Frank, 1993). Hyperplastic follicles contained germinal centres and lingible bodies. The pararteriol area of spleen were well developed and showed no depletion of lymphocyte (Chandra and Merovitch, 1985).

The present study revealed immuno-pathological changes not only in intestine where the parasite lives but also in lymphoid organ. The combined effects of cadmium toxicity and parasitic infection induce severe hypersensitivity reactions causing various immunopathological changes. The present study showed inflammatory response of embryonated *A. galli* eggs infection and cadmium acetate on lymphopoietic system and other biological system causing hypoxic edema and degenerative changing in spleen of chick and disturb the physiological and metabolic activities of the host.

REFERENCES

Chandra, R.K. and Merovitch 1985. Immune response in parasitic disease. Part B. Mechanism. *Internet. Symp. Bellagio* (Italy), 4 (4): 756-762.

Frank, R.R. 1993. Comparative histology of wngicallum infection in the alimenhy tract and spleen of Acethopagneus australis. *Intl. J. Parasitol.*, 23(3): 391-394.

Rogers, W.P. and Summ-Erville, P.I. 1975. The infection stage of nematode parasite and its significance in parasitism. *Adv. Parasitol.*, 1: 107-109.

Stoev, S.W., Anguelov, G., Ivanov, I and Paviov, D. 2000. Influence of Ochratoxin-A and an extract of archichoke on the vaccinal immunity and health in broiler chicks. *Exp. Toxicol. Pathol.*, 52(1): 43-55.

Tanigueri, T., Yuasa, N., Sato, S. and Hnriuchi, T. 1977. Pathological changes in chickens in oculated with reticulo-endotheliosis virus/contaminated marks disease Vaccine Nat & Inst. (Complete the Reference)

Vincent, A.L. and Ash, L.R. 1978. The lymphatic pathology of *Brugia pahangi* in the Mongolianjird. *J. Parasitol.*, 66: 613-620.

Wedderburn, N. 1974. Immuno-depression produced by national (check) infection in mice. In Parasite in immunized host. Mechanism of survival. *Ciba Foundation Symposium*, 25: 123-125.

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INTEGRATED MANAGEMENT OF SEED BORNE FUNGI OF SOYBEAN BY ADENOCALYMMA ALLIACEUM MIERS.

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ABSTRACT

Seeds of soybean are infested with numerous fungi as *Alternaria alternata, Aspergillus niger, Cercospora kikuchii, Chaetomium spp., Cladosporium cladosporioides, Curvularia lunata, Fusarium roseum, Macrophomina phaseolina, Penicillium itaclicum, Phomopsis spp., Rhizoctonia solani* and *Sclerotium rolfsii.* Management of these pathogens becomes quite difficult by their nature of survival in the seed. Aqueous extract from the leaves of garlic creeper *(Adenocalymma alliaceum* Miers.*)* was investigated for their antifungal activity against these pathogens by seed treatment. The extract demonstrated wide spectrum fungitoxicty. The seed were treated with aqueous leaf extract for 5 minutes to 2 h. It is evident that the treatment with leaf extract for 1.30 h inhibited the growth of dominant fungi as *Aspergillus niger, Penicillium itaclicum, Rhizoctonia solani* and *Sclerotium rolfsii.* The result indicated that the longer duration of seed treatments with plants extracts was effective in controlling the growth of the entire surface borne seed mycoflora. So it can be utilize for the biological control of seed borne fungi of soybean.

Key words: *Adencoalymma alliaceum Miers, Soybean seed, Seed mycoflora, antifungal activity.*

Soybean seeds (*Glycine max* L.) are very high in nutritional value. It contains about 20 per cent oil and 40 per cent high quality protein (as against 7.0 per cent in rice, 12 per cent in wheat, 10 per cent in maize and 20-25 per cent in other pulses). Soybean protein is rich in valuable amino acid lysine (5%) in which most of the cereals is deficient. In addition, it contains a good amount of minerals, salts and vitamins (thiamine and riboflavin) and its sprouting grains contain a considerable amount of Vitamin C, Vitamin A is present in the form of precursor carotene, which is converted into vitamin A in the intestine. A large number of Indian and western dishes such as bread, 'chapatti', milk, sweets, pastries etc., can be prepared with soybean. Wheat flour fortified with soybean flour makes good quality and more nutritious 'chapatti'. Soybean oil is used for manufacturing vanaspati ghee and several other industrial products. Soybean is used for making high protein food for children. It is widely used in the industrial production of different antibiotics. Soybean builds up the soil fertility by fixing large amounts of atmospheric nitrogen through the root nodules, and also through leaf fall on the ground at maturity. It can be used as fodder; forage can be made into hay, silage etc. Its forage and cake are excellent nutritive foods for livestock and poultry. Soybean being the richest, cheapest and easiest source of best quality proteins and fats and having a vast multiplicity of uses as food and industrial products is sometimes called a wonder crop.

More than 40 species of phytopathogenic fungi, bacteria and viruses may infest soybean seed causing various diseases, out of which 15 can result in significant economical losses, reducing yield and deteriorating quality of seed crop. Infected seed can provide primary inoculums for infestation of new crop and seed-borne pathogens may be dispersed for long distances with it (Vidić *et al.*, 2003; Ignjatov *et al.*, 2006; Medić-Pap,. 2007).

[illegible] of plants have been used in [illegible] for many years due to their [illegible] properties (Sofowora, 1993). [illegible] the medicinal value of these plants lies in [illegible] chemical substances that produce a definite [illegible] action on the human or animal body [illegible] 2005). The most important of these [illegible] constituents which are mainly secondary [illegible] are alkaloids, flavonoids, tannins and [illegible] compounds. These phytochemicals are toxic [illegible] cells.

[illegible] creeper (*Adenocalymma* [illegible] family Bignoniaceae, is a wide spread [illegible] North Brazil know as "Ipod alho" [illegible] because it has pungent garlic like smell. [illegible] as well as the flower smell alliaceous and [illegible] substitute for garlic by natives, when the [illegible] inaccessible in the interior regions. (Rao *et* [illegible] *Adenocalymma alliaceum* is one of the [illegible] important commercial cultivars because it [illegible] source of several compounds which are [illegible] antispasmodics, diuretics, anesthetics, and [illegible].

[illegible] present study has therefore been [illegible] with the objective to evaluate the efficacy [illegible] extract of *Adenocalymma alliaceum* against the major seed-borne pathogens of soybean.

METHODS AND MATERIALS

Seed source. Seed samples were obtained from the Daliganj market; Lucknow .A total number 5 varities of soybean were used viz. VL Soya 21, VL Soya 1, RS 1092, MAUS 61-2 and Kalitur.

Isolation Technique. Two generalised isolation procedures were employed for the isolation of pathogenic and saprophytic fungi (Neergaard, 1977). The two methods were the moist blotter and the potato dextrose agar (PDA) method.

Isolation on Moist Blotting Paper. Ten non-sterilized seeds were evenly placed on three layers of moistened 9 cm diameter filter paper (Whatman No.1) in plastic Petri dishes to allow for the penetration of light. A total of 10 seeds were used for each sample. Total 50 seeds are taken. The plates were incubated at 27 ± 2°C for 4 to 5 days in an alternating cycle of 12 hour s NUV (near ultra violet light) and 12 hours darkness regime. Fungi developing on seeds were examined and transferred to PDA for identification and pathogenicity studies.

Incubation on PDA. Ten seeds, surface sterilized for 10 minutes in 1 percent solution of sodium hypochlorite as a pre – treatment, were evenly spaced on PDA plate. The plates were incubated at 27 ± 2°C for 4 to 5 days in an alternating cycle of 12 hours NUV and 12 hours darkness. A total of 10 seeds per sample were used. Fungi developing on seeds were identified as in the previous experiment.

Plant Material. During the month of January and February the fresh leaves (100g) of *Adenocalymma alliaceum* Miers were collected from the Botany Department, Lucknow University, Lucknow. The studies were carried out in the Mycology and Plant Pathology Laboratory, Lucknow.

Preparation of Aqueous Extract of Leaves of Garlic Creeper. Collected leaves were properly washed with 70% ethanol and water separately. Final wash with distilled/sterilized water removes the trace of ethanol. An aqueous extract was prepared by blending fresh leaves with distilled water (10ml/g fresh weight) i.e.10%.in a blender for 2 minutes at 27 ±2°C. The homogenate was filtered through a layer of muslin cloth. The filtrate was centrifuged at 12000 rpm for 20 minutes. The supernatant was sterilized through a Millipore filter (0.2µm) and served as the stock solution. All dilution were made with sterile distil water when necessary (Rana *et al.*, 1999).

Seed treatment. The different soybean seed cultivar were treated with leaf extract of *Adenocalymma alliaceum* (2 %) by soaking seed in it for different time period as 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 1.15 hour, 1.30 hour, 1.45 hour, and 2 hour.

Study of mycoflora of treated seeds. Treated seed with plant extract for above time intervals were incubated in Potato dextrose agar (PDA) at 27 ± 2°C for 4 to 5 days in an alternating cycle of 12 hour s NUV (near ultra violet light) and 12 hours darkness regime. They were studied for the growth of fungi from the seed surface.

RESULTS AND DISCUSSION

The most common fungi found to be growing on all untreated seed were *Alternaria alternate(Aa), Aspergillus niger(An), Cercospora kikuchii(Ck), Chaetomium spp(Cspp)., Cladosporium cladosporioides(Cc), Curvularia lunata(Cl), Fusarium roseum(Fr), Macrophomina phaseolina(Mp), Penicillium italicum(Pi), Phomopsis spp(Pspp.), Rhizoctonia solani(Rs),* and *Sclerotium rolfsii(Sr).* (Table 1)

Table 1: Mycoflora of Soybean Varity on untreated seed.

Name of pathogens	Varity of soybeen				
	VL Soya 21	VL Soya 1	PS 1092	MAUS 61-2	Kalitur
Alternaria alternata	+++	++	+	-	-
Aspergillus niger	++	+	++	+++	++
Cercospora kikuchii	++	-	+	-	-
Chaetomium spp.	-	-	-	+	++
Cladosporium cladosporioides	-	+++	++	+	-
Curvularia lunata	++	++	-	+	-
Fusarium roseum	+	++	-	+++	+
Macrophomina phaseolina	+	++	+	-	++
Penicillium itaclicum	-	-	+	-	++
Phomopsis spp	++	+	+	+++	-
Rhizoctonia solani	+++	++	-	+	+
Sclerotium rolfsii	++	+	-	+++	-

Symbols used in table 1

+ = presence on 10 % seeds

++ = presence on 20% seeds

+++ = presence on 30 % seeds

- = absence on seeds

Table: 2 Effect of the Duration of Exposure of the Leaf Extract on seed of soybean mycoflora.

Sr no.	Soybean variety	Mycoflora on treated seed with *Adenocalymma alliaceum* Miers leave extracts Exposure Time								
		5 mit	15mit	30mit	45mit	1 h	1.15h	1.30h	1.45h	2h
1	VL Soya 21	*Aa*	*Aa*	*Aa*	*Aa*	*Aa*	-	-	-	-
		An	*An*	*An*	*An*	*An*	*An*	-	-	-
		Ck	*Ck*	*Ck*	*Ck*	-	-	-	-	-
		Cl	*Cl*	*Cl*	*Cl*	-	-	-	-	-
		Fr	*Fr*	*Fr*	*Fr*	*Fr*	-	-	-	-
		Mp	*Mp*	*Mp*	-	-	-	-	-	-
		Pspp.	*Pspp.*	*Pspp.*	*Pspp.*	*Pspp.*	-	-	-	-
		Rs	*Rs*	*Rs*	*Rs*	*Rs*	*Rs*	-	-	-
		Sr	*Sr*	*Sr*	*Sr*	*Sr*	*Sr*	-	-	-
2	VL Soya 1	*Aa*	*Aa*	*Aa*	*Aa*	*Aa*	-	-	-	-
		An	*An*	*An*	*An*	*An*	*An*	-	-	-
		Cc	*Cc*	*Cc*	*Cc*	*Cc*	-	-	-	-
		Cl	*Cl*	*Cl*	*Cl*	-	-	-	-	-
		Fr	*Fr*	*Fr*	*Fr*	*Fr*	-	-	-	-
		Mp	*Mp*	*Mp*	-	-	-	-	-	-
		Pspp	*Pspp.*	*Pspp.*	*Pspp.*	*Pspp.*	-	-	-	-
		Rs	*Rs*	*Rs*	*Rs*	*Rs*	*Rs*	-	-	-
		Sr	*Sr*	*Sr*	*Sr*	*Sr*	*Sr*	-	-	-
3	PS 1092	*Aa*	*Aa*	*Aa*	*Aa*	*Aa*	-	-	-	-
		An	*An*	*An*	*An*	*An*	*An*	-	-	-
		Ck	*Ck*	*Ck*	*Ck*	-	-	-	-	-
		Cc	*Cc*	*Cc*	*Cc*	*Cc*	-	-	-	-
		Mp	*Mp*	*Mp*	-	-	-	-	-	-
		Pi	*Pi*	*Pi*	*Pi*	*Pi*	*Pi*	*Pi*	-	-
		Pspp	*Pspp.*	*Pspp.*	*Pspp.*	*Pspp.*	-	-	-	-
4	MAUS 61-2	*An*	*An*	*An*	*An*	*An*	*An*	-	-	-
		Cspp	*Cspp*	*Cspp*	*Cspp*	-	-	-	-	-
		Cc	*Cc*	*Cc*	*Cc*	*Cc*	-	-	-	-
		Cl	*Cl*	*Cl*	*Cl*	*Cl*	-	-	-	-
		Fr	*Fr*	*Fr*	*Fr*	*Fr*	-	-	-	-
		Pspp	*Pspp*	*Pspp*	*Pspp*	*Pspp*	-	-	-	-
		Rs	*Rs*	*Rs*	*Rs*	*Rs*	*Rs*	-	-	-
		Sr	*Sr*	*Sr*	*Sr*	*Sr*	*Sr*	-	-	-
5	Kalitur	*An*	*An*	*An*	*An*	*An*	*An*	-	-	-
		Cspp	*Cspp*	*Cspp*	*Cspp*	-	-	-	-	-
		Fr	*Fr*	*Fr*	*Fr*	*Fr*	-	-	-	-
		Mp	*Mp*	*Mp*	-	-	-	-	-	-
		Pi	*Pi*	*Pi*	*Pi*	*Pi*	*Pi*	*Pi*	-	-
		Rs	*Rs*	*Rs*	*Rs*	*Rs*	*Rs*	-	-	-

The result indicated that as the duration of seed treatments with plants extracts increased the number of surface mycoflora decreased (Table 2). The inhibition of fungi growth was observed when the seeds were soaked in the garlic creeper leaf extract for 1.30 h

Application of plat extracts for the control of seed borne disease is method devoid of any health hazard problem. Hill bunt of Wheat (Tilletia *foetida*) was effectively controlled by seed treatment with plants extract of *Datura stramonium, Thuja sp.* and *Eucalyptus* (Shing *et al.*, 1980)

The antifungal effect of selected medicinal extracts can be applied at a larger scale to treat the seed before sowing them in the field. The seed treatment with plants extract does not have any adverse effect on the germination of seed even after the treatments for 1.30 h. Thus, on the other hand the seed treated with plants extract will also not create any problem of pollution as the biochemical's of plants extract will easily degrade in the soil.

Leaf extracts of *Adenocalymma alliaceum* showed a wide spectrum of fungicidal activity.Results signifies the potentiality of *Adenocalymma alliaceum* as a source of antifungal therapies. Hence, further work is required to evaluate its potentially active principle on other pathogens as this biofungicidal botanics is environmentally safe and could replace the toxic and hazardous synthetic compounds. Simultaneously investigations are also needed to characterize and formulate the active principles of the extract which may provide lead for the discovery of a novel antifungal compound from *Adenocalymma alliaceum.*

REFERENCES

Edeoga, H.O.; Okwu, D.E. and Mbaeble, B.O. 2005. Phytochemical Constituents of some Nigerian Medicinal Plants. *African Journal of Biotechnology*, 4: 685-688.

Hazan, A.G. and Atta, R. 2005. Trends in Ethnopharmacology. *Journal of Ethnopharmacology*, 100:43-49.

Ignjatov M., Balaž J., Milošević M., Popović T., 2006: Bakteriozna pegavost soje. *Arh. Poljopr. Nauke* 67, 239: 11–17.

Medić-Pap, S. 2007: Mycoflora – the cause of soybean seed diseases. Typescript. University of Novi Sad, Novi Sad.

Neergaard , P. 1977 . Seed pathlogy vol I and II McMillan Press Itl. Londaon :Pp 1187

Rana, B.K.; Tanja, V. and Singh, U.P. 1999. Antifungal activity of an aqueous extract of leaves of garlic creeper (*Adenocalymnma alliaceum* Miers.) .*Pharmaceutical biology*. 37: 13-16

Rao, L.J.M.; Srinivas, P. and Gurudutt, K.M. 1999. Chemical composition of the volatile oil from garlic creeper (Adenoalymma *alliaceoum). J. of Medicinal and Aromatic Plant Sciences*. 21,987-989.

Shing, A. K.; Diekhit, A.; Sharma, M. l. and Dixit, S. N. 1980 . Fungitoxic activity of some essential oils. *Economic botany*, 34 :186-190

Sofowora, A. 1993. *Medicinal plants and Traditional Medicine in Africa.* Spectrum Books Limited, Ibadan, Nigeria.346pp.

Vidić, M.;Jasnić, S. and Dorović, V. 2003: Bolesti semena soje. Biljni Lekar 6: 585–591.

Webser, N. 1962. Webster New Twentieth Century Dictionary. The World Pub Co., Clevelant & New Yark

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DIVERSITY OF FISH NEMATODE – *INDOCUCULLANUS FAUSTII N. SP.* (NEMATODA: CUCULLANIDAE) FROM MEERUT- DELHI REGION, INDIA

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ABSTRACT

The present species identified as *Indocucullanus faustii n.sp.* obtained from the intestine of the freshwater fish, *Mystus seenghala*. Nematodes constitute one of the most important group of animals. Some of them were free living in soil, water and other parasites were of plants and animals. The nematodes parasitizing the animals including man cause a number of diseases which may sometime results in the death of their hosts. In the present studies, *Indocucullanus faustii n.sp.* no distinction lips, papillae and conspicuous teeth while in *Indocucullanus alii* was demarcated.

***Key words**: Nematoda,* Indocucullanus, fish, diversity, Mystus seenghala, *Scanning electron microscopy.*

Two female specimens of the nematode belonging to the genus *Indocucullanus* identified as *Indocucullanus faustii n.sp.* were obtained from the intestine of freshwater fish, *Mystus seenghala.* According to a World Health Organization (WHO) Expert Committee (1987) the overall impact of parasitic infection constitutes a significant health and social problem. Recent global estimate indicate that intestinal helminth infections were the most common infection in the world (Pawlowski 1984). Infection with intestinal helminthes is a significant cause of morbidity and places avoidable burdens on the health care resources of the state. Helminth infection also affects the socio-economic structure of a society through its harmful effects on the physical and intellectual development of children as well as on the productivity of adult members of society (Stephenson, 1987).

The number of nematode species in the world was uncertain. According to Steiner (1960) about 9000 species has been describe but these were doubtless only a fraction of those actually existing. Hyman (1951) thought that there must be at least 5,00,000 species in the world. Kates (1965) estimated that the 3,212,000,000 from the pet animals in United States had some 7,750,000,000 helminthes infections this may be compared with 44,000,000 human helminthes infections estimated by Stoll (1947). There were about 57 times as many helminthes infections in the large domestic animals as in man and about 176 times as many in all farm animals and pets as in man. Nematodes are not only parasitic to animals but also to plants. Ooestenbrink (1960) estimated that plant nematodes cause an annual crop loss of 10% in United States. Allen (1959) estimated the loss for California alone was $90 to $ 140 million per year.

Diversity of species were extremely important relationship with virulence and hypersensitivity reactions in the host. The disease spectrum entirely depends on species. But in the present species they were harmful to human being which may sometime results in the death of their hosts. So the symptoms of the disease and virulence of the disease in case of both micro-parasites as well as macro-parasite was strictly species oriented and hence it was essential to study the diversity of each form and each kind of parasite. Therefore, the collection and identification of parasites was very important to study from disease point of view and also from formulations of the anti-parasitic agents against specific parasites. Taxonomy of parasitic nematodes was based primarily on their morphological variations invariably found between

individuals within the same species study of such variations was therefore of great importance to taxonomists.

MATERIALS AND METHODS

Fishes were collected from fish market from the Meerut, Delhi region, India. The fishes were taken out from water and then chloroformed. The alimentary canal was cut open in to the normal saline. The parasites were recovered from the intestine of fish *Mystus seenghala*. Parasites were transferred to normal saline (0.75% NaCl). After removing the saline with the help of a dropper 70 % alcohol was poured in the petri dish to kill and fix the parasite in 90% alcohol with 2 percent glycerine. The preserved parasites were clear in lactophenol for 15-24 hours and mounted in the same medium for appropriate observations for enface-view study. The head of parasite was cut with a sharp blade and brought into desired position under the cover glass. All measurements are given in millimeters.

Scanning Electron Microscopic Studies

After *in vitro* treatment, the parasites were kept into the modified Karnovskys fluid (1965), separately according to the concentration of drug and exposure hours which were used as a fixative. The parasites were kept in modified Karnovskys fluid at 4°C for 5-6 hours after that they were transferred into 0.1 M cacodylate buffer solution then subsequent dehydration.

Dehydration: Absolute dry acetone was used as a dehydrating agent. This was found to be advantage as it was miscible with liquid carbon dioxide freon-13 used for critical point drying for dehydration. Acetone was prepared which different percentage as 30, 50, 70, 80, 90 and 95. Dehydration was carried out in gradual steps. After dehydration worms were subjected to critical point drying.

Critical point drying: The drying apparatus were properly installed and run with cold water circulation to cool the chamber about 20°C. The parasites were kept in loading baskets with dry acetone. The parasites were removed in boats and placed into the frying apparatus. The inlet valve connected to the CO_2 cylinders were opened to fill the liquid gas rapidly. To avoid the back pressure the vent valve were opened. The vent valves were slightly opened to maintain the level of the liquid. The drain valves were opened to remove acetone. Flushing were carried out for 3 to 5 minutes. After flushing were completed the loading basket were filled with the liquid CO_2 for impregnation in parasites. The steps were again repeated. The inlet valve were closed to allow the level of liquid CO_2 to fall to about the level of the top to the boat.

The chamber were warmed by running warm water (36°C-38°C) and when the temperature attained 32.5°C the CO_2 were evaporated and the drying were completed.

The specimens were removed and mounted on the SEM stubs with double adhesive tape. After that they were coated with gold approximately 350°A and the stubs were subjected to scanning electron microscopic studied and SEM microphotography.

RESULTS AND DISCUSSION

Family: Cucullanidae; Genus: *Indocucullanus* and species: *Indocucullanus faustii n.sp.* (Fig. 1 Camera lucida diagram of *I. faustii n.sp.* female anterior region in 150x. Fig. 2 S.E.M. of *I. faustii n.sp.* female anterior region in 216x. Fig. 3 Camera lucida diagram of *I. faustii n.sp.* female posterior region in 150x. Fig. 4 S.E.M. of *I. faustii n.sp.* female posterior region in 235x).

Material: Two females; Host: *Mystus seenghala;* Location: Intestine; Locality: Meerut-Delhi region; Number of fish examined: 28; Number of fish infected: 11; Type specimen: Halo type and paratype specimens deposited in the Department of Zoology, Meerut College Meerut, C.C.S. University. Meerut, India. (Figs. 1, 2, 3, 4).

Thick, cylindrical worm with a broadly rounded head, mouth terminal, bounded by two lips, each bearing three papillae. A pair of conspicuous teeth. Mouth lead into pseudobuccal capsule. Oesophagus divided into an anterior narrow and posterior slightly bulbous part. vulva post-equatorial (Fig. 4).

Female: The females measured 11.43-15.3 (13.365) mm and 0.235-0.4457 (0.34035) mm in length and

width respectively. The buccal capsule measured 0.242-0.3 (0.27) mm in length. The oesophagus was 0.825-1.05 (0.9375) mm in length. The width of interior narrow oesophagus was 0.075-0.0675 (0.07125) mm. The width of bulb was 0.08-0.27 (0.225) mm. The nerve ring was situated at the distance of 0.22-0.58 (0.4) mm from the anterior extremity. The length of tail was 0.225-0.135 (0.18) mm and was conical.

SEM studies of *Indocucullanus faustii n. sp.*: In scanning electron microscopic studies of *Indocucullanus faustii n.sp.*, anterior region showed the presence of large number of distinct rounded concricenisis and protuberances. The cuticular striations were very indistinct. The rim of buccal capsule revealed the concentric folding of the cuticle. The folds of the outer cortical region was very distinct on the right side of the parasite. The tail region showed indistinct cuticular striations. The anus was revealed a pair of large papillae. Extremity of the tail showed presence of a pair of very small papillae posteriorly. The posterior region of the tail was contained rounded protuberances and concricenisis.

Ali (1956) reported key to the species of *Indocucullanus* from fishes in South Asia. The detailed study of the present material belong to the genus *Indocucullanus*. The present nematode was showing closeness with *I. alii* (Kalyankar, 1971) but from the latter it differed in the present species as there were no distinction lips, papillae and conspicuous teeth whereas in *I. alii* they were demarcated.

Females *Indocucullanus faustii n.sp.* measured 11.43-15.3 (13.365) mm whereas female of *I.alii* measured 4.18 mm in length. Maximum thickness of the present parasite was 0.235-0.4457 (0.34035) mm whereas in *I. alii* width measured 0.23 mm. Oesophagus of *I. alii* 0.55 mm long whereas in present species was 0.825-1.05 (0.9375) mm long.

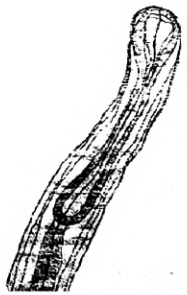

Fig. 1. Indocucullanus faustii n.sp @&

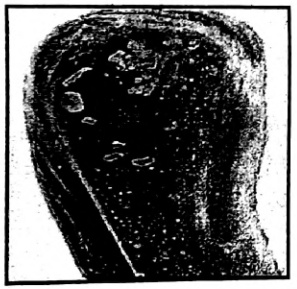

Fig 2. *Indocucullanus faustii n.sp @&*

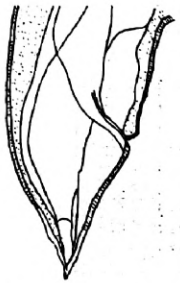

Fig. 3. *Indocucullanus faustii n.sp @&*

Table - 1 : Comparative measurements of male and female studied by different authors of genus *Indocucullanus* (All measurements recorded in millimeters)

Parameters		*I. jaiswali* All (1956)		*I. arablansae* All and Kalyankar (1966)		*I. longispiculum* Khan (1969)		*I. alili* Kalyankar (1971)		*I. jalnaensis* Kalyankar (1971)		*I. malvanae* Kalyankar (1971)		*I. calcariferli* Zaidi and Khan (1975)		*I. guerreroi* Arya and Johnson (1975)		*I. karachli* Zaidi and Khan (1975)	
		Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Total length		8.90	9.51	14.72	13.94-18.06	3.236-3.604	4.448-5.004	3.95	4.18	13.03		17.11		2.58-2.98	3.103-4.147	12-13	14-15	4.350-4.405	
Total width		0.41	0.44	0.36	0.4	0.24-0.34	0.380-0.476	0.22	0.23	0.55		0.64		0.203-0.260	0.340-0.370	0.18-0.20	0.21-0.24	0.319-0.320	
Length of oesophagus		0.94	1.0	1.66	1.73-1.74	0.503-0.598	0.612-0.693	0.53	0.55	1.30		0.94		0.542-0.611	0.669-0.738	0.75-0.79	0.85-0.95	0.099	
Width of anterior narrow oesophagus						0.095-0.136											0.07-0.13	0.098	
Width of bulb																			
Length of tail		0.25	0.23	0.62	0.22-0.26	0.018-0.122	0.095-0.163			0.38		0.28				0.16-0.18	0.18-0.19		
Length of spicule	Small	0.30		0.42		1.057				0.50		0.4		0.319-0.344				0.64	
	Large	0.30		0.42		1.078		0.1		0.55		0.77							
Nerve ring from anterior end		0.3	0.32	0.51		0.285-0.291	0.272	0.98	0.26	0.42		0.57		0.275-0.287		0.26-0.28	0.30-4.6	0.246	
Length of tail process								1.12											
Length of buccal capsule							0.122-0.136	0.24											
Distance between striations																			

Table - 1a : Comparative measurements of male and female studied by different authors of genus *Indocucullanus* (All measurements recorded in millimeters)

Parameters		*I. ariusi* Srivastava and Gupta (1976)		*I. puriensis* Srivastava and Gupta (1976)		*I. wertheimae* Gupta and Garg (1976)		*I. sclaenai* Gupta and Gupta (1977)		*I. simhai* Gupta and Naqvi (1983)		*I. trichiuri* Gupta and Naqvi (1983)		*I. indica* Gupta and Srivastava (1984)		*I. thaparl* Gupta and Srivastava (1984)		*I. faustil n.sp.*	
		Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Total length		2.52		8.9	15.13-16.38	4.95	5.87-7.11	9.25	5.87-7.11	11.65-14.20	13.70-17.25	10.70	17.15	7.8-8.4	2.5-4.00	4.45-5.20	7.22		11.42-15.3
Total width		0.64		0.81	0.43-0.61	0.17	0.21-0.23	0.29	0.21-0.23	0.28-0.36	0.27-0.33	0.46	0.55	0.375-0.40	0.22-0.30	0.50-0.625	0.575		0.235-0.4457
Length of oesophagus		0.61		1.0	1.37-1.52	0.64	0.65-0.76	1.7	0.65-0.76		0.68-0.72	1.10	1.53	0.856-0.900	0.40-0.55	0.65-0.72	0.82		0.825-1.05
Width of anterior narrow oesophagus		0.14		0.18	0.25-0.32			0.2			0.140-0.165	0.24	0.30						0.075-0.0675
Width of bulb																			0.18-0.27
Length of tail		0.154		0.25	0.31-0.49	0.12	0.12	0.45	0.12		0.14-0.21		0.33	0.230-0.275	0.175-0.320	0.15-0.17	0.35		0.225-0.135
Length of spicule	Small			0.31		0.19		0.27		0.36-0.43		0.64		0.30-0.33		0.30-0.33			
	Large			0.332		0.19		0.27		0.37-0.44		0.68		0.350-0.362		0.430-0.475			
Nerve ring from anterior end		0.35		0.3	0.5-0.69			0.45		0.21-0.28	0.20-0.24	0.47	0.46	0.30-0.35	0.20-0.25	0.25-0.31	0.375		0.22-0.58
Length of tail process																			
Length of buccal capsule						0.16	0.16-0.21		0.16-0.21							0.120-0.135	0.140		0.24-0.3
Distance between striations																			

Length of tail measured 0.23 mm as in *I. alii* whereas in *Indocucullanus faustii n.sp.*it measured 0.225-0.135 (0.18) mm. The length of buccal capsule was not measured in *I. alii* whereas in present study species measured 0.24-0.3 (0.27) mm. The nerve ring was situated at a distance of 0.26 mm from the anterior extremity whereas in present species it was found 0.22-0.58 (0.4) mm from the anterior extremity. On the basis of these variations present species differed from all the reported species of the genus *Indocucullanus* new and old species and it revealed new diversity of species. Comparison of *Indocucullanus faustii n.sp.* with different authors of the genus *Indocucullanus* is given in Table 1 and 2.

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REFERENCES

Ali. S.M. 1956. Studies on the nematode parasites of fishes and birds found in Hyderabad State. *Indian J. Helminth.*, 8(I): 1-83.

Ali, S.M. and Kalyankar, S.D. 1966. *Indocucullanus arabianse n.sp.* from the intestine of *Tachysurus maculates* (catfish) in India. *Indian J. Helminthol.*, 18 (Seminar Suppl.): 74-76.

Arya, S.N. and Jhonson, S. 1975. A new cucullanid nematode from the fish, *Cybium guttatum* from Indian waters (Spiruroidea: Cucullanidae). *Memoria de la Sociedad de Ciencias Naturales La Salle*. 35(102): 291-295.

Gupta, N.K. and Garg, V.K. 1976. On two new spiruroid nematodes from marine food fishes in India. *Rev. Iberica Parasitol.* (Spain), 36(3-4): 181-188.

Gupta, S.P. and Naqvi, N.H. 1983. Nematode parasites of fishes. VII. On two new species of the genus *Indocucullanus* Ali (1956) from fishes. *Indian J. Helminthol.*, 34(1): 78-85.

Gupta, S.P. and Srivastava, A.B. 1984. On some nematode parasites of marine fishes from Puri, Orissa. *Indian J. Helminthol.*, 36(2): 97-110.

Hyman, L. 1951. The invertebrates: Acanthocephala, Aschelminthes and Entoprocta. The pseudocoelomate Bilateria. Vol. III. Mc Graw Hill Books, New York.Kalyankar, S.D. 1971. Studies on a known and some new nematode parasites of fishes from India. *Marathwada Univ. J. Sci.*, 10(3): 89-107.

Kates, K.C. 1965. Cited from nematode parasites of domesticated animals of man by (Levine, N.D. 1968). Bruges Publishing Company Minneapolis, Minnosota, Anim. Zool., 5: 95-130.

Khan, D. 1969. A new species of *Indocucullanus* Ali (1957) from Pakistan. *Pakistan J. Zool.*, 1(1): 77-79.

Pawlowski, Z.S. 1984. Implications of parasitic nutrition interactions from a world perspective. *Fed. Proc.*, 43: 256.

Srivastava, A.B. and Gupta, S.P. 1976. Nematode parasites of fishes. 2. On two new species of the genus *Indocucullanus* Ali (1956). *Jap. J. Parasitol.*, 26(1): 46-48.

Steiner, G. 1960. In: Sasser, J.N. and Jenkins, W.R. (eds.) Nematology. Univ. North Carolina Press, Chapel Hill. Pp. 3-7.

Stephenson, L.S. 1987. Impact of helminthes infection on human nutrition: Human malnutrition and helminthes. Taylor & Francis London. 189: 1-20.

W.H.O. 1987. Prevention and control of intestinal parasitic infections. Report of a WHO Expert Committee, Geneva, W.H.O. Technical Report Series.

Zaidi, D.A. and Khan, D. 1975. Nematode parasites from fishes of Pakistan. *Pakistan J. Zool.*, 7(1): 51-73.

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VISCOMETRIC INVESTIGATION AND MOLECULAR INTERACTIONS OF CURCUMIN AND METHNOL AT DIFFERENT TEMPERATURES

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ABSTRACT

The density and viscosity have been measured for the binary mixtures of curcumin and methanol at 303.15, 308.15 313.15, 318.15 and 323.15 K. From the experimental data, relative viscosity, Gibbs's free energy (ΔG) and thermodynamic parameters have been computed . Viscosities of the binary mixtures have been correlated successfully using Jones-Doles, Vand's , Moullik, equation. The excess values of some of the above parameters were also evaluated and discussed by Redlich kister eqn.in the light of molecular interactions in the mixture.

Keywords: *Molecular interactions, density, viscosity, binary liquid mixture, thermodynamic parameters*

Curcumin is the active constituent of turmeric(Curcuma longa). For centuries turmeric has been used as dietary spice, as coloring agent in foods and textiles and as medicine for numerous diseases. The rhizome(modified stem) is the most useful part of the plant. The characteristic yellow colour of turmeric is due to curcumin. Extensive investigations over last five decades has indicated that curcumin is a potent antioxidant (Pandey *et al.* 2011), which are important naturally occurring nutrients helps in maintaining health by slowing the destructive ageing process of cellular molecules. They are found naturally in body & in plants such as fruits and vegetables and is used in diabetes, leishmanisis, psoriasis and apoptosis (Pandey *et al.* 2010). The characteristics of living organisms such as selective permeability across cell membrane , muscle contraction, hearing and memory processes & nerve conduction etc. can be interpreted in terms of interactions of molecules. In some recent publications, efforts have been made to correlate biological activity with calculated physical parameters with the help of densities (Akhtar 2007) and viscosity (Pal and Mandal 2006, Ali *et al.* 2008) for binary (Banipal *et al.* 2007) and ternary (Wanchoo and Narayan 1994, Krishnan *et al.* 1994) mixtures at different temperatures. These antioxidants are important food additives and have many applications in the pharmaceutical industries. Antioxidants are chosen for the molecular interaction studies because they have great potential and is gaining popularity This type of work with therapeutic active antioxidant curcumin has not been reported so far. We will be first to perform this type of studies.

MATERIALS AND METHODS

Curcumin and methanol of high grade purity (> 99%) were purchased from BDH (India) in 2008. A double stem calibrated pyknometer purchased from M/S Science Corporation, Allahabad, has been used to determine the density of solvent and solutions. The viscosity was measured by Ostwald's viscometer. The temperature was maintained by thermostatic water bath (JULABO, model ME-3) . The experimental uncertaintity in density is nearly 0.01%.

3. THEORY AND CALCULATIONS

Energy of Viscous flow $E\eta = Ae^{(E\eta/RT)}$ (1)

For solvent $\mu_1^0 = RT \ln\eta_0(v_1^0/hN)$ (2)

For Solute $\mu_2^0 = \mu_1^0 + (RT/\bar{v}_1^0)(1000 B - (\bar{v}_1^0 - \bar{v}_2^0)$ (3)

$\Delta G = RT \ln (\eta V_m / h N)$ (4)

$\Delta G = \Delta H - T \Delta S$ (5)

Excess Gibb's free energy of activation

$G^E/RT = \ln(V/\eta_2 v_2) - X_1 \ln(\eta_1 v_1/\eta_2 V_2)$ (6)

Excess viscosity $\eta^E = \eta - (X_1\eta_1 + X_2\eta_2)$ (7)

Redlich kister Eqn. $Y^E = X_1(1-X_1)(2X_1-1)$ (8)

RESULTS AND DISCUSSION

The values of excess viscosity (η^E), free energy (G^E) for all systems containing curcumin and methanol at different temperatures 303.15, 308.15, 313.15, 318.15 and 323.15K are given in Table (1). It is seen that density and viscosity increases with concentration of curcumin and decreases with increase in temperature, The increasing value of density and viscosity value shows that there is moderate attraction with solute and solvent molecules. The decrease of

Table 1. Values of excess viscosity (η^E), excess free energy (G^E) and Vand's coefficient (Q) of system at different temperature (K)

Conc.	303.15	308.15	313.15	318.15	323.15
			$\eta^E x\ 10^{-1}$ (kgm^{-1}s^{-1})		
1.012	4.104	3.813	4.320	5.763	7.936
2.023	6.401	7.588	6.248	7.623	10.120
4.047	9.326	9.635	8.557	10.350	12.663
6.070	11.269	11.467	10.185	12.223	13.857
8.094	12.775	12.538	11.364	13.061	15.095
10.117	13.598	13.313	12.069	13.742	13.758
			$G^E x\ 10^{-1}$ (KJ mol^{-1})		
1.012	-1.573	-1.583	-1.600	-1.591	-1.595
2.023	-1.582	-1.592	-1.612	-1.603	-1.610
4.047	-1.593	-1.604	-1.623	-1.614	-1.621
6.070	-1.602	-1.612	-1.632	-1.623	-1.630
8.094	-1.609	-1.619	-1.639	-1.630	-1.637
10.117	-1.614	-1.625	-1.644	-1.630	-1.644
			Q x 10^{-4}		
1.012	2.515	2.498	2.474	2.459	2.436
2.023	2.608	2.595	2.590	2.576	2.573
4.047	2.718	2.718	2.706	2.690	2.685
6.070	2.824	2.802	2.799	2.781	2.778
8.094	1.898	2.875	2.872	2.856	2.848
10.117	2.954	2.944	2.927	2.921	2.917

Table 2. Value of μ_1^0, μ_2^0, A, B, ∇_1^0, M amd K for system at different temperatures

	303.15 K	308.15 K	313.15 K	313.15 K	323.15 K
B (m^{3}mol^{-1})	-4.6836	-7.2147	-11.4418	-21.4931	-42.9656
A (m$^{3/2}$mol$^{-1/2}$)	4.7444	6.1183	6.4746	12.1890	21.0561
∇_1^0 (m^3 mol^{-1})	6.0466	6.1344	6.0333	6.4639	6.9065
K x 10^{-9} (m^6 mol^{-2})	1.0869	0.9830	0.7041	1.3669	1.5698
M	1.1210	1.1607	1.1394	1.2724	1.4524
μ_1^0 (KJ mol^{-1})	56.1631	69.6086	69.7628	73.0944	73.8387
μ_2^0 (KJ mol^{-1})	44.3554	51.1204	39.9722	16.2481	-11.5710

Table 3. Values of Eη, A, ΔH and ΔS for system at different temperatures (K)

Conc. mol.m^{-3}	Eη KJ mol^{-1}	A	ΔH KJ mol^{-1}	ΔS KJ mol^{-1}K^{-1}
1.012	7.6829	24.3156	6.3715	0.4689
2.023	7.8902	22.9038	7.3299	0.4722
4.047	7.3477	28.7866	6.7580	0.4705
6.070	7.4499	27.9352	6.7617	0.4708
8.094	7.4260	28.3362	6.7151	0.4708
10.117	7.3826	28.8439	6.8180	0.4713

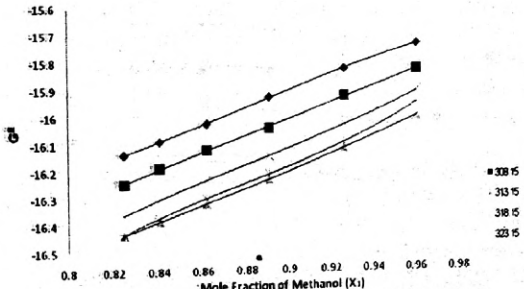

Fig.1 Variation of excess free energy (G^E) with mole fraction (X_1) for Methnol + curcumin at 303.15, 308.15, 313.15, 318.15, 323.15 K

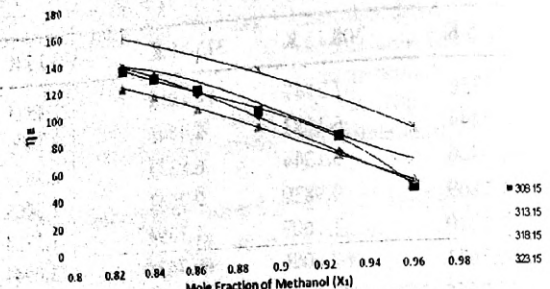

Fig 2 Variation of excess viscosity (ηE) with mole fraction (X1) for Methnol – curcumin at 303.15, 308.15, 313.15, 318.15, 323.15 K

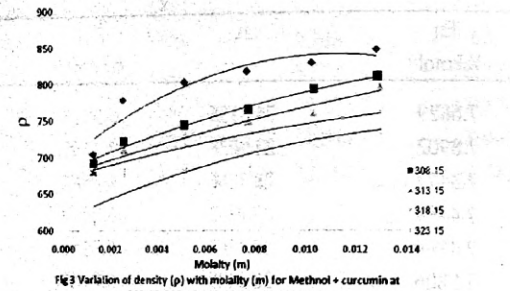

Fig 3 Variation of density (ρ) with molality (m) for Methnol + curcumin at 303.15, 308.15, 313.15, 318.15, 323.15 K

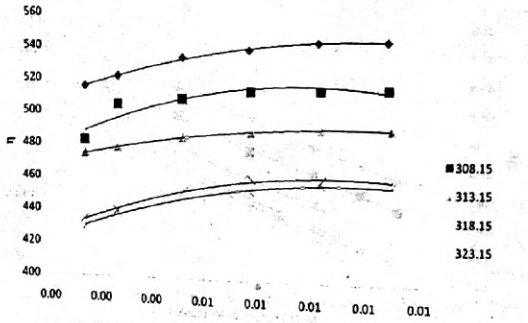

Fig 4 Variation of viscosity (η) with molality (m) for Methnol + curcumin at 303.15, 308.15, 313.15, 318.15, 323.15 K

values with temperature shows a decrease in intermolecular forces due to increasing thermal energy of system. The (η^E) values are positive throughout increases with increase in temperature as well as concentration shows presence of strong interactions whereas (G^E) values are negative throughout increases with increase in temperature as well as concentration shows dominance of dispersion forces.

These viscosity results have been explained by the well known equations (Einstein and Physik, 1901; Vands, 1948; Moullik, 1972 and Jones –Dole, 1929) which have been successfully applied to explain the results of viscosity measurements and the viscometric parameters shown by anti oxidant curcumin and methnol and in terms of transition state treatment. At different temperatures various thermodynamic parameters ΔH, ΔG and ΔS have been calculated for binary mixtures given in Table (2) evaluated with the help of graph plotted in between $\ln(\eta V_m/hN)$ and T . The free energy of activation ΔG is almost constant for binary mixtures indicating charge transfer complexes in other systems. The sign dB/ dT is an indicative of structure making/breaking ability of solute rather than size or sign of B-coefficient. The values of (η^E, G^E) for binary mixtures have been plotted against mole fractions of methanol, the plots have been presented in Fig. (1&2) respectively.

The values of Eη were calculated from the slope of linear plot of log Eη vs. 1/T and are given in Table(3) . Viscosity data has also been analyzed on the basis of Transition state theory of relative viscosity of electrolytic solutions as suggested by feakins *et al.* The values of μ_1^0 (free energy of activation per mole of solvent) and μ_2^0 free energy of activation per mole of soute) were calculated and is given in Table(4).It is suggested for structure maker, $\mu_1^0 > \mu_2^0$. due to increase in interaction of solute ions by the solvent molecules as a result of weakening of forces among molecules at transition state.

REFERENCES

Pandey, A.; Gupta, R.K. and Srivastava, R. 2011. Curcumin – The yellow magic, *Asian Journal of Applied Science*. 4 : 343 – 354.

Pandey, A.; Dwivedi, V.and Misra, K. 2010. Drug sensitivity of curcumin analogues and bioconjugates. In structural modification and drug development. *Studium Press*, LLC US. 7 : 267 - 298

Akhtar, Y. 2007. Volumeteric and viscometeric behaviour of amino acids in 308K. *Fluid Phase Equilibria*, 258 : 125-130.

Pal, S. and Mandal B. 2006. Densities and viscosities of aqueous solution of alcohols at different temperatures, *J. Chem. Eng. Data*, 51 : 1406-1410.

Ali, A.; Sabir, S. and Hyder, S.S. 2008. Physico-chemical of amino acids in aqueous Caffeine Solution at 25, 30, 35 and 40 °C. *Chinese Journal of Chemistry*, 24 (10) : 1547-1553.

Banipal, T.S.; Kaur, D.; Banipal, P.K. and Singh, G. 2007. Thermodynamic and transport properties of l-serine and l-threonine in aqueous sodium acetate and magnesium acetate solutions at *T* = 298.15 K, *The Journal of Chemical Thermodynamics, 39 (3)* : 371-384.

Wanchoo, R. K.; Narayan, J. 1994. Some Physical Properties of Binary Liquid Systems: (2-Butanone *n*-Propionic Acid or *n*-Butyric Acid). *Physics and Chemistry of Liquids*. 27(3): 159- 167.

Krishnan, K.M.; Ramachandran, D.; Venkateswarlu, P. and Raman, G.K. 1994. Viscosities of Binary Liquid Mixtures of Acetophenone with Aromatic Alcohols at 303.15 K , *Physics and Chemistry of Liquids*, 28(3) : 195 – 199.

Einstein, A. and Physik, A.D. 1911, 34591 Einstein's Annalen Papers: The Complete Collection 1901-1922 *by Jurgen Renn(Editor)*, Bestimmung der search.barnesandnoble.com/ Einsteins-Annalen.../9783527405640 Moleküldimensionen

Vand, 1948. J. Physical Colloid Chem, ,52,277, Transport characteristics of suspension: VIII linkinghub.elsevier.com/retrieve/pii/0095852265900164

Moullik, S.P. 1972. Electrolyte conductance in solutions of non-electrolytes—urea, sucrose, glycerol and mannitol. *Electrochimica Acta.* 17(8): 1491-149720.

Jonnes, G. and Dole, M. J. American chem.Soc., 1929,51,2950., Viscosity of Electrolytes and Related Properties. ...linkinghub.elsevier.com/retrieve/pii/0013468667800628

Gupta, R. and Singh, M. 2005. Viscometric and thermodynamic studies of interactions in ternary solutions containing sucrose and aqueous alkali metal halides at 293.15, 303.15 and 313-15 K. *Journal of chemical sciences.* 117(3): 275-282

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IDENTIFICATION OF SUSTAINABLE AGRICULTURAL DEVELOPMENT PLANNING ZONES: A CASE STUDY OF GAURIGANJ BLOCK, SULTANPUR DISTRICT (U.P.)

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ABSTRACT

Present paper is an attempt to identify and map out the sustainable agricultural development planning zones (SADPZs) selecting a part of Sultanpur district (Gauriganj block- laying between 26°7'5"N to26°19'5"N latitudes and 81°36'45"E to 81° 45'18"E longitudes, area-20481.5 ha., total number of villages-100), Uttar Pradesh. .In order to achieve the above objective, environmental and socio-economic parameters were analyzed and integrated on criterion basis. The data pertaining environment and socio-economic parameters at village level were generated/collected from satellite imagery (IRS P6, LISS III, Jan.2006), NIC, and block headquarter. Four SADPZs were identified in the study area. These SADPZs may be taken up for agricultural development planning on sustainable basis in a phased manner.

Key words: *Sustainable agricultural development planning zones (SADPZs), criterion basis, satellite imagery.*

Sustainable agricultural growth and development in India is the greatest challenge of this century. Today, India is facing crucial problem of population pressure on increasingly limited land resources, in addition to malnutrition and wide spread poverty . According to the latest reports of Department of Agriculture and Co-operation (DAC, 1994) 107 million hectares of area was found under various types of degraded lands. It should be noted that today the population has exceeded one billion and by 2025 at the current growth rate of 1.6 per cent, it would be 1.32 billion (Shafi, 2001). Four hundred million tons of food grain would be needed to feed this population (Patil, 2003). Agricultural development planning on sustainable basis is an important key to meet the above challenge of the present century. This is also the need of the hour, which must be preceded by a thorough and careful functional survey of present position and its scientific interpretation (Harmsen & Nidhumolu,2002).To Remote sensing and GIS can play a vital role in identification and mapping of planning zones for sustainable agricultural development in speedy and cost-effective manner in areas having diverse physico-cultural conditions. Remote sensing (RS) coupled with Geographic Information System (GIS) provides a powerful mechanism, not only to monitor land resources and environmental changes but also permit the analysis of information of other environmental variables (Marble, 1983). Keeping these aspect in view , the SADPZs were identified and mapped in Gauriganj block, Sultanpur district (laying between 26°7'5"N to26°19'5"N and 81°36'45"Eto81° 45'18"E-area-20481.5 ha., total number of villages-100), Uttar Pradesh. The study area is a part of the vast Indo-Gangatic plain and represents a typical tropical, semiarid, monsoonal type of climate. The average annual rainfall is 977 mm, mainly received between July and September (Sharma et al., 2001). The soils of the study area are largely affected by land degradation processes. The block is economically backward and majority of the population (78.50 % of the working force) earns livelihood from agriculture and allied activities.

MATERIALS AND METHODS

The identification of SADPZs in this particular area has been made mainly on the basis of eleven indicators viz. i. Strongly salt affected lands (as % to total area) ii. Area affected by soil wetness (as % to total area) iii. Surface ponding (as % to total area) iv .Eroded area (as % to total area) v. Vegetated area (as % of area under 0.2 and above NDVI value) vi. Density of rural population (persons per km²) vii.Rural literacy (as % to total population) viii.Irrigated area (as % to total agricultural area) ix. SC/ST population (as % to total population) x . Population below poverty line (as % to total population) and xi . Working population (as % to total population).The data pertaining above indicators were generated/collected from the following sources-

1. IRS-P6, LISS III data (path-101 and row-53, Band- Green, Red, NIR and SWIR, Resolution-23.5 M, January, 2006).
2. The Google Earth high resolution images, http// www. Google Earth.com.
3. Field check/ training data, collected through field work in the month of January, 2009.
4. Thematic maps prepared by *Sharda Sahayak* C.A.D. Project,1988, Lucknow (U.P.).
5. Survey of India topographical sheets numbered 63 F (1:250000), 63 F/11, 63 F/12, and 63 F/ 16, 1974, and village boundary map prepared by NNRMS, Sultanpur (U.P.)
6. Census of India, NIC, Sultanpur,U.P.
7. Revenue records, Tahsil Headquarter, Gauriganj.
8. Chief Development Office, Sultanpur, U.P.
9. ERDAS IMAGINE Version 9.1 and Arc View, Version 3.2a. Software.

The methodological steps followed for identification and mapping of SADPZs are as under:

i. The base map of study area was imported into image processing software ERDAS IMAGINE 9.1 and geo-referenced by taking various control points .The projection type used is polyconic with the spheroid and datum an Everest.

ii. The satellite image was registered through image to image registration and processed using Iterative Self-Organizing Data Analysis –ISODATA (Tou & Gonzalez, 1974; ERDAS, 1999) algorithm of unsupervised classification approach in ERDAS Imagine 9.1 for on land use / land cover mapping.

iii. To evaluate the classified image, files (thematic raster layer) accuracy of classified map was assessed taking random sample 250 points on reference image and analyzed in ERADAS IMAGINE software using accuracy assessment option in the classification dialog. The classified layers were compared with ground truth data and Google earth high resolution image. On the basis of comparison of classified map with ground truth data and Google earth high resolution image the error matrix was prepared.

iv. To generate the data on vegetation cover, band rationing technique of Normalized Difference Vegetation Index (NDVI) was attempted using a set of transformation as follows (Tucker, 1979):

NDVI = (NIR-R) / (NIR+R)

Where -NDVI= Normalized Difference Vegetation Index; NIR = Near Infrared band; R= Red band

By design, the NDVI varies between -1.0 and +1.0, but vegetation values typically range between 0.1 and 0.7. Higher index values are associated with higher level of healthy vegetation cover, whereas index values near zero indicates the less green vegetation. The NDVI image was analyzed in image processing software of ERDAS Imagine version 9.1.

v. Land use / land cover and NDVI raster layers were vectorized using ERDAS IMAGINE vector utility option and exported to GIS environment (ARC VEIW 3.2a) for data base generation and analysis at village level.

vi. The criterion based analysis was performed for integration of the all types of data pertaining selected indicators. The polygons corresponding strongly salt affected lands, wet soil area and surface ponding were taken directly from the land use / land cover map and analyzed in ARC VIEW 3.2a environment. The socio-economic parameters such as rural population density, irrigated area, SC/ST population and population below poverty line were also analyzed at village level. According to spatial extent of each parameter, all villages of the study area were grouped into four categories using natural breaks option of legend editor

Table-1. Land use / land cover statistics in the study area
(Based on unsupervised classification of IRS P6, LISS III data, January, 2006)

Sl.No.	LU/LC classes	No. of Pixels	Area (ha.)	Area (%)
1	Salt affected lands	41072	2665.75	12.56
2	Wet soil area	72245	4449.31	20.97
3	Surface ponding /water bodies	34576	1991.58	9.38
4	Dense vegetation	12174	701.28	3.3
5	Agricultural area	69347	3994.39	18.82
6	Bare soils / built-up area	133924	7714.04	36.35
	Total	**368339**	**21216.33**	**100**

Table-2. SADPZs in Gauriganj block, Sultanpur district (U.P)

SADPZs	No. of Villages	Area (In %)	Population (In %)	Name of the villages
Zone I	18	Zone I	19.08	Benipurbaldev, Gulalpur, Bastidei, Majhawara, Bhatgawan, Mau, Gopalipur, Shujanpur, Guwawan, Sarai Hirdai Shah, Jethauna, Chhitepur, Saintha, Lugri, Katra Lalganj, Madhopur, Sultanpur and Jaymalpur.
Zone II	55	52.06	51.28	Rauza, Bhanmatipur, Tulsipur,Basupur, Oripur, Narauli, Rohnikhurd, Gauripur, Jagdishpur, Anapur, Bisundaspur, Dostpur, Harakhpur, Saraibhagmani, Mahimapur, Garhamafi, Khajuri, Jethumawai, Tikaria, Misrauli, Asaidapur, Babupur, Gudur, Gudunpur, Lalshahpur, Pandri, Pathanpur, Aintha, Ismailpur, Saripur, Antanagar, Bhanpur, Sakarwan, Ramaipur, Argawan, Amiya, Palia, Paharpur, Raghipur, Rohnibuzurg, Bhawanshahpur, Chandaipur, Biswan, Pachehri, Dharupur, Sogrha, Gujartola, Painga, BarnaTikar, Rampur, Kanarwa, Kazipatti, Sembhui, Pure Fazil, Sarauli, Itaunza and Pachhim.
Zone III	24	18.57	16.29	Shahbajpur, Saraibakhandsingh, Behta, Darpipur, Baburitola, Lilatikar, mednamawai, Belkhaur, Basaikpur, Annibaizal, Sambhawan, Pure Udhau, Paharganj, Kharawan, Chauhanpur, Banwaripur, Dhanapur, Asura, Balipur, Khurdawn, Rajgarha, Sehipur, Madhpur, Dhani Jalalpur and Basthan.
Zone IV	03	2.45	0.43	Sujapur,Rajapatti, and Mohanpur.
Total	**100**	**207.56** (km2)	**120892**	-

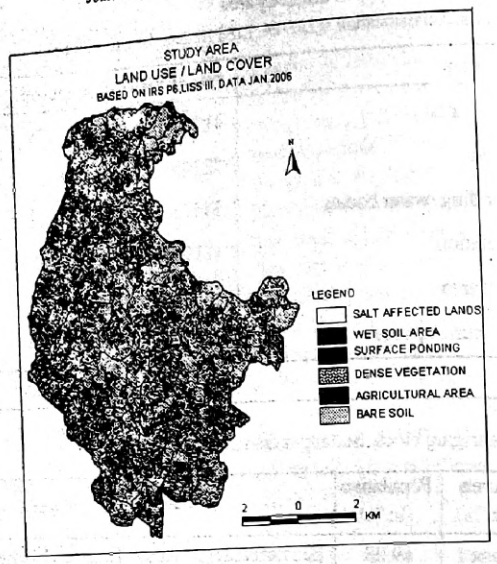

Fig. 1

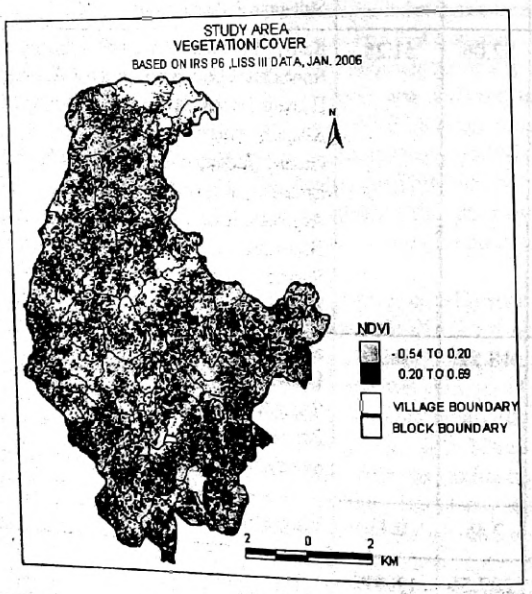

Fig. 2

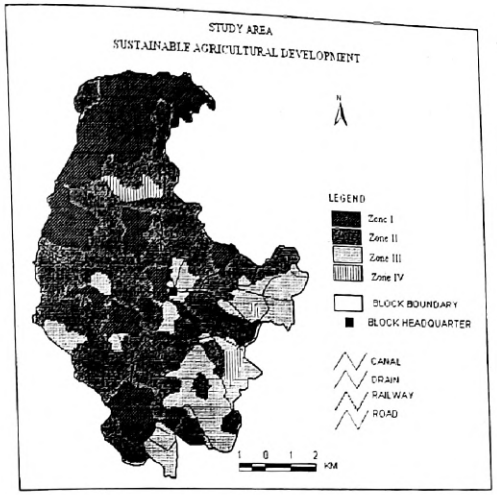

Fig. 3

in ARC VEIW 3.2a.and each category was assigned different weighted value (i.e.1,2,3&4). The higher weighted values are given to the villages falling within higher categories of the parameters of salt affected lands, wet soil, surface ponding, eroded soil, rural population density, SC/ST population, and population below poverty line while, higher weighted values are assigned to the villages falling within lower categories of the parameters of irrigated lands, literacy, vegetation cover and vice-versa .

vii. Total weighted score for each village has been computed by aggregating the weighted values of all parameters and finally, all villages were grouped in to SADPZs on the basis of total weighted score. The higher total weighted score indicates the lower agricultural sustainability while, lower weighted score represents the higher level of agricultural sustainability.

RESULTS AND DISCUSSION

In the study area, six land use /land cover categories i.e. i. Salt affected lands(12.56% area) ii. Wet soil area (20.97% area) iii. Surface ponding/ water bodies(9.38% area) iv. Dense vegetation cover(3.3% area) v. Agricultural area (18.82% area) and vi. Bare soils/built-up(36.35% area) were mapped using unsupervised classification (ISODATA algorithm) approach (Table 1& Fig.1).The accuracy assessment result is obtained about 0.7983 overall Kappa and 88.32 percent of overall accuracy. The land use /land cover analysis results have shown that out of total classified image area of 21216.33 ha., 42.91 % fall under wet soil , salt affected lands and surface ponding .

NDVI values are found in range of -0.54 to 0.69, having mean value of 0.07 and standard deviation of 0.36 for January, 2006 image. The study area was mapped classifying NDVI into two broad categories using threshold of index 0.20(Fig.2). Below 0.20 NDVI values are observed over salt affected lands, built-up area, water bodies and open soil while above 0.20 NDVI values are seen over agricultural, sparse vegetated and dense vegetated area. The study reveals that the salt affected poor soil cover, soil wetness, surface ponding, sparse vegetation cover, low level of literacy; wide spared poverty and improper utilization of natural resources characterize the area under study. The study emphasized on identification of village clusters for the agricultural development

planning on a sustainable basis, based on environmental and socio- economic conditions.

According to the total weighted score, the study area was divided into four SADPZs (Table 2,Fig-3). Out of 100 villages, 18 villages are fall under zone I. The villages falling in this zone showing very low level of agricultural development sustainability due to various agro-ecological constraints. They can be taken up for the agricultural development planning on sustainable basis with immediate effect. A large number of villages (55) of the block fall under the zone of low level of sustainability (zone II) which covers 52.06 % area and 51.28 % population of the study area. Out of remaining 27 villages ,24 fall in the zone III(moderate sustainability) while only 03 villages fall in the zone IV(high sustainability) .The study demonstrate that the majority of the villages (73%) of the study area fall under lower level of agricultural development sustainability. (SADPZs I and II) which indicates the severity of the sustainability problem. It would be therefore be necessary to plan for sustainable agricultural development in these villages on priority basis considering overall agro-ecological conditions.

REFERENCES

DAC 1994. *Draft Report on Status of Land Degradation in India*, Department of Agriculture and Co-operation.

ERDAS, Inc. 1999.*Erdas Imagine Tour Guides*, Atlanta ,Georgia,USA, p. 671.

Harmsen K. and Nidhumolu, B. N. 2002.Land use planning for sustainable development in South Asia: An example from India, *Resource and Environment Monitoring*, 34(7): 1467-1473.

Marble,D.F.,Penquet,D.J.,Boyle,A.R.,Bryant, N.,Calkins,H.W.,Johnson,J.and obrist ,A. 1983. GIS and remote sensing, *Manual of Remote Sensing*, American Soc. of Photogrammetry, 1:.923-958.

Patil,J. 2003.Sustainable agricultural Development : Issues and Programs, *Financing Agriculture*, 35(1): 35-38.

Shafi,M. 2001 .Land use change, environment and sustainable agriculture, *NGJI*, BHU ,Varanasi, 47 (1-4):1-4.

Sharma SD, Khan GH, Prasad KG. 2001. Selection of suitable provenances of Dalbergia sissoo for sodic lands. *Indian Journal of Forestry* ,24:58- 64.

Tou, J.T and Gonzalez, R.C. 1974. *Pattern Recognition Principles*, Addison-Wesley Pub. Co.

Tucker,C. 1979. Red and photographic near infra-red linear combination for monitoring green vegetation.*Remote Sensing Environ.*, 8:127-150.

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BENEFITS OF SOCIAL FORESTRY SCHEMES TO THE FARMERS OF DISTRICT JAUNPUR

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ABSTRACT

An impact analysis was made on the respondents of social forestry schemes in district Jaunpur. Farmers were benefited directly and indirectly from the forest programmes running in the district Jaunpur. They have also gained the knowledge of selection of suitable plant varieties for plantation on their lands.

Keywords : *Adoption, Ex-Post facto research, Participants*

The Government forest development programmes lay emphasis on productive forestry and social forestry. Productive forestry seeks to raise man-made forest to meet the long term requirements to forests products in general, and material for wood-base industries in particular.

It is apparent from what has been stated earlier years that the special feature of the social forestry programme was primarily to meet the day to day demand of firewood, grass, fodder etc. and to assist in providing the opportunity of regular additional income (Bhatt and Mishra 2003, Kumar et. al. 1998). The impact of social forestry programme as a ground relating understudy was worked out with following dimensions.

1 Indirect Benefits
2 Direct Benefits and
3 Choice of different tree species

MARERIALS AND METHODS

Ex-post-facto research design was used for the study. Multistage sampling technique was used for the selection of respondents. The study was conducted in 5 each randomly selected villages of purposive selected two C.D. blocks of purposive selected Jaunpur district of Uttar Pradesh. The respondents were selected on the basis of stratified random sampling method, where 60, 60 and 80 respondents were randomly selected from the three strata big, medium and big as per possession of land of farmers on the basis of government classification. Therefore a total of 200 respondents were selected. Extent of adoption was measured with the help of developed scales and indexes specially developed for the study. The collected data was classified, tabulated and processed with the help of percentage.

RESULTS AND DISCUSSION

1 Indirect Benefits

The reactions of farmer respondents were recorded to study the impact of various social forestry schemes on fodder and fuelwood supply, employment and income generation in rural areas. The summary of responses is presented in Table 1.

About 61.5 per cent of the respondents had informed that there was no change in the fodder supply situation while 22.0 per cent of them felt that the fodder availability was improved since the initiation of social forestry schemes. About 69.0 per cent farmers were of the opinion that there was no change in the price of fodder and only 2.5 per cent felt that the fodder price has decreased after initiating social forestry" schemes.

Table 1 depicts that villagers have been using agricultural waste, tree branches, twigs and leaves,

dung cake, biogas and electricity as energy for cooking. It was difficult to estimate the proportion of different sources of this energy, but most of the respondents did not consider the supply of fuelwood as a serious problem. People did cut the trees grown on road sides, but did not feel the urge for planting fuelwood species in their own field. Under such a situation, 61.0 per cent respondents observed no change in fuelwood availability while only 14.0 per cent beneficiaries have felt that the availability has improved since the initiation of social forestry schemes. About 52.5 per cent of farmers observed no change

Table 1 Indirect Benefits of Social Forestry

Indirect Benefits	Opinion of the respondents				Total
	No Effect	Increase	Decrease	Not Aware	
Fodder availability	123(61.5)	44(22.0)	2(1.0)	31(15.5)	200(100.0)
Fodder price	138(69.0)	5(2.5)	9(4.5)	48(24.0)	200(100.0)
Fuel-wood availability	122(61.00)	28(14.0)	6(3.0)	44(22.0)	200(100.0)
Fuel-wood price	105(52.5)	24(12.0)	7(3.5)	64(32.0)	200(100.0)
Employment situation	05(2.5)	144(82.0)	4(2.0)	27(13.5)	200(100.0)
Wage rate	13(6.5)	156(78.0)	8(4.0)	23(11.5)	200(100.0)

Figure in parentheses indicate percentage

Table-2. Number of Seedlings Received by the Respondents under Different Social Forestry Scheme

No. of Seedlings	No. of Recipients		
	Free of Cost	At subsidised price	Total
Upto 50	62(31.0)	8(04.0)	70(35.0)
51–200	41(20.5)	11(05.5)	52(26.0)
201–500	30(15.0)	5(2.5)	35(17.5)
501–1000	12(06.0)	3(1.5)	15(07.5)
1001–2000	07(03.5)	2(1.0)	09(04.5)
2001–5000	09(04.5)	5(2.5)	14(07.0)
5001 and above	(0.0)	5(2.5)	05(2.5)
Total	161(80.5)	39(19.5)	200(100.0)

Figure in parentheses indicate percentage

Table 3 Choice of tree species among the respondents

Name of species	Common Name	Use	Frequency of respondents	
			Frequency	Percentage
Mangifera indica	Mango	Fruit	129	64.5
Psidium guajava	Guava	Fruit	118	59.0
Emblica officinalis	Anola	Fruit	104	52.0
Aegle marmelos	Bael	Fruit	38	19.0
Syzygium cumini	Jamun	Fruit	88	44.0
Azadirachta indica	Neem	Oil	23	11.5
Aalbergia sissoo	Shisham	Timber	103	51.5
Eucalyptus tereticornis	Eucalyptus	Timber	52	26.0
Tectona grandis	Sagwan	Timber	44	22.0
Terminalia arjuna	Arjun	Fodder Medicine	93	46.5
Acacia nilotica	Babul	Fuel	27	13.5
Bambusa arundenacea	Bamboo	Timber	89	44.5
Delonix regia	Goldmohar	Ornamental	104	52.0
Ficus religiosa	Pipal	Fodder	21	10.5
Ficus bengalensis (Moraceae)	Banyan	Fodder Ornamental	48	24.0

in the price of fuelwood, while about 12.0 per cent and 3.5 per cent respondents have felt that there is a increase and decrease in fuelwood price after the introduction of social forestry schemes, respectively.

Most of the participants of community plantation and recipients of seedlings informed that the social forestry schemes did not affect either the availability or price of fuelwood and fodder in their villages. Similar observations were made by Sood 2006. While the community plantations could not make significant impact on fodder and fuelwood supply due to coverage of smaller areas and poor rate of survival, most of the plantations established on private lands were yet to be harvested.

2. The direct benefits

The direct benefits of social forestry schemes were receipt of wages, supply of inputs and income generated in the form of outputs.

It was observed from table 2 that wages were paid to the participants of schemes such as community plantation, establishment of farm forestry under the Western Ghats Development project and Gramayan. Partial wages were paid to the participants of 'kisan nursery' scheme. Other inputs like seeds, fertilizers, pesticides and watering equipment were also provided under the Western Ghats Development scheme. However, a majority of the respondents who had participated in other schemes had received only seedlings either free or at a subsidised price.

It was observed from the data presented in Table 2 that all the respondents had received seedlings of different tree species, out of which 80.5 per cent had received free of cost, 19.5 per cent had procured at a subsidised price.

The number of seedlings received by the beneficiaries ranged between 10 and 5000. About 35.0 per cent beneficiaries had received upto 50 seedlings followed by 26.0 per cent, and 17.5 per cent respondents had received 51 to 200 seedlings and 201 to 500 seedlings, respectively. Remaining 21.5 per cent respondents had received 501 seedlings to more than 5000 seedlings, whereas 2.5 per cent respondents had received more than 5000 seedlings on subsidised rates.

3 Choice of different tree species

It is clear from table 3 that selection of suitable tree species is a critical factor influencing people's participation and profitability of the programme. As most of the tree plantations established under various social forestry schemes were yet to be harvested, it was too early to get field data on the economics for different species. However, based on initial growth rates and limited market information, most of the respondents were able to indicate their choice upto a maximum of five species. These species have been listed in Table 6.3.3. Out of 15 species preferred the farmers, 05 were grown for food, 04 for timber, 03 for fodder, 02 for ornamental and one each for oil and fuelwood purpose. The most preferred species among these were mango, guava, anola, goldmohar, shisham, arjuna, bamboo and jamun, where the frequency of respondents were 129, 118, 104, 104, 103, 93, 89 and 88, respectively.

REFERENCES

Bhatt, B.P. and Mishra, L.K. (2003). Production potential and cost benefit analysis of agrihorticulture agroforestry systems in North-East India. *J. Sustain, Agric.*, 22 : 99-108.

Kumar, A., Hooda, M.S. and Baradur, R. (1998). Impact of multipurpose trees on productivity of barley in arid ecosystems. *Ann. Arid Jone*, 37 : 153-157.

Sood, K.K. (2006). The influence of Household Economics and Farming Aspects on Adoption of Traditional Agroforestry in Western Himalaya. *Mountain Res. And Dev.*, 26(2) : 124-130.

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IMPACT OF RUCHAMAX HERBAL PREPARATION ON MILK COMPOSITION AND YIELD IN CROSSBRED COWS

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ABSTRACT

The impact of Ruchamax a herbal preparation was tested on milk composition and yield, Twenty five lactating cows of 1st to 5th lactation were taken in experiment for full lactation and divided into 5 uniform groups of 5 each, according to their milk yield and lactation number. Cows in group I was fed seasonal roughage with concentrate as control. In group II (normal nutrition –400 gm concentrate/liter milk) and remain in general herd and III (better nutrition –600 gm concentrate/liter milk) and cows of T-II & III also kept in open paddock. Cows in group IV (normal nutrition) and V (better nutrition) were kept inside paddock and all these 4 groups (II, III, IV and V) were provided Ruchamax @ 30 gm/day for 7 consecutive days commencing from 6th, 18th and 30th week after calving. Highest milk yield (3305.95) was recorded in T-III, medium in II (2954.04), I (2329.55), V (2678.78) and lowest (1807.92 liter) in T-IV in full lactation. The fat and total solids percentage difference were significant (P>0.01) and protein per cent was significant different (P>0.05).

Keywords : *Milk composition, yield, ruchamax*

In India cattle rearing and the utilization of livestock resources has occupied an important place in the economic scenario. Our mythologies, epics and legends are replete with references to cattle signifying its importance. Other countries have made great advances in livestock development because of various reasons. India too, has recently emerged as the largest milk producing country in the world with its annual production at approximately 74 million tonnes, surpassing United States of America, where annual milk production is approximately 71 million tonnes (Banerjee, 1999). It is because the saturation point has been reached in the cereal grain production, and no further increase per unit land area is possible. Therefore, the landless, marginal and small farmers have started paying attention to livestock mainly towards milk production. Livestock represents the only way in which natural vegetation that covers large parts of India can be converted into products that can be used by man as food i.e. milk, meat and fiber. It provides draught power, particularly needed by the small and marginal farmers and manure to the crop enterprise and this in turn provides feed and fodder in the form of crop residue and by products. The value of output from the livestock sector was Rs. 79864.00 crores in 1994-95 which was 9.3 per cent of the total gross domestic production.

Fortunately India is blessed with tremendous livestock wealth. It has the largest population of cattle and buffaloes in the world and its breeds are admired for their heat tolerance, inherent resistance to disease and their ability to thrive under different climatic conditions. Even so, the productivity of Indian cattle is very low as compared to other countries. With increasing population pressure, the priority we must give to ensure basic nutrition for people vary little land is available for forage production. The annual requirement of feed and fodder in India has been estimated as about 766 million tonnes for green fodder, 650 million tonnes for dry fodder and 79.4

million tonnes for concentrate (Ranjhan, 1997). However, only 530 million tonnes of dry fodder, 573 million tonnes of green fodder and 42 million tonnes of concentrate are available. The annual requirement of digestible crude protein and total digestible nutrients is estimated as 40.9 and 454.8 million tonnes against the availability of only 22.01 and 253.9 million tonnes, respectively (Jain *et al.*, 1996). Cattle depend largely on green grasses growing by the side of fields and on crop residues. As cereal straws have low nitrogen content and they are composed of cell wall components, with little soluble cell contents, to be digested by microbial fermentation in the rumen, hence, either the animal should be able to consume higher quantity of straw or some alteration in the fore-stomach functioning must be changed favorably.

MATERIALS AND METHODS

The present experiment was conducted on 25 crossbred lactating cows, divided into 5 groups (containing 5 animals in each treatment) for full lactation. Observations were taken at Livestock Research Centre, (LRC) and Department of Animal Science, College of Agriculture of G.B. Pant University of Agriculture and Technology, Pantnagar (Udham Singh Nagar) Uttaranchal. This is situated at the foothill of Kumaon hills of shivalik range at the 29°N latitude and 70° 30E longitude with humid sub-tropical climate, in tarai belt of Uttaranchal. Details of the materials and techniques used during the experiments presented areas under-

Selection of animal:

Twenty five crossbred healthy lactating cows 7 days after calving were selected from the lactating herd of Livestock Research Center, Nagla. They were divided in five groups of five cows each on the basis of their lactation number and stage of lactation.

Feeding and management:

All the animals were fed *ad libitum* seasonally chopped available roughage twice a day at around 7 a.m. in the morning and 6 p.m. in the evening during the lactation period. Concentrate mixture to the animals was given as follows. The treatment groups were divided into 2 groups i.e. Normal Nutrition and Better Nutrition. In the Normal Nutrition group the concentrate mixture was given according to LRC norms (400 g/lit) and Better Nutrition group was given (600 g/lit) based on their milk production. The total amount of concentrate was divided into two halves and each half was offered to cows at the time of two milking done at 3 a.m. in the morning and 3 p.m. in the evening. Milking was done manually in milking shed. Cows were kept in further sub grouping was done to keep the parallel treatment group in an open and inside enclosure having feeding and watering facilities. The floor was washed and cleaned daily in the morning and evening.

The cows in group I represented control group and were not given any medicine. The cows in group II were fed Ruchamax and remain in general herd in open paddock and in III group (better nutrition) were given Ruchamax @ 30g/day for 7 consecutive days commencing from 6th week, 18th week and 30th week after calving, and the animals were kept in open paddock. The cows in group IV and V (Better Nutrition) were kept inside paddock and given Ruchamax @ 30g/day for 7 consecutive days commencing from 6th week, 18th week and 30th week after calving. As and when animal calved it was allotted to one of the treatment group to have five animals each in a treatment and medicine was given for seven consecutive days.

Analysis of milk samples:

During the trial monthly collection of milk samples were made for their analysis. A 100 ml milk sample was collected from the thoroughly mixed milk sample and analysis was done.

Determination of milk fat:

The fat percent was estimated by Gerber's method (ISI, 1962). Ten ml concentrated sulphuric acid (10:1) was taken in the butyrometer and 10.9 ml (11 0ml) milk with a pipette was added to butyrometer slowly from the side. One ml amyl alcohol was added and was closed with stopper key, mixed carefully and were centrifuged for four minutes at 1000 rpm and fat percentage was read from there to the lowest part of the upper meniscus.

Determination of total solids:

Total solids in milk were estimated by oven dry method (AOAC, 1970). It was based on the principle of removal of moisture from milk. It can be removed at atmospheric pressure or at reduced pressure. Some sand was taken at the bottom of weighed silica dish (this helps in uniform distribution; and quick removal of moisture, because it provides porosity). To it added 10 ml of milk in silica dish and kept it in the oven at 80-90°C for 5-6 hours. After complete removal of moisture silica dish was cooled in desiccators. It was then weighed and a total solid was calculated by the following formula:

$$\text{Percent total solids} = \frac{W_3 - W_2}{W_3 - W_1} \times 100$$

W_1 = Weight of empty silica dish (g)
W_2 = Weight of silica dish + milk + sand (g)
W_3 = Weight of silica dish + residue + sand (g)

RESULTS AND DISCUSSION

The present study was conducted to observe the effect of Ruchamax feeding and management practices on milk production, milk composition. The results obtained during the course of investigation are presented as-

Lactation trial:

Lactation trial was conducted on fifteen crossbred lactating cows. A monthly collection of blood samples, milk samples and rumen fluid was made from all the experimental animals along with daily milk yield (liter).

Total milk production:

Total milk production (lit) is presented in Table 1. Table depicts that five animals in each treatment (I and II) had completed 300 days of lactation, whereas in treatment III, IV and V also not any animal had dried. And the lactation length of the animals of treatment II was 300 days and for treatment III and V was also 300 days. For the sake of comparison, milk yield was standardized in 300 days basis. Total milk yield recorded was 16689 liter in treatment III, which was the highest yield in all five treatments, while in treatment II total milk yields was 15018 liters and in treatment V it was 13500 liters and for treatment I and IV it was 12858 and 9237 liters respectively. Average milk yield (lit/day) was highest in treatment III and the value was 11.12 lit/day. Average milk yield in treatment II, V, I and IV was 10.01, 9.00, 8.57 and 6.15 lit/day respectively.

Average milk production:

Average milk production (lit/day), recorded during the lactation trial is presented in Table 2 and 3. This shows that initial milk yields were 7.64, 8.93, 8.92, 7.29 and 6.65 liters in treatment I, II, III, IV and V, respectively and then it reached up to 9.66, 13.45, 13.16, 7.05 and 8.94 lit/day in treatment I, II, III, IV and V, respectively. The peak yield was (9.66) recorded in 12 weeks and then a smooth decline was observed in treatment I, whereas in treatment II peak yield was obtained also in 12 weeks and it persisted up to 28 weeks and then decreased smoothly. In treatment III the peak yield was obtained in 12 week and it persisted up to 24 weeks and then decreased slightly. The lowest peak yield (8.33 lit/day) was recorded in treatment IV and it was obtained at 4 weeks, and decline was very slow, whereas in treatment V peak yield was obtained in 36 weeks and it persisted from 8 to 40 weeks. Average values of fat, protein and total solids are presented in Table 2and3. The table shows that maximum fat per cent was observed in treatment IV followed by treatment V, I, II and III and differed significantly. Protein content was maximum in treatment IV followed by treatment V, III, II and slightly less in treatment I and these values are significantly different ($P>0.01$). But total solids were highest in treatment III and lowest in treatment I ($P>0.01$).

It may be concluded that Ruchamax feeding to lactating crossbred cows @ 30 gm/ day for 7 days is cost effective. It increases milk yield without adversely affecting the animals. The animals belonging to 2nd lactation showed better performance in comparison to other lactation indicating younger cows could give

Table 1: Average milk production (lit) in lactating crossbred cows during lactation trial

Lactation length (weeks)	Treatment				
	T-I	T-II	T-III	T-IV	T-V
Initial yield	7.64	8.93	8.92	7.29	6.65
4	8.30	10.18	9.76	8.33	7.67
8	8.91	13.30	11.43	7.98	9.31
12	9.66	13.45	13.16	7.05	8.94
16	9.52	11.69	12.90	6.56	7.93
20	8.73	10.43	11.99	5.94	8.25
24	8.39	10.01	11.97	5.19	9.43
28	7.18	10.12	10.59	5.77	9.73
32	6.77	9.72	10.61	5.67	9.89
36	6.97	8.20	10.57	5.18	10.21
40	5.57	7.02	10.57	5.00	9.42
43	4.28	3.51	6.06	3.56	5.75
Average	6.96	9.71	10.71	6.13	8.60

Table 2: Effect of Ruchamax on milk production and its composition during lactation

Particulars	Treatments					Level of significance	CD at 5%	CD at 1%
	I	II	III	IV	V			
Milk yield (lit/day)	8.57 ± 1.16	10.01 ± 1.16	11.12 ± 1.16	6.15 ± 1.16	9.00 ± 1.16	NS	3.49	4.82
Fat %	3.77^{b} ± 0.013	3.74^{a} ± 0.013	3.70^{a} ± 0.013	3.81^{b} ± 0.013	3.77^{b} ±0.013	**	0.041	0.057
Protein %	3.62^{a} ± 0.009	3.64^{a} ± 0.009	3.65^{b} ± 0.009	3.67^{b} ± 0.009	3.66^{b} ± 0.009	*	0.027	0.037
Total solids %	11. ± 0.022	11.58 ± 0.022	11.83^{d} ± 0.022	11.65 ± 0.022	11.82^{d} ±0.022	**	0.066	0.091

** - (P<0.01)
* - (P<0.05)
Figures bearing different superscripts in a row differ significantly (P<0.05)

Table 3: Effect of Ruchamax on milk yield

	(T-IV) Inside paddock (Ruchamax with normal nutrition)			(T-V) Inside paddock (Ruchamax with better nutrition)		
	Total milk yield (lit)	Lactation (days)	Average milk yield (lit)	Total milk yield (lit)	Lactation (days)	Average milk yield (lit)
1.	117.60	112+	1.05	3258.00	300*	10.86
2.	2550.00	300*	8.50	2115.00	300*	7.05
3.	2250.00	300*	7.50	3444.00	300*	11.48
4.	1884.00	300*	6.28	2341.92	287+	8.16
5.	2238.00	300*	7.46	2235.00	300*	7.45
Total	9039.60	1312	30.79	13393.92	1487	45.00
Avg.	1807.92	262.4	6.15	2678.78	297.4	9.00

* Continuing
\+ Dried

Table continued……..

	(T-I) Open pedock control			(T-II) Open pedock (Ruchamax with normal nutrition)			(T-III) Open pedock (Ruchamax with better nutrition)		
	Total milk yield (lit)	Lactation (days)	Average milk yield (lit)	Total milk yield (lit)	Lactation (days)	Average milk yield (lit)	Total milk yield (lit)	Lactation (days)	Average milk yield (lit)
1.	3588.00	300*	11.96	3558.00	300*	11.86	2532.00	300*	8.44
2.	2949.00	300*	9.83	3093.00	300*	10.31	3515.75	287+	12.25
3.	411.04	112+	3.67	3317.72	287+	11.56	3756.00	300*	12.52
4.	2382.00	300*	7.94	2152.50	287+	7.50	3468.00	300*	11.56
5.	2317.70	245+	9.46	2649.00	300*	8.83	3258.00	300*	10.86
Total	11647.74	1257	42.86	14770.22	1474	50.06	16529.75	1487	55.63
Avg.	2329.55	251.4	8.57	2954.04	294.8	10.01	3305.95	297.4	11.12

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\+ Drie

better economic advantage due to Ruchamax feeding.

REFERENCE

A.O.A.C.(1965), Official methods of analysis, 10th ed. Association of Official Analytical Chemists. Washington, D.C

Banerjee, A.(1999), Export potential of Indian dairy products. Indian Dairyman, 51: 24-29

ISI(1962), IS-2051 Indian Standards Institution, New Delhi

Jain, D.K. Sharma, K.N.S, Walli, T .K. and Rai, S.N,(1996). Estimates and Availability of Bovine Population across Major States in India. Publication No. 281, NDRI, Karnal, India

Ranjhan, S.K.(1997). National Symposium on Feeding strategies for eco-friendly animal production in India, Izatnagar, Feeding strategies for supporting livestock and poultry production. Animal Nutrition Association, Indian Veterinary Research Institute. 65-75 p.

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SOIL ADDED CADMIUM AND ZINC ACCUMULATION IN DIFFERENT PARTS OF SOME LEAFY VEGETABLES

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ABSTRACT

A pot experiment was conducted to investigate the uptake and accumulation of Cd and Zn in different parts of some leafy vegetables. The soil sample used was alluvial collected from SDI experimental farm (Texture: silty clay loam, clay 36.6%, CEC 20.7 Cmol (P^+) kg^{-1}, organic C: 0.50% and DTPA-Cd 0.36 ppm). It had initial pH of 7.5 which increased to 7.7 after irrigation. Plastic pots (each containing 5 kg of soil) were used. Results indicated that in spinach and radish grown on cadmium (Cd) and Zn treated soil, the production was decreased. Addition of Cd (25-50 mg Kg^{-1}) reduced the production by 34.3-46.8% in root and 5.6-14.3% in leaves of spinach. Similarly the reduction magnitudes were 17.6-28.9% in roots and 3.9-12.1% in leaves of radish after addition of the same concentration of Cd. The production of vegetables did not decrease with addition of Cd and Zn in combination. The Cd and Zn contents and their distribution in vegetables tissues varied with the Cd and Zn addition levels. The results indicated that Cd moved more easily into the aerial parts whereas Zn accumulated in the roots of the plants tested.

Key words: Cadmium, Zinc, Spinach, Radish, Accumulation.

Growing vegetable by using city wastewater, industrial effluent etc. as a sole source of irrigation and as fertilizer supplement is a common practice in the vicinity of big cities. Such a practice leads to accumulation of toxic metals like Ni, Cd, and Cr etc. in the soil as well as the crop plants, which affect the food chain. Green leafy vegetables by roots, tubers and lowest in cereal crops (Setia et al., 1998). A constant anthropogenic release of Cd to the environment has resulted in a continuous build up of Cd in soils. Some heavily contaminated soils are confined to areas of non-ferrous metal mining and production (Shenker et al., 2001). Vegetables constitute an important part of the human diet since they contain carbohydrates, proteins as well as vitamins, minerals and trace elements (Abdola and Chmtelnicka, 1990). Sewage sludge is a product of waste water treatment processes which tends to concentrate potential contaminants such as pesticides, metals, pathogens, industrial solvents, dyes, plasticizers and other organic chemical residues (Gibson et al., 2005). The Heavy metals uptake by plant strongly depends on several soil and plant factors. The use of sewage-sludge has received much attention due to enrichment of heavy metals in soils which impacts human health and social problems (Gholamabbas et al., 2010; Angelova et al., 2005; Yusuf et al., 2003).

Long-term use of this waste water, which is mainly used for cultivation of leafy and other vegetables, has resulted in the accumulation of heavy metals in the soil and their transfer to the various crops under cultivation, with levels of contamination that exceed permissible limits (Nrgholi, 2007).

The ability of plants to accumulate trace elements in their edible parts varies between plant species and among genotypes within species

(Peterson, 1972; Welch, 1986). Thus there are genetic controls over the trace elements concentrations found in edible portions of higher plants, these genetic differences can affect the nutritional status of animals and the geographic distribution of trace elements problem areas (Allaway, 1986).

Higher concentrations of heavy metals exhibit severe bleaching, reduction in cell size, shrunken chloroplast, fragmentation, loss of cellular contents, cell lysis, tetrad cells, clumping of cells, etc. The high concentration of heavy metals is due to stagnation of the metals which precipitate out as insoluble metal complex and which are deposited on the soil surface (Imam Kasim, 1989).

When absorbed by the root, Cadmium can be easily transferred to other parts such as stem and leaves. If excess quantity is accumulated in the plant body, Cadmium will adversely affect the plant growths and metabolism. (Barber, A. and Brennan, E. 1974).

Since Cadmium has some similar chemical characteristics as zinc and manganese, it sometimes replaces the latter at the reactive site resulting in inhibited enzymatic activities thus leading to plant withering, yellowing and retarding growth. (Taiz, L. and Zeiger, E. 2002).

Vegetables, take up heavy metals from the soil as well as from surface deposits on the parts of vegetables exposed to polluted air. Moreover, the presence of heavy metals in fertilizer contributes an additional source of metal pollution for vegetables, considering the importance. This study was conducted to investigate the levels of absorption and accumulation of Pb & Cd in different part of vegetables.

MATERIALS AND METHODS

A silty clay loam, from sewage-sludge irrigated soils of SDI farm situated on the confluence of Ganga and Yamuna alluvial deposit, was sampled from Allahabad city, India. The properties of the soil were: pH 7.5, EC 0.16 dSm^{-1}, organic C: 0.50% ($K_2Cr_2O_7$ oxidation) total N 0.05%, total P 0.04%, CEC 20.7 Cmol (P^+) kg^{-1}, DTPA-Cd 0.36 mg kg^{-1} and DTPA-Pb 0.34 mg kg^{-1}. The texture was sand (>0.2 mm) 36.7%, silt (0.002-0.2 mm) 28.5% and clay (<0.002 mm) 34.7%. Soil reaction and conductivity were measured by with pH and EC maters. The organic matter of soil was determined by the wet oxidation method (Walkley & Black, 1934).

CEC was determined by standard method prescribed by Nelson & Sommers 1982. Soil texture was (Sand, silt and clay) determined by the hydrometer method (Goe and Bauder, 1986). The initial pH of the soil was 7.5 which increased to 7.7 after irrigation. Plastic pots of 5 litre capacity (each containing 5 kg of soil) were used. The soil was ground to pass through a 2-mm sieve. An amount of 2 kg soil was put into the pots for plant growth. Fertilizers added per kg soil was 0.8 g calcium ammonium nitrate, 0.5 g di-ammonium phosphate, 0.367 g potassium sulphate. Nine treatments were designed as follows:

(I) control (II) Cd 25 mg kg^{-1} (abbreviated as Cd 25); (III) Cd 50; (IV) Zn 100; (V) Zn 125; (VI) Cd 25 + Zn 100; (VII) Cd 25 + Zn 125; (VIII) Cd 50 + Zn 100; (IX) Cd 50 + Zn 125. Cd was added as $CdCl_2$ and Zn as $ZnSO_4$. After 24 h of the treatments application seeds were sown, rotationally planted in the pots and kept at 20-25% moisture. All treatments were replicated 3 times. Soil moisture was maintained by irrigating the crops at interval of 5-6 days. Spinach was harvested after 45 days. After harvest, fresh weight was measured and some samples were used for quality analysis immediately. Other samples were oven-dried at 70-80°C for further mineral analysis.

Plant samples were digested in tri-acid mixture (750 ml conc. HNO_3, 150ml conc. H_2SO_4 and 300 ml $HClO_4$). Cd and Zn were determined by Atomic Absorption Spectrophotometer. Perkin Elemar make Model ANALYST- 100. All data were statistically analyzed using Excel 2000 software. R.B.D. design was used to the assessment of distinct.

RESULTS AND DISCUSSION

Effects of Cd and Zn on spinach and radish production

Spinach and radish show the great ability to accumulate heavy metals due to the assimilation of these elements from soil by the roots of plants. These elements gradually get accumulated in different parts of the plants including leaf and roots. Table 1shows that the yield of Spinach and radish was reduced remarkably by the Addition of Cd (25-50 mg Kg^{-1}). It reduced the production magnitudes by 34.3-46% in root and 5.6-14.3% in leaves of spinach. Similarly the reduction magnitudes were 17.6-28.9 % in roots and 3.9-12.1% in leaves of radish after addition of the same concentration of Cd. Addition of Cd (25-50 $mgkg^{-1}$) compared with control treatment. The yield of spinach marginally increased by the addition of Zn compared with control treatment, the values being 3.7-28.1% and 6.5-12.2% in spinach and radish. Although the amount of Cd addition was rather less than that of Zn, The influence of Cd was greater than that of Zn. Cd thus led to higher yield reduction of vegetables (Pandey & Sharma, 2002), as compared to Zn, The production of vegetables did not decrease in the combinational addition of Cd and Zn albeit and there was slight increase in produced compared to control.

Cd and Zn contents in vegetables grown in Cd and Zn polluted soil:

The results presented in Table 2 indicates that the treatment of soil for spinach caused increase in the content of Cd in the different parts of plants .When 25 mg kg^{-1} Cd was added, the content of Cd in spinach increased up to 14 fold over control treatment. The fact that there was no distinct difference among treatments of Nos.1, 4 and 5 meaning that the Zn addition did not affect the Cd contents in spinach leaves. On the contrary, only No.7 treatment (Cd 25 + Zn 125) caused a relatively higher Zn content (Table 3), and there were no significant difference among others, although small variations were present. These might suggest that Cd would be transferred to the leaves of spinach more easily than Zn. For radish. The contents of Cd in roots were positively correlated with the amount of Cd addition. Meanwhile, the addition of Zn increased Cd contents in radish roots if they were combined. There were remarkably different Zn contents among treatments, however only treatment 5 with (Zn) had a relatively higher Zn content. At this treatment the content of Zn was 5-fold higher in comparison with the control. It appears that Zn addition does not affect Zn accumulation in radish to that extent as compared to Cd accumulation in plants.

Cd contents in leaves of spinach were generally much greater than in the other tissues (Table 2). Cadmium is easily assimilated through both the root system and the aerial parts. The highest Cd content, over 25 mg kg^{-1}, was found to be present in spinach's leaves in the treatments Nos. 3 and 7 Compared to treatments No. 2, 6 and 7 (the amount of Cd addition was 25 mg kg^{-1}), it was likely that Zn addition would influence Cd accumulation in spinach's leaves. At lower Zn addition, Cd accumulation was obviously decreased as compared to control. Cd accumulation was dramatically increased if compared with control. For radish roots, the contents of Cd increased with the Zn addition in the combination. Cd could be transferred to the aerial parts of the plants. For spinach, when Cd was added, Cd contents in the leaf were greater than those in the roots, but at combinatorial treatment the contents of Cd in the leaf were lower than other tissues. Radish roots were main tissues in which Cd was stored if the soil is added with Cd. The percentage of Cd in radish roots increased with increasing Cd addition if it was added in gradual manner. For the combinatorial addition, both root and leaf had greater Cd but the increase in leaves was more significant than in roots of spinach. On the other hand the increase in roots was more significant than in leaves of radish. From Table 3 it is clear that Zn accumulation in spinach roots was highest due to Zn treatment in isolation (Treatments 4 and 5). There was noticeably higher Zn content in vegetables roots due to treatment 3 as compared to control and Treatment 2, although no Zn was added to the soil. There was more Zn accumulation in radish root than in the leaf almost for all treatments. However, enrichment of Zn in root and shoot was observed to be 100%, and 250%, respectively, after treatment 5 over the control.

Table 1: Effect of different treatment of heavy metals on the fresh weight (g fresh weight/ pot) of spinach and radish.

Plant	Tissue	Treatment										
		I	II	III	IV	V	VI	VII	VIII	IX	SE	CD
Spinach((*Spinacea oleracea* L.)	Root	16	10.5	8.5	19.4	20.5	19.3	21.0	19.0	18.1	2.61	5.54
	Leaf	55.4	52.0	47.2	57.5	61.0	53.0	60.5	56.4	54.0	4.32	9.17
Radish (*Raphanus sativus* L.)	Root	35.2	29.0	25.0	35.0	39.5	34.0	45.0	31.1	29.3	22.2	47.1
	Leaf	40.4	42.0	35.5	43.0	48.4	41.0	48.0	44.2	43.3	5.37	11.3

I Control : II Cd 25 mg kg^{-1} (abbreviated as Cd 25, and the similar meaning was for the follows); III Cd 50; IV Zn 100; V Zn125; VI Cd 25 + Zn100; VII Cd 25 + Zn125; VIII Cd 50 + Zn 100; IX Cd 50 + Zn125.

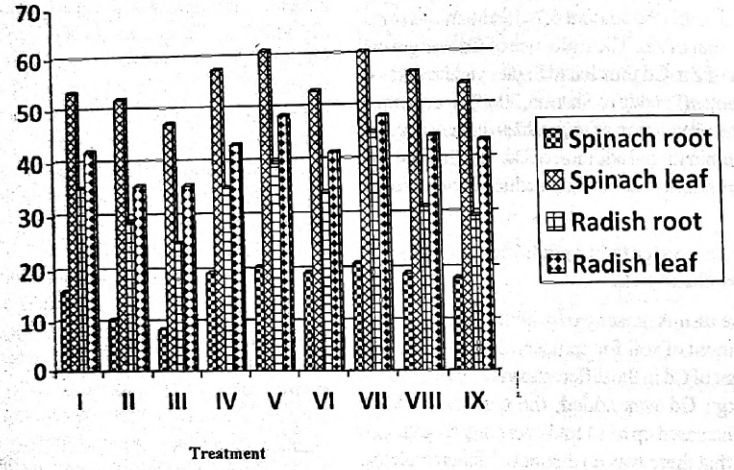

Fig 1 Effect of different treatment of heavy metals on the fresh weight (g fresh weight/ pot) of spinach and radish.

Table 2: Contents of Cd in different tissues of spinach and radish (mg kg^{-1}) after defferent treatments.

Plant	Tissue	Treatment										
		I	II	III	IV	V	VI	VII	VIII	IX	SE	CD
Spinach((*Spinacea oleracea* L.)	Root	0.22	3.1	10.1	0.24	0.23	3.0	10.1	10.3	10.1	1.50	3.19
	Leaf	0.31	4.1	15.4	0.20	0.22	3.2	14.3	16.2	16.4	1.79	3.79
Radish (*Raphanus sativus* L.)	Root	0.12	3 .3	6.4	0.25	0.22	2.2	2.1	4.2	4.3	0.76	1.60
	Leaf	0.31	1.0	1.3	0.33	0.40	1.0	1.1	1.2	1.3	0.25	0.54

Footnote are same as table 1: The level of cd in different parts of the plant of the plants were determined by Atomic absorption spectrometer as described in m

aterial and methods.

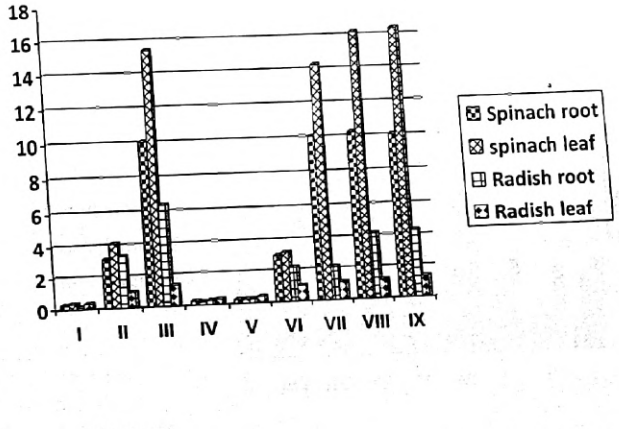

Fig 2. Contents of Cd in different tissues of spinach and radish (mg kg^{-1}) after defferent treatments.

Table 3: Contents of Zn in different tissue of spinach and radish (mg kg^{-1}) after different treatments.

Plant	Tissue	Treatment										
		I	II	III	IV	V	VI	VII	VIII	IX	SE	CD
Spinach((*Spinacea oleracea* L.))	Root	1.2	1.1	1.2	2.4	3.8	2.3	3.3	2.2	2.4	0.62	1.30
	Leaf	0.4	0.4	0.5	2.1	2.4	2.0	2.4	1.5	2.1	0.50	1.13
Radish (*Raphanus sativus* L.)	Root	1.0	0.6	1.0	1.1	2.1	1.1	1.2	1.0	1.2	0.32	0.69
	Leaf	0.4	0.4	0.4	0.6	1.4	0.4	0.5	0.4	0.4	0.29	0.62

Footnote are same as table 2: The level of cd in different parts of the plant of the plants were determined by Atomic absorption spectrometer as described in material and methods.

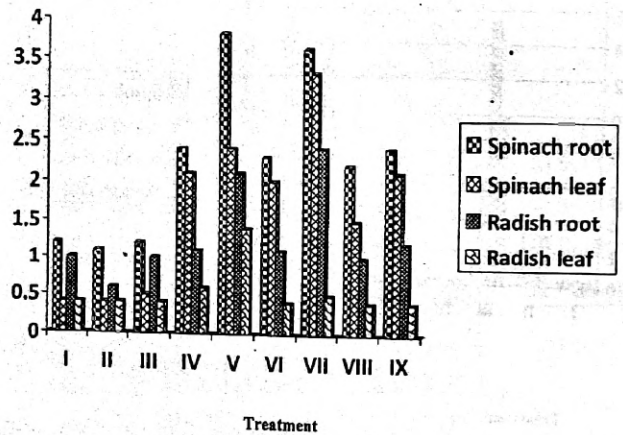

Fig. 3 Contents of Zn different tissue of spinach and radish (mg kg^{-1}) after different treatments.

This percentage was relatively lower than that of Cd in radish roots. The Zn was found to be enriched in different tissues for spinach and radish. This was not only dependent on plant species but also on Zn addition levels and presence of Cd. Significant bioavailability of cadmium to vegetables in soils previously treated with sewage- sludge (Chaney, 1994) industrial effluents and organic substances (Mani et al., 2007; Shuman, 1998) has already been shown.

CONCLUSIONS

Cadmium alone reduced the yield of both spinach and radish. Addition of Cd (25-50 mg Kg^{-1}) reduced the production by 34.3-46% in root and 5.6-14.3% in leaves of spinach. Similarly the reduction magnitudes were 17.6-28.9% in roots and 3.9-12.1% in leaves of radish after addition of the same concentration of Cd. The production of vegetables did not decrease with addition of Cd and Zn in combination. The contents of Cd and Zn in different tissues of vegetables changed with various ways of treatments.

The results of presented study showed that Zinc can effectively immobilize Cd in the soil. Zinc has potential to reduce Cd accumulation in both root and shoot of the spinach and radish.

The application of Zn to the soil possibly reduces Cd in the edible parts of the plants and helps to reduce the risk to the health of people living in metal contaminated areas. Amore detailed study is required to grow spinach, radish or other vegetable crops in metals- contaminated areas and evaluate their growth and distribution of heavy metals in different edible parts of plants.

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REFERENCES

Abdola, M. and Chmtelnicka, J., 1990. New Aspects on the Distribution and metabolism of Essential Trace Elements after Dietary Exposure to Toxic Metals. *Biol. Trace Element Res.* 23, 25-53.

Allaway, W. H., 1986. Soil, Plant, Animal and Human Interrelationships in Trace Element Nutrition. In: Nmertz., w. (Ed). Trace elements in Human and Animal Nutrition 5 th Edn.,) Academic Press, New Yark, pp–465-488

Angelova, V.; Ivanova, A. and Ivanova, K., 2004. Effect of chemical forms of lead, cadmium, and zinc in polluted soils on their uptake by tobcco. *J. Plant Nutri.* 27 (5), 7571-773

Barber, A. and Brennan, E., 1974. Phytotoxic effect of cadmium. Phytopathology, 64: 578-586.

Chaney, R. L., 1994. Trace metal movement: Soil-plant systems and bioavailability of biosolids applied methods: In: Clapp, C.E., Larson, W.E., Dowdy, R.H. (eds): Sewage-Sludge: Land utilization and the Environment. Soil Sci. Soc. Am., Miscellaneous Publ., Madison: 27-31

Gholamabbas, S.; Majid, A.; Sayed, A.; Sayed, F. M.; Karim, C. A.; Brian, K. R. and Rainer, S., 2010. Transport of Cd, Cu, Pb and Zn in a calcareous soil under wheat and safflower cultivation- A column study. *Geoderma* 154, 311-320.

Gibson, R.; Wang, M. J.; Padgett, E. and Beck, Aj., 2005. Analysis of 4- nonylphenols, phthalates and polychlorinated biphenyls in soils and biosolids, Chemosphere. 61,1336-44

Goe, G.W. and Bauder, J. W., 1986. Particle size analysis. In: Klute, A.(Ed.). Methods of soil Analysis. Part 1, 2nd ed., Agron. Monogr. 9., ASA and SSSA, Madison, Wisconsin. pp. 383-411

Imam Kasim, D.; Nanda Kumar, N. V. and Hussain, R. C., 1989. Environmental Contamination of chromium in agricultural and animal products near a chromate factory. *Bull. Environ Con. Toxicol.* 43, 742-746

Mani, D.; Kumar, Chitranjan and Srivastava, R. K., 2007. Effect of calcium, zinc and organic

matter on the uptake of cadmium in *Brassica rapa* L. (Turnip) PROC. NAT. ACAD. SCI. INDIA, 77 (B III) 263-276

Mani, D. Sharma, B. and Kumar, Chitranjan., 2007. Phytoaccumulation. Interaction, Toxicity and Remediation of Cadmium from *Helianthus annuus* L. (sunflower). *Bull. Environ. Con. Toxicol.* 79 (1), 71-79

Nelson, D. W. and Sommers, L. E., 1982. Methods of soil analysis, In; page, A. (Ed.), LASA and SSSA, Madison, WI, 2nd ed , pp. 539-579. Part 2.

Nrgholi, B., 2007. Investigation of the Firozabad waste water quality-quantity variation for agriculture use. Final report. Iranian Agril. Eng. Res. Institute.

Pandey, N. and Sharma, C. P., 2002. Effect of heavy metals C^{o2+}, N^{i2+} and C^{d2+} on growth and metabolism of cabbage. *Plant Sci.* 163, 753-758

Peterson, P. J., 1972. Unusual accumulations of elements by plants animals. *Sci. prog.* (Oxford), 59, 505-526

Setia, K. Kawatra, B. L. Hira, C. K. and Mann, S. K., 1998. Toxic heavy metal contents of food material consumed by the population in tube well and sewage water irrigated areas. *J. Food Sci. Technol.* 35: 543-46.

Shenker, M. Fan, T. W. M. and Crowley, D. E., 2001. Phytosiderophores influence of Cd mobilization and uptake by wheat and barley plants. *J. Environ Qual.* 30: 2019-98.

Shuman, L. M., 1998. Effect of organic waste amendments on cadmium and lead in soil fraction of two soils. Communication in Soil Sci. *Plant Anal.* 29, 2939-2952

Taiz, L. and Zeiger, E., 2002. "Plant Physiology". Sinaner Associations. Massachertis.

Walkley, A. and Black, C. A., 1934. An examination of the Degtareff method for determining soil organic matter and a proposed modification of the chromic acid titration. *Method. Soil Sci.* 37, 29-38.

Welch, R. M., 1986. Effects of nutrient deficiencies on seed production and quality. *Adv. Plant Nutri.* 2, 205-247

Yusuf, A. A.; Arowolo, T. A. and Bamgbose, O., 2003. Cadmium, copper and nickel levels in vegetables from industrial and residential areas of Lagos City, Nigeria. *Food Chem. and Toxicol.* 41, 375-378

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A REVIEW ON CATTLE BREEDING PRACTICES IN ANCIENT INDIA

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People realized the economic importance of the domestic animal and as such various animal including cattle were tamed and reared for various economic purposes.

However, cattle breeding appeared to be one of the important aspects of the animal husbandry practices in ancient India. "They (Aryans) Gods are invoked to protect and feed the cows, to increase the herds, to make the cows full of milk and satisfy the horses, to lead the herds to good pasture, and protect them from misfortune in the way".[1]

Typology of cattle :

The Vedic texts refers to cows of different colours and varieties, [2] such as, *rohini (red), syemi (white),* krsna (black), brown and yellow. Some were of similar colour and some of different ones. *Kuta* was the hornless cow, *slona* was the lame cow, *banda* was the maimed cow, and *kana* was the one-eyed cow[3]. Satapatha Brahman[4] mentions that the animals belonging to the family of cow like *Rsabha* (special bull). *Babhru gau* (brown ox) *Prsan gau* (pie-bald bullock). *Vasa* (sterile cow), *sitiprhsaht gau* (white-blacked bullock). Vasa-prsni (spotted sterile cow), syama gau (dark-grey bull), dhenu (milch cow), *syeta anadvan* (reddish-white draught bullock) *anadvan* (draught bullock), *yama gau* (twin bullocks), *dvirupa gau* (bicoloured bullock), *krsna parimurni parimurni paryariri gau* (black, decripit diseases bullock), *napumsaka gau* (castrated bull), *pasthava* (ox), *uksa* (bull) calves of different ages, such as *tryavi* (of 18 months) *dityavad* (two years old), *pancavi* or 2 and half years old, *trivatsa* or three years of age and *turyavad* of four year old, *Krans-sukla vatsa* (a black cow which has a white calf) and *anaduhi vahala* (yoke trained cow). Kautilya[5] prescribed one of the roles of sum to maintain a record (register) classifying the cattle as male calves, steers, tamable cows, drought oxen, yoke and breeding bulls, fit for slaughter, buffaloes, female calves, heifers, pregnant cows, milch cow, barren cattle etc.

The Selection of Cow :

"Domesticated animals show certain characteristics which can be statistically recorded with in the overall range of variation. The environment of domestic animals and man brings about the selection of such combinations of characteristics. Selected breeds created by men through selection of animals according to definite breeding objectives and through sexual isolation show a narrow range of variation".6

The selection of cow for higher productivity was a specialized subject even in the days of Matsya Purana and Brihat Samita Which gives the instruction for the selection of a cow. According to Matsya first of all the cow is to be examined; there should be a cow of gentle temper free from ailment and diseases, strong, of nice colour, having beautiful hoofs (especially curls turning to the left on the right side and turning to the right on the left side) having all the lucky signs, with extensive thighs, red lips, neck and tongue, with eyes clear and beautiful (not red or having many hair) and hoofs large, having eyes of the lustre of Vaidurya with lovely corners, having seven and seven teeth and bright palate, with lovely sides and thighs with si parts elevated, five parts and eight capacious an wide. A cow having these qualifications is said to hav auspicious signs".

Matsya said: "The following six parts of the cow's body should be elevated viz. – chest, back, head, belly, loins".

"A cow with the following level parts of the body is said to be fine one, viz.- ears, eyes, forehead, and the following eight parts should capacious viz.- tail, dewlap, udders, thighs; and extensive head and neck are also desirable".[9]

Regarding, the features of cow's Parasara[10] observed as under:

1. Cows whose eyes are dim and with ears and disagreeable to look at and whose eyes resemble those of the mouse, will bring misery. Cow whose horns are moving and flat, whose bodies are rough and whose color resembles that of the ass, will also bring on evil.
2. Cows having ten, seven or four teeth and those whose heads are hanging and hairless, whose backs are bent or depressed, necks short and thick, hip resembling a grain of barley in shape, whose hoofs are broken likely to bring evil.
3. The cow's with long, red, black tongues with small ankles, with a large hump on the back, with thin slider bodies and with either defective or excessive organs, will bring on evil.

The Selection of Bull/Oxen :

All the scientific breeding depended on the sturdy and physically fit bull, so at the time of performance of the ceremony of setting it at liberty, it was ensured that the bull possessed "all marks" i.e. not deficient in any limbs. Vishnu11 says that after washing and decorating the bull, it was brought along with four young well bedecked cows. Apart from other religious and purificatory acts in the said cow, the mantra of "the father of the calves" was pronounced in the right ear of the bull[12]. It shows that for healthy breeding it was considered as the husband of the cow and the father of the young calves.[13]

Often a physically fit bull [14] was set at liberty for breeding purposes. It is laid down that it should be imposed on the person who set it at liberty for the damage done by it.[15] In the Matsya Purana[16] it is laid down the this *brahmani bull* must have elevated shoulders and hump, a soft and straight tail, tender cheeks, broad back, shining eyes, sharp horns, thick and long hair or the tail, eighteen healthy teeth and eyes like Atallika flowers.

The Brahmanas should set free the following class of bulls, viz.-red, tawny or reddish, white or black in colour, tawny reddish back, of variegated colours, with long ears and shoulders, with glossy hair, red eyes or having nutbrown colour near the horns, with white stomach, or black sides. The Kshatriyas should set free a bull of red and beautiful colour; the Vaisyas of golden colour and the Sudras of black colour. The bull with its horns pointing forward towards the eyebrows ought to be set free by the men of all classes. The bull is having its feet like those of the white cat, with tawny or reddish in colour, with eyes shining like a jewel having white feet or with only two feet white, or of the colour of a pigeon or a partridge, is also said to be a good one. It is called Karat.

The bull whose face is white or reddish up to ears and whose body is especially or red colour is called Nandimukha cow. The bull whose stomach and back are white is called Samudra and increases the progeny of the family. The bull of the colour of jasmine or having variegated circular spots is considered to increase the wealth of the donor.

The bull having circles like lotus increase the fortune; the one of the colour of Atasi flower increases prosperity. All these kinds of bulls are good. Now I shall tell you the kind of bulls that are of bad signs and should neither be set at liberty nor kept in the house. Those are the bulls that have black palate, lips and mouth, and rugged horns and hoofs, indistinct colour, mouth resembling that of a wolf or a tiger, and colour like that of a crow, vulture, or a form like that of a rat, weak, having no teeth, squinteyed, one-eyed, lame, with half of the white feet, and having restive eyes. Further, the kind of bulls that ought to be set liberty or kept in the household.

Those should be well-built, roaring like the thunder clouds, high in stature, walking like an infuriated elephant, with broad chest and very powerful.

The white bull having its head, ears, forehead, tuft of hair at the tail-end, feet and eyes black, is described to be very excellent.

Similarly a black bull having all those things white is said to be the same. The bull whose tuft of hair at the tail-end may be long enough toughning the ground and the hairs of the tail long and thick, such a Nila bull is said to be especially good. The bulls having the signs of pearl, a banner, etc, are exceptionally good. They are the givers of wonderful, siddhle and victory. The when obstructed in their motion stop, and whose head and neck are elevated, are excellent. Those that have their forepart of the horns and the eyes red, body white, hoofs resplendent like coral, are said to be the best of all. These are to be kept in the house or set at liberty. These increase grains and wealth. The bulls whose four feet, face and tail are white, and whose colour is red like the juice of lac or red dye is known as Nila-Vrisabha. It should be set free; it should never be kept in the household. It is a saying amongst the household that one should desire many sons for even if one son out of an many goes to Gaya or offers a Gauri (virgin) or sets a Nila-Vrisabha at liberty his family is blessed.

In selecting a breeding bull similarly advice is given by Varahamihira. Kautilya's time, the administrative authorities appeared to be fully aware of its importance. The King was enjoined to preserve the breed of the cattle in the country and a government officer called superintendent of cattle was entrusted with the exclusive task of supervising the livestock in the country, keeping census and ensuring their proper breeding and management .17

The selection of cattle for higher productivity was a specialized subject even in the days of Brihat Samhita. This gives instructions regarding good features of cattle and the selection of a bull. Regarding the features of oxen 18 Parasara observed as under:

1. The ox whose testicle is large and hangs down, whose breast is covered with muscles whose cheek is large and marked with Sinews, and whose penis is thick in three places, will bring on evil.
2. The ox, whose eyes resemble those of the cat, whose colour is brown, whose body is rough, whose lips, jaws and tongue are black, whose breathing is like snoring and which causes annoyance to the herd of cows, is not fit for the Brahmanas.
3. The ox that posses excrement in large lumps, whose horns not grow, whose belly is white, whose body is of the colour of the deer and which causes annoyance to the herd of cows is to be rejected though it may be a home-bred one.
4. The ox which is black, of ashy colour, or of the colour of the sun, and whose eyes resemble those of the cat, is not fit for Brahmanas.
5. The ox, which, while at work lifts up its leg as if from a mire, whose neck is thin and dyes fearful, is an animal of inferior kind. Such animals cannot carry heavy burdens.
6. The ox whose lips are soft, close and red, whose ears are small, short and raised up, whose belly breast is strong, the ankles are prominent; whose hoofs are red and close, whose breast is strong, the hump on whose back is large, whose skin and hair are fine, whose horns are red at the ends, whose breathing is loud whose shoulders are like those of the lion, whose dew-lap is soft and small and whose gait is beautiful, is of an excellent kind.
7. The ox, the curl of hair or ringlet on whose left side is from right to left and the one on the right side is from left to right, and whose ankles resemble those of the deer, is an excellent one.
8. The ox whose eyes are like vaidurga or the jasmine or the water bubble, whose eye-lids are thick and whose hoofs are close is also excellent and will carry heavy burdens.
9. The ox with a wrinkle at the end of its nose, whose face is liked that of the cat, whose right side is white, whose colour is that of the white or blue lotus or red cotton, whose tail is fine and whose speed is that of the horse. Whose testicle is hanging, whose belly is like that of the goat and whose buttocks and breast are contracted, will bring on prosperity, carry heavy burdens and go great distances.
10. The ox which is white, whose eyes are of brown colour, whose horns are red and whose face is large, is known as Hamasa. It brings on prosperity and an increase of the herd.

11. The ox, whose tail hangs to the ground, whose buttocks and eyes are red, and the hump on whose back is fine, and whose body is variously colored, will make his master the lord of wealth at once.
12. The ox, one of whose legs are white is also a good one, whatever may be his color. If oxen of excellent sort cannot be had, those of middle importance may be procured and used.

As the bull was likely to sire many of the calves born in the area, and as it was in a sense public property, its careful choice was a form of selective, and of value to the economy of the locality.19 The gifting away of the bull to a Brahmana was a meritorious deed as the giver saw in his own cow-pen growth and increase of his cows.20 However, the gift of own ox means the giving away of ten cows and is equal to the gift of the whole earth. Therefore, no other animal than an ox deserves veneration.21 Moreover, the nostrils of an ox should never be pierce for three or four days after it has become strong-limbed and should never be pierced that time as long as he is weak. Professionals should make the nose-pins, twelve angulas in length from the khadira or sheesham wood and should have pair or three of them.

In Mahabharata cattle breeding was a regular profession. Sahadeva acts as a cowherd under Virata. "Under me" Cows do not multiply in number in a short time; nor does any disease appear among them. I know the marks of oxen which are fit be prized and by smelling whose urine even a barren woman brings forth a child". Similarly Nakula acted as a housekeeper. "I know the character of horses and how to break them. I know how to correct their vices and to treat their diseases. A horse under me shall never feel shy. In my hands, no mare evil: what need then to speak of horses"? Narada's question shows the importance attached to the scientific rearing of animals: "Do you study the various sutras, including the elephant-sutra, the horse-sutra, and the ratha-sutra?" The importance of kine is fully recognized. "kine are always the root of prosperity. There is no fault in kine. Kine always afford the best food in the form of havi, unto the deities. The sacred mantras Swaha and Vashat are always established upon kine. kine are the chief conductresses of sacrifice. They constitute the mouth of sacrifice. They bear and yield excellent and strength-giving nectar". "Kine benefit human beings with milk, ghee, curds, dung, skin, bones, horns and hair, O Bharata". The bullocks too should be normally treated with kindness. "Formerly, the deities, while tilting the earth whereon they performed a sacrifice, used the goad for striking the bullocks yoked to the plough. Hence in tilling earth for such a purpose, one may without incurring censure or sin, apply the goad to bullocks. In other acts, however, bullocks should never be struck with the goad or the whip."

Livestock-breeding and hunting retained important place in ancient place in ancient Indian economy, especially in areas with favorable climatic conditions and a lower level of socio-economic development than in the Ganges Valley. It is noteworthy that the suttanipata describes cattle as bringing grain (food), strength, beauty and happiness. The Jatakas also often mention the high value of livestock. It is interesting that Ashoka's edicts contain a long list of animal whose slaughtering was prohibited. Along with the farm households, where a small number of cattle were kept, there were large cattle-breeding farms. Buddhaghosa's commentary on the Suttanipata, for instance, mentions an owner of 30,000 head of cattle including 27,000 milch cows. Although these figures are obviously unrealistic, they do provide evidence of considerable concentrations of heard in private hands. Herdsmen were hired for a fixed wage to graze private owners' cattle, which they drove to the pastures or into the forests in the morning. Ashoka's edicts show that the state paid much attention to promoting livestock-breeding along with such officials as the Dharmamahamatras, they mention special officials supervising pastures-Vrajabhumikas.

There were certain restrictions to castrating bulls-emperor Ashoka issued an order that:

".....Athamipakhaye
Dasaye Pumnadasaye tisaye puna
Vasume tisa catumasisu sudivasaye
Gone no nilakhilaviye ajake edake".

That is to say, a bull, a goat or a ram must not be castrated on the 8th, 10th, 15th, and 13th day of each fortnight neither on the Punarvasu day, on a festival day and in every fourth month of the year. Brahmani bulls were objects of special attention on certain festive occasions. They were marked on the right flank with a discuss and on the left flank with a trident. In the Brsotsanga ceremony which was to take place on the day of the full moon in month of kartika or Asvina, the bull was set at liberty. It was of marked as above and then washed, adorned and brought near with four young cows which were also washed and decorated. To the right ear of the bull, the mantra "The father of calves" was pronounced and also the mantra "this young bull I give you as husband" was uttered into the ears of the cows. Visnu Puranas directs that the bull must be the offspring of a milch cow having young ones living. It must not be deficient in any limb and it must be one who protects the herd. In matsyapurana, it was instruction that the bull must have elevated shoulders and hump, a shoft and straight tail, tender cheeks, broad back, shining eyes, sharp horns, thick hair on the tail and eighteen nice teeth. Further, the bull must be well-built, roaring like the thunder clouds, high in stature and walking like an infuriated elephant.

The bulls so set at liberty were public property. They were the breeding bulls and that is why the ancients were so particular as to their physical fitness. The Arthasastra says that a herd of ten heads of either cows or buffaloes shall contain four male animals (and in Sukadum Nask of Dinkard, Book III, we find particulars about the time of allowing admission of the bull to the female). Similarly a scholar revealed that cattle should be grouped in herds of ten each of similar colour, while they were being grazed. A certain proportion of cattle must be males; thus in every herd of ten cows or buffaloes there must be four males".

Rationale of Breeding

The second point of Brihat Samhita focused that the symptoms of cow indicate ill health. Obviously, unhealthy cow will not be profitable to the owner or may cause even death of the cow. Both the situations will reduce the profit from cow keeping. Therefore, ancient writers were knowledgeable about the health and disease symptoms of the cows. Similarly, cows with loose and flat horns as these are genetically determined traits. In ancient India the main locomotive power was bullocks were used to drive carts, pull plough, and take out water from deep wells. Such bullocks were tied in yokes which used to rest on their soldiers and the bullocks with flat and loose horns which were reluctant to perform the work used to take out their heads from the rope tied to the yokes easily which otherwise would not pull their heads they possessed tight and upright horns. This fact was known to the ancient Indians and is also suitable while selecting a draught bullock. The ancient Indians wrote this feature of the bullock in the form of evil so that people may not commit mistake in purchasing a bullock.

However, as evidenced from the ancient literature even now a days the best males were used as breeding bull and others were castrated to be used as oxen. In present days also only best males are permitted in the herd to produce future offspring's and the rest are culled for various purposes.

In ancient times the best young breeding males were physically examined for any deviation from the breed characteristics, identifiable disease occurrence; old age, poor libido and if these characters were present, the animals were preferred for oxen or were sold for other purposes, except breeding males.

As far as the scientific inference can be drawn with literature is concerned, no inference can be drawn with regard to size and shape of the horn and its relationship with milk production traits. The reason for not choosing black, brown or dark coloured oxen may be the fact that as per the scientific laws/principles the black bodies will absorb greater amount of solar radiation as compared the white/light coloured animals and thus, the efficiency of darker animals will be adversely affected in the field during sunny hours when most of the agricultural operations are performed in animal driven systems.

Similar to ancient practice during Asoka's reign, a separate animal husbandry & dairying depts. under government control (State/Central) is responsible for organizing all animal husbandry

activities for betterment and improvement of livestock wealth of nation.

REFERENCES

Buch, A.A. 1945. Economic life in Ancient Indian. Baroda, pp.29.

Atharvaveda, mandal 18, Chapter 4, Hymn 33.

Atharvaveda, mandal 12, Chapter 4, Hymn 3.

Satapatha Brahman, Kand 13, Prapatahk 3, Brahamna 2, Hymn 3.

Mandokhot, U.V. 1987. Breeding practices and selection criteria for domestication of animals. Indian Journal of History science, vol.22 (2), pp. 127-135.

Roy, B.P. 1984. The Later Vedic economy. Janaki Prakashan, Delhi, pp.261.

Matsya Purana, Chapter 207, verse 2 to 8.

Matsya Purana, Chapter 207, verse10.

Matsya Purana, Chapter 207, verse11 to 12.

Brihat Samhita. Features of cows and oxen, Chapter 61, verse 2 to 4

Vishnu Purana, Chapter LXXXVI, verse 12. Cited form Kher, N.N. (1973). Agrarian and Fiscal Economy. Motilal Banarasidass, pp.224-225.

Vishnu Purana, Chapter LXXXVI, verse 13.

Atharvaveda, Mandal 9, Chapter 4, Hymn 2 17.

Amar. 9 62, P.213; 9.70; p.215 cited from maity, S.K. (1957). Economic life of Northern India. The World Press Pvt. Ltd. Nar. XI 33.

Matsya Purana, Chapter 207, verse 13-14.

Shamasastry, R. 1929. The superintendent of /cows. Kautilya's Arthasastra, Book II, Chapter XXXIV, Wesleyan Mission Press, Mysore 146-142.

Brihat Samhita,. Features of cows and oxen, Chapter 61, verse 5 to 19.

Maity, S.K. (1957). Economic Life of Northern India. The World Press Pvt. Ltd.

Atharvaveda, mandal 9 Chapter 4, Hymn 19.

Brihat-Parasara Samhita. Vol. II, Slok 51 cited in Raychaudhuri (1964). Agriculture in Ancient India. ICATR, New Delhi, pp. 125.

Brihat-Parasara Sambita. Vol II, Slok 53

Brihat-Parasara Sambita. Vol II, Slok 54

Mahbharata, (Virata Parva), 10.

Mahbharata, (Virata Parva), 12.

Mahbharata, (Anu), Chapter 51.

Mahbharata, (Anu), Chapter 66.

Mahbharata, (Anu), Chapter 69.

Suttanipata, pp. 296-297.

Jatak, VI. pp. 180-181.

Cited in A. Bose, Social and rural economy..... Vol.I, pp.63.

Vide P.E.V.

Buch, M.A (1945). Economic Life in Ancient India. Baroda, pp. 41-42.