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ABSORPTION OF PROTEIN IN THE GASTROINTESTINAL TRACT OF TELEOST FISH

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Abstract ■ Teleost fish larvae, considered to be the smallest vertebrates on earth, are extremely well adapted for nutrient utilization (absorption and assimilation). Intestinal transepithelial transport of proteins, peptides and amino acids are of greater quantitative significance in fish and fish growth. The intestine is involving specific transport system that carry sugars, amino acids, peptides, proteins, macromolecules, lipids and other substances into the body through circulation. Individual amino acids are readily absorbed against concentration gradient. Protein and peptides in the intestinal contents are taken upto some extent, without previous degradation by pinocytosis or related processes. It is also observed that a specialized region of gut is responsible for the massive absorption of protein and of protein fragments, most of which undergo intracellular lyses. Further, there is a declining proximal to distal gradient of absorption along the post gastric intestinal tract.

Keywords : amino acid, peptide, protein, absorption, GI tract, teleost fish.

INTRODUCTION

It is known that both fresh and salt water fish have high protein needs, provided that 'need' is defined as the percentage of dietary protein assuring best growth (Guillaume, 1987). Just as body lipids and proteins cover maintenance energy needs, dietary fatty acids and amino acids are used as energy sources in preferences to glucose in metabolic processes which are involved in the synthesis of proteins, required for growth. As growth is continuous in fish, so it is the equilibrium between crude proteins 30-60%; (Wilson, 1985; Weatherley and Gill, 1987; Patra 1993,1994,1995; Patra et al.,1999) and lipids (8-25%; Henderson and Tocher, 1987; Sargent et al., 1989; Patra, 1993) in the diet which plays the fundamental factor. Teleost can be divided into two broad groups on the basis of their simple anatomical criterion that is the presence or absence of a stomach. Among the species of interest in fish farming in India, members of the family Cyprinidae are examples of agastric fish while family Siluridae are representatives of the gastric teleost. Kapoor et al. (1975), however, mentioned that the presence or absence of a stomach may be encountered among species of the same family, for example the Bleniidae and Cobitidae.

The nutritional needs of fish and the way in which these needs are covered present highly original characters. Fish inhabit a milieu, which imposes special constraints, including the viscosity of the fluid in which they move, low oxygen availability in comparison to air and high thermal dependence. On the positive side, this medium frees them of problems related to posture regulation and to body weight and thus the mass - energy reserves.

In contrast to these differences, gastric histology is remarkably uniform, especially in terms of the epithelium, which contains three cell types: i) Oxyntic cells of the gastric glands, present at least in the anterior region and secreting both pepsin and HCl, ii) Mucosal cells lining the central lumen and iii) the Endocrine cells. The gastric retention times of ingesta affects subsequent intestinal steps, a priori in particular for the hydrolysis of proteins.

Stomach morphology presents considerable variations, related to the volume and the nature of ingesta (Smith, 1989). In general, the gastric capacity of fish continuously feeding on small particles is minimal, while that of fish feeding intermittently or ingesting large prey or food particles.

The 'herbivorous fish', which accounts for only about 5% of the total number of species (20,000), utilize varying strategies and the absence of a stomach can be compensated by the development of a more or less complex buccal or pharyngeal masticator apparatus. Alimentary yield in herbivores is generally lower than in omnivores and even lower than the carnivores, where they have a longer gut.

Cells of the intestinal mucosa of most teleost fish secrete a number of enzymes, like carboxy and amino peptidages, amylases, lipases, lecithinase, nuclease and others. However, the qualitative importance of intestinal brush border enzymes to digestion in fish is known very less. Regardless of the details, a basic character shared by all teleost fish is the presence of at least two intestinal segments. The first assures the absorption of lipids (Sheridan, 1988) and the second is responsible for the pinocytotic uptake of macromolecules (Gas and Noaillac-Depeyre, 1981). What is true for the adult is also true for all larval forms, starting at the first feeding (Alliot, 1981). Specific ultrastructural features are associated with these 'anterior' and 'posterior' segment, in particular in terms of absorbing cells (figure 1). Similar observations also represented by Vernier (1990).

GASTROLUMINAL HYDROLYSIS AND ABSORPTION OF AMINO ACID

In gastric teleost, proteolysis in the stomach depends on the volume of gastric juice (HCl and pepsinogen), on the specific activity of the pepsin(s) formed, on gastric retention time, on the nature and volume of the ingesta etc. (Brett and Higgs, 1970). The capacity of the Cyprinidae to digest proteins is comparable to that determined in the gastric species. The essential contribution of stomach to the process of assimilation of proteins and thus to the control of nutritive flow through the gut lies in its role as a reservoir (Ash, 1985). The study of the total proteolytic activity of the digestive tract in various species has shown that as an average it is higher in carnivorous than in the herbivorous species. If, however we take into account the greater digestive volume and the large number of daily filling in non-carnivorous species, it can be opined that the effective duration of proteolysis is higher than that in carnivorous one. Thus, Hofer (1982) showed that the daily production of luminal proteolytic enzymes in grass feeding roach, and omnivorous Cyprinid, was higher than that calculated for the same fish feeding on meal worms. In addition, there is a progressive decrease in proteolytic activity along the length of the gut. If it is admitted that proteolytic

enzymes are resistant to autolysis, then this decrease could correspond to the absorption of these enzymes by intestinal cells in the posterior region. In herbivorous fish, elongation of the posterior segment is commonly observed to take part in reabsorption. This would correspond to adaptive characters associated with active secretion of these enzymes and with their effective contact with the dietary proteins. This compensates for the lower protein content in the feed (Patra, 1995).

Smith (1970) showed that the averted intestinal sac of gold fish could carry out the active transport of 18 amino acids from the mucosal to the serous side, against their respective gradients. Since then, *in vitro* and *in vivo* experimentation with different amino acids has confirmed that the process is active and thus energy consuming, stereo specific and sodium dependent (Boge et al., 1979). Smith (1983) measured *in vitro* the serous transport of different amino acids in gold fish. Similar observations on the absorption of amino acids & protein reported by Buddington et al (2001), Futoshi et al. (2004), Kvale et al. (2007), Martine et al. (2003), Morais et al. (2005), Refstie (2006), Stroband and Veen (2005), Zhang et al. (2006), Zhou et al. (2007), Patra (2008), and Lovell (2009) in the intestinal serosal layer of different teleost species. The results obtained with all amino acids tested were similar at 8°C water temperature. The same type of study at 30° C temperature showed that the transport of short neutral amino acids (Thr, Ser, Pro, Gly, Ala, Val) and basic amino acids (Lys, Arg) were lower than that of long chain neutral amino acids (Met, Tyr, Phe, His), whose transport capacity was unchanged and which may correspond to the adaptation temperature. Even though the glucose and amino acid transport systems have comparable characteristics, there is apparently no mutual interference. Marcotte and de la Noue (1984) showed that supplementing with D-glucose in rainbow trout *in vitro* had no effect on cell accumulation or on serosal transfer of glycine and L-alanine.

The study of Bell et al. (1987) in the Atlantic salmon did not confirm the involvement of cycle and ã-glutanyl transferase in the transport of amino acids, in particular that of glutamine and cystine. However it is generally assumed that there is less amino acid absorption in the posterior region of gut. (Marcotte and de la Noue, 1984). Habibi and Ince (1984) showed that sex difference had no effect on the intestinal transport of leucine in rainbow trout.

There are relatively very few data on the absorption of dipeptides. Boge et al. (1981) showed that the dipeptide is apparently partially hydrolyzed by brush border membrane enzymes to a greater extent than by cytoplasmic enzymes. Dabrowski et al. (2007) reported that the concentration of amino acids released by hydrolysis in the intestinal lumen was several times higher than that reported for other vertebrates and that their absorption was thus essentially a passive phenomenon.

Regardless of the differences discussed above, all teleost species studied so far require the same 10 essential amino acids Viz. arginine, histidine, leucine, isoleucine, threonine, lysine, methionine, phenylalanine, tryptophan and valine. Patra (2004, 2007 & 2008) observed that both cyprinidae and siluridae require to absorb 26 (20 natural and 6 unnatural) amino acids through internal serosal layer (Table 1). It is recommended to refer to the review of Wilson (1989) for quantitative needs and others.

Table 1. Different classes of amino acids absorbed through intestinal serosal layer.

Classes of amino acid	Name of the amino acid
Non-polar R groups 8	Alanine (Ala)
	Valine (Val)
	Leucine (Leu)
	Isoleucine (Ile)
	Proline (Pro)
	Phenylalanine (Phe)
	Tryptophan (Trp)
	Methionin (Met)
Polar R groups 9	Glycine (Gly)
	Serine(Ser)
	Threonine (Thr)
	Cysteine (Cys)
	Tyrosine (Tyr)
	Asparagine (Asn)
	Glutamine (Gln)
	Aspertis acid (Asp)
	Glutamic acid (Glu)
Basic amino acid 3	Lysine (Lys)
	Arginine (Arg)
	Histidine (His)
Other amino acids 6	2 amine n-butaric acid
	DL DOPA
	Cystine
	Nor Leucine
	Hydroxy proline
	Ornithine HCl

INTRACELLULAR DIGESTION AND ABSORPTION OF PROTEINS

Tanaka (1972) showed that intestinal cells of the posterior segment of 22 teleost species were characterized microscopically by the presence of acidophilic granules in their supranuclear region. This observation was valid for fish collected from the wild or from farms inhabiting with salt and freshwater and with or without a stomach. This acidophilic granules absorbed by fish at the onset of their exotrophic feeding phase. Gauthier and Landis (1972) followed by Noaillac –Depeyre and Gas (1973) showed that the vacuoles containing the acidophilic granules were capped by a vast tubulovesicular endocytosis complex. In 1975 (Krementz and Chapman), this complex (complex of endocytosic vacuoles) was also described in young adult channel cat fish which is a gastric species.

A large number of structural studies subsequently confirmed the consistency of these elements in the posterior gut (lower gut, hind gut, ileum): in larvae of *Chaenogobius annularis* (Govoni et al., 1986), in larvae and adult of various species of mugilidae (Albertine-Berhaut, 1987), in juvenile sea-brum (Cataldi et al., 1987), in young adult dover sole (Mac Donald, 1987), in adult rainbow trout (Ezeasor and Stokoe, 1981) and in adult black mollie (Caceci and Hrubec, 1990).

Endocytosis of proteins by the cells of the posterior segment of intestine in various species was first demonstrated with native or cationized ferritin (MW: 4,80,000 da) and HRP (MW: 40,000 da). Ferritn can be visualized directly ultrasturcturally, while HRP is detected with light and electron microscopy by its catalytic properties (Figure 2 a, b & figure 3)

The supranuclear cytoplsmic structures of intestinal cells involved in endocytosis has been established in many cases : **i)** adult agastric Cyprinadae, eg. Gold fish (Iida et al., 1986), carp (Noaillac-Depeyre and Gas, 1973); tench (Noaillac-Depeyre and Gas, 1976), barbell (Rombout, 1977) and the herbivorous carp (Stroband and Van der Veen, 1981), but also a number of gastric species, e.g. perch (Noaillac-Depeyre and Gas, 1979), *Clarias* (Stroband and Kroon, 1981), cat fish (Noaillac-Depeyre and Gas, 1983), cod (Lied and Solbakken, 1984) and rainbow trout (Fujino et al., 1987); **ii)** the juvenile

of various salmonid species (Escaffre et al., 1989) the larvae of various salt water and freshwater species (Escaffre et al., 1989).

Thus, at all stages of teleost development, from the first feeding by the larvae or juvenile upto the adult, ingested protein regardless of their molecular weight are segregated in the supranuclear vacuolar system and are often transmitted by the tubulovesicular system of the apical cytoplasm.

Lysosomal activity in the vacuolar system. Gautheir and Landis (1972) with the help of light microscopy were the first to report a lead phosphate deposit in gold fish. The reaction product of acid phosphatase was localized at sites coinciding with regions containing 'supranuclear bodies', in other words, vacuoles. The same phenomenon has been supported by Georgopoulou et al., (1985) in rainbow trout and Iida et al. (1986) in gold fsh. Lysosomal protease, cathepsin D and cathepsin B, have the same localization (Georgopoulou et al., 1986).

Intracellular digestion of absorbed protein. Immunological methods can be used to elegantly follow the fate of a particular ingested protein, e.g. human IgG (IgGh, MW: 150,000 da) or the hepatitis B virus surface antigen (HBs Ag, MW: 2 X 10^6 da), its penetration into cells and its degradation. These experiments were done in rainbow trout (Georgopoulou et al., 1986). Using anti-F and anti-Fc antibodies, and a monoclonal antibody, specific to the native conformation of HBs. Ag fluorescence was employed to visualize the penetration of these proteins (IgGh and HBsAg) into epithelial cells of the posterior gut. In conclusion the posterior gut of teleost fish assures the absorption and intracellular digestion of ingested proteins (Figure 4)

TRANS EPITHELIAL PROTEIN TRANSPORT

Pathways followed during transfer. A para cellular pathway involving the direct passage of molecules between epithelial cells can not be ruled out, but in the light of the presence of the junction complex of the apical terminal bar ensuring the linkage of cells with its neighbours, this situation would correspond to particular situations, Noaillac-Depeyre and Gas, (1973) administrated HRP to carp and subsequently detected the enzyme in the intracellular spaces and above the sub epithelial basal membrane in lymphocytes having infiltered the epithelium and in the capillaries of the lamina propria. Tracer proteins have been used to show that proteins or peptides absorbed by epithelial cells of the posterior segment can escape degradation by the vacuolar system and pass in to cells, using pathway called transcellular. Considerable controversy exist concerning the common or respective trajectories of soluble proteins absorbed in the fluid phase (HRP, native ferritin) and proteins which can be absorbed on the apical plasma membrane. Regardless of the actual situation, this transcellular pathway has been histochemically demonstrated in carp (Rombout et al., 1985) and rainbow trout (Georgopoulou et al., 1988), as well as in the tophotaeniae of Goodeidae (Schindler and de Vries, 1987b). Immunological methods have been used to follow the intracellular trajectory of transepithelial transport of the bovine growth hormone (bGH) added to the lumen of the posterior gut (Le Bail et al., 1989).

'Intestinal' immune response to transfer. The pronephros and spleen of fish are the major sites of hematopoises. In the absence of groups of lymphoid cells, comparable to Peyer's patches, and of M cells, a large number of leucocytes and macrophages are observed in the connective tissue of the gut, forming the gut associated lymphoid tissue (GALT). Temkin and McMillan (1986) described the different cells constituents of the GALT in gold fish as did Rombout et al. (1989a) in carp. The

lymphocyte population heterogenous, including primary lymphoblast plasmocytes and macrophages, as well as several types of granulocytes. The stratum compactum and stratum granulosum are absent in young fish which subsequently in the course of their development (Eseasor and Mikrosk 1986), advanced the idea according to which the stratum compactum and granular cells constitute a system of composite defense, mechanical and humoral and which develops in response to the pressures from the environment. These granular cells have been observed in gills, skin and nostril of salmonids, i.e. in those organs that are in direct contact with the environment (Roberts et al., 1973). In conclusion the GALT of fish resembles a diffuse lymphoid tissue and could be involved in an intestinal immune response.

Response to the transfer of antigenic proteins. The transfer of antigen through the intestinal epithelial cells of Cyprinidae, agastric teleost, has been shown from the lumen of the posterior gut to the intra epithelial leukocytes or macrophages. This suggests the immunological involvement such as the induction of a local response (Rombout and Van den Berg, 1989; Rombout et al., 1989). Davina et al. (1982) administered Vibrio bactrin orally to *Barbus conchonius* and observed an increase in the number of intraepithelial leukocytes 30 minutes later, primarily in the second intestinal segment. Rombout and Van den Berg (1989) reported a very high increase in the number of intraepithelial macrophages 24h after the anal incubation of ferritin or saline solution. Sire et al. (1988) and Le Bail et al. (1989) found antigens (bGH and protein A) to be 'captured' by the granular cells, which behave as immune cells. The results lead to the conclusion that teleost fish like mammals possess local immune system.

TRANSFER TO THE CIRCULATORY SYSTEM

Systemic immune response and vaccination. Intact antigenic proteins and / or macromolecules having escaped intracellular degradation are either phagocytosed by intraepithelial lymphoid cells or those by the laminal propria (Rombout and Van den Berg, 1989; Le Bail et al., 1989) or enter the circulatory system (Ash, 1985; Le Bail et al., 1989). Fujino and Nagai (1988) administered BSA to *Onchorhynchus keta* and reported the appearance of the protein in the serum of the salmon several hours after incubation. After reaching the circulatory system, proteins such as HRP are taken up by a variety of organs, such as pronephros, spleen and liver (McLean et al., 1989). A 'systemic' immune response after the oral or anal administration of an antigen is thus possible (Mor and Avtalion, 1990). It can be concluded that the oral vaccination of teleost in fish farms can be envisages, replacing all other treatment methods, vaccinating fish against bacterial infection by intra peritoneal injection or by immersion has resulted in considerable commercial success but it is difficult to employ it in fish farming practice.

TRANSFER OF MOLECULES WITH BIOLOGICAL INTEREST

Some experimentation has addressed zootechnical problems, concerning the transfer of molecules with biological interest e.g. peptide hormones. The oral administration of salmon pituitary extract to male gold fish (Suzuki et al., 1988a) has resulted in high plasma levels of salmon gonadotropin (sGtH), which were maximal 6-12 h after ingestion. Increased plasma level of testosterone and of 17á-20â dihydroxy-4-pregnan-3-one as well as increased production of milt, suggested that sGtH retained its biological activity. Suzuki et at. (1988b) again administered salmon pituitary extract to the

female gold fish and noted the appearance of GtH in the plasma as well as its induction of ovulation in treated fish. Similar observation was recorded with spotted sea trout (*Cynoscion nebulosus*) and Solar et al. (1990) with sable fish (*Anoplopoma fimbria*) indicate that the oral delivery of leutinizing hormone super active analog (LHRHa) can induce spawning and ovulation in teleost.

Bovine growth hormone (bGH) 20,000 da MW peptide was introduced into the digestive tract of juvenile rainbow trout and was partially transferred to the general circulations (Sire et al., 1988; Le Bail et al., 1989). Results obtained by radioimmunoassay (RIA) and radio receptor assay showed that the protein was well preserved during its transfer, since antigenic epitops and binding sites to specific receptors remained functional. These results were confirmed by Moriyama et al. (1990). Hertz et al. (1991) who reported that recombinant human growth hormone administered orally to carp underwent only a very slight loss of bioactivity, as shown by plasma RIA.

In terms of fish farming applications, the desired goals of vaccination and of the use of peptides with biological interest is contradictory. In case of vaccination, the antigenic molecules passing through the epithelium must stimulate immune defenses. The first line response, the GALT will be difficult to analyze, since, IgM formed are transferred to the mucous could subsequently interfere with antigens during the antigen absorption process.

In the case of peptides with biological interest, on the other hand, such as various pituitary and hypothalamic hormones whose action on target cells are desired, their antigenicity should be as low as possible. Thus, the comparison of results obtained in rainbow trout for GH indicate that plasma transfer is much higher with rsGH (Moriyama et al., 1990) than with bGH (Le Bail et al., 1989). In conclusion the use of a recombinant hormones from the species treated should avoid interference with the components of the GALT and with the granulocytes of the lamina propria, which forms the stratum granulosum in salmonidae. Results obtained with a heterologus hormone will be better if it is used in juveniles before this line of defense is installed.

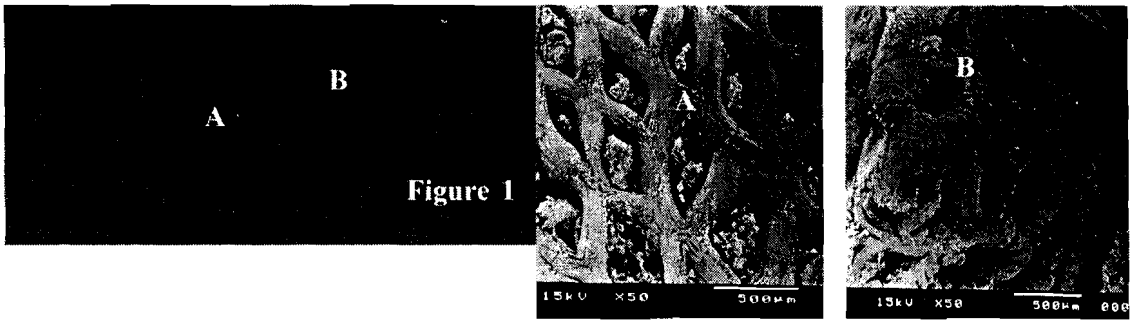

Figure 1. Absorption site of protein in the digestive tract of Indian major carp ('__' boundary of protein absorbing epithelium), A – Scanning Electron Micrograph of intestinal bulb of *Labeo rohita,*; B - Scanning Electron Micrograph of intestinal absorbing epithelium of *Labeo rohita.* [Source : Patra, 2008]

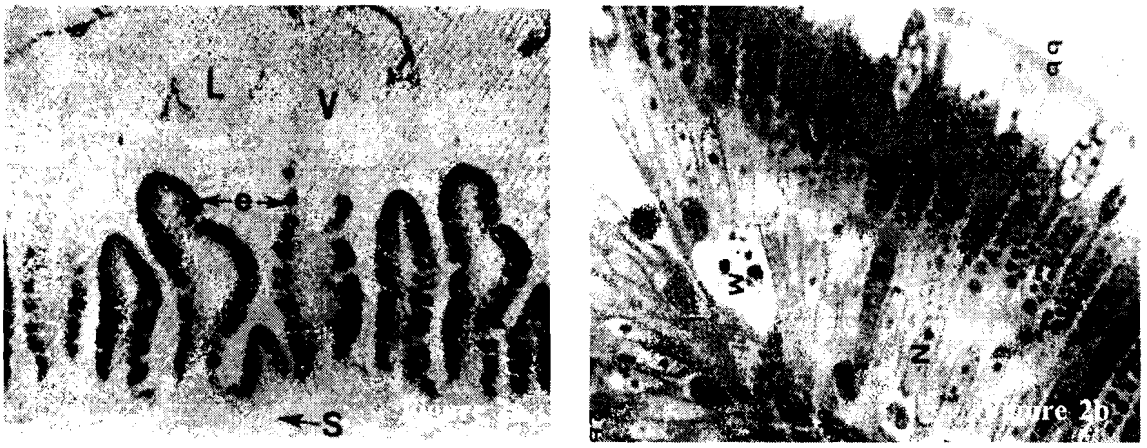

Figure 2. Absorption site of protein macromolecules in the posterior intestine (a) 6 h after anal administration of horse spleen ferritin (magn. X 150); (b) semithin section of a fold of rainbow trout posterior intestine, 24 h after anal administration of native ferritin (magn. X 900). Absorption site of macromolecules in the posterior intestine of trout fry individuals (e - epithelium; bb – brush border; L - intestinal lumen; V– spiral valves; S - serosa). [Source: Escaffre et al., 1989; Sire and Vernier, 1992].

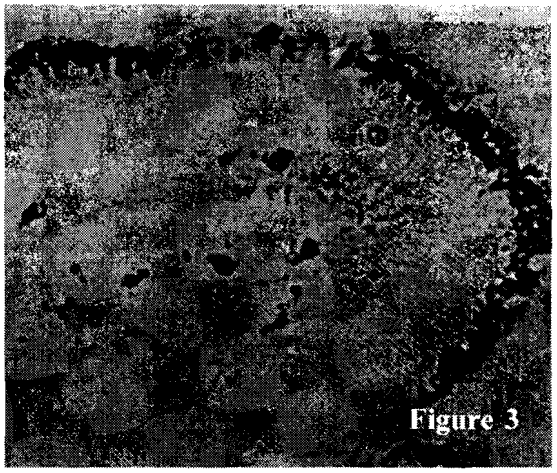

Figure 3. Absorption site of protein macromolecules in the posterior intestine. 1 h after anal administration of horse radish peroxidase (magn. X 810). Absorption site of macromolecules in the posterior intestine of trout fry individuals (e - epithelium; L - intestinal lumen; V - spiral valves; S -serosa). [Source: Escaffre et al., 1989].

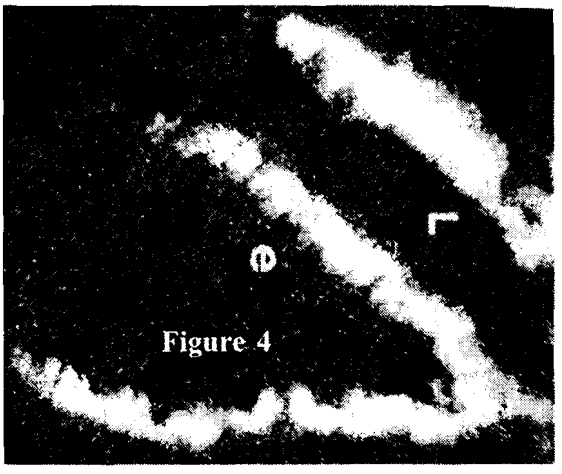

Figure 4. Absorption site of macromolecules in the posterior intestine. 8 h after anal administration of rabbit immunoserum anti-human Ig (magn. X 810). Absorption site of macromolecules in the posterior intestine (e - epithelium; L – intestinal lumen; V - spiral valves; S - serosa). [Source : Escaffre et al., 1989].

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EFFECT OF PLANT EXTRACTS ON ALLEVIATION OF DETERIORATION OF SEED GERMINATION AND ENHANCEMENT OF PLANT POTENTIAL OF A GRAM SPECIES

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Abstract ■ Gram (*Cicer arietinum* L.) seeds lost viability at a rapid pace under accelerated ageing condition. Pretreatment of the seeds with leaf extracts of bel (*Aegle marmelos*) and kalmegh (*Andrographis paniculata*) 50g in 500ml distilled water of each for 8 hours before accelerated ageing treatment (100% RH and 30±2°C) for different durations for 45 days under the accelerated ageing condition slowed down the ageing-induced rapid loss of seed germination. Plant performance was found to be much better in comparison of seeds, underwent plant extract pretreatments and this was measured in terms of field emergence capacity, root length, shoot length, fresh weight and dry weight of the plants. Again, plant potential was also higher in the pretreatments as evidenced from the treatment-induced higher chlorophyll and protein levels as well as catalase and peroxidase activity. Results, therefore, revealed that in spite of having accelerated ageing treatment, pretreated seeds with the plant extnoets retained higher seed vigour and produced healthier plants.

Key words : Gram, accelerated ageing, bel, kalmegh, seed germination, plant potentiation.

INTRODUCTION

Seed storage is a serious problem in tropical and subtropical countries like India where high temperature and high relative humidity greatly accelerate seed ageing phenomenon causing consequent deterioration and non-viability of seeds. The problem of retention of seed vigour in India is much more acute because of extremely high relative humidity prevailing during the major part of a year and which is very conducive to the growth of microorganisms, particularly fungi. since most crop seeds require storage for either one or several planting seasons, agriculturists and horticulturists of this region are often handicapped with respect to maintenance of standard seed vigour under ambient storage environment. Keeping this problem of seed germination, storage and production of healthier plants in mind, an attempt was made in this investigation for the retention of the seed viability and enhancement of plant potential of a gram seed species having viability problems. Present experiment was performed under accelerated ageing condition by imposing high relative humidity with a view to maintain the uniform

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adverse storage condition and also to obtain expeditious results. In fact, accelerated ageing treatment provides a powerful manipulative tool which makes it possible to study the process of seed deterioration over a very short period and this mimics the natural ageing process (Heydecker, 1972).

Although efficacy of several classes of chemicals *viz.* hormones, retardants, redox chemicals, phenols, vitamins and salts on maintenance of seed health under storage has been established (Bhattacharyya and Basu 1990, Chhetri *et al.*,993, Basu 1994, Rai 2000), this field of seed physiology still remains relatively less explored. Thus, the major objective of this work was to test the efficacy of a leaf extracts of bel (*Aegel marmelos*) and kalmegh (*Andrographis paniculata*) on the alleviation of deterioration and viability retention and enhancement of plant potential of a gram seed species.

MATERIALS AND METHODS

The seeds of gram (*Cicer arietinum* L.) after surface sterilization (0.1% $HgCl_2$ for 90 seconds) were separately presoaked in aqueous solutions of leaf extracts of bel (*Aegel marmelos*) and kalmegh (*Andrographis paniculata*) 50g in 500 ml distilled water of each for 8 hours and then dried back to the original dry weight of the seeds. The pretreated seed lots (250 g each) were taken in separate porous cloth bags and thus stored in a desiccator in which 100% relative humidity (RH) was preimposed by keeping distilled water within it. This experimental set-up was kept at 30±2°C for 45 days allowing the seeds to experience forced ageing treatment and distilled water was changed at 15-day intervals to restore the desired RH within the desiccators for 45 days. From the seed lots germinability and field emergence capacity of seeds were made after 0, 15, 30 and 45 days of accelerated ageing treatment.

To analyse the percentage germination, four groups of 100 seeds (total 400 seeds) were transferred to separate Petri dishes containing filter paper moistened with distilled water. Germination data were recorded after 120 h of seed soaking following the International Rules for Seed Testing, ISTA 1976 and field emergence capacity was recorded after 15 days of seed sowing. Some growth and biochemical parameters were recorded from the leaves of the 30 and 60 days old plants raised from the 0 and 45 days of accelerated ageing seeds.

Extraction and estimation of chlorophyll and protein from leaves were done by the method of Arnon, 1949 and Lowry *et al.*, 1951 respectively. Activity of catalase was analysed following the method of Snell and Snell, 1971 as modified by Biswas and Choudhuri, 1978 and that of peroxidase was analysed as per the method of Kar and Mishra, 1976.The assaying of the enzymes were done as per the methods of Fick and Qualset, 1975.

Statistical analysis of the data was done in terms of least significant difference (LSD) which was calculated at 95% confidence limits and as per the method of Panse and Sukhatme 1967.

RESULTS AND DISCUSSION

Results showed that pretreatment of gram seeds with bel and kalmegh leaf extracts significantly alleviated the ageing-induced loss of germination and enhanced field emergence capacity under accelerated ageing environment (Table-1). Reduced seed germinability and field emergence capacity are considered to be the important visible criteria for the evaluation of poor seed vigour (Anderson 1970,Halder *et al.*,1983, Rai,2000). In this investigation, the

Table 1. Effect of seed pretreatment with leaf extracts of bel and kalmegh (50g in 500 ml of water of each) on germination and field emergence capacity of gram seeds stored under accelerated ageing condition for 45 days.

Seeds were presoaked with leaf extracts of bel and kalmegh or distilled water for 8h and then sun dried. The seed samples were then separately allowed to experience accelerated ageing treatment (100% RH) in a desiccator. Data were recorded after 0, 15, 30 and 45 days of accelerated ageing seeds.

Treatments	Percentage germination				Field emergence capacity (%)			
	Accelerated ageing (days)							
	0	15	30	45	0	15	30	45
Control	100	88.0	77.5	37.00	92.9	76.5	63.4	31.0
Bel	100	94.7	87.3	51.20	94.2	83.1	75.0	47.2
Kalmegh	100	92.4	84.9	50.12	93.2	81.2	74.1	43.4
LSD (P=0.05)	NC	4.15	5.81	2.23	NS	5.70	4.50	2.12

NC: Not calculated, NS: Not significant

plant extracts-induced arrestation of loss of seed germination and field emergence capacity are indicative of retention of seed viability property of the experimental plant extracts. Accelerated ageing treatment impaired field performance of experimental plants as evident from the reduction of root length and shoot length (Table 2), fresh weight and dry weight (Table 3) levels of chlorophyll and protein (Table 4) as well as activities of catalase and

Table 2. . Effect of seed pretreatment with leaf extracts of bel and kalmegh (50g in 500 ml of water of each) followed by accelerated ageing treatment for 45 days on changes of root length and shoot length of gram plants.

Data were recorded from 30 and 60 days old uniformly grown plants raised from the 0 and 45 days of accelerated ageing seeds.

Treatments	Root length (cm)				Shoot length (cm)			
	Plant age (days)							
	30		60		30		60	
	Accelerated ageing (days)							
	0	45	0	45	0	45	0	45
Control	5.19	1.23	15.18	1.29	37.91	12.90	77.86	18.33
Bel	7.96	2.92	18.98	3.10	59.32	14.60	98.29	21.55
Kalmegh	7.50	2.09	18.39	2.99	57.63	15.70	97.67	20.51
LSD (P=0.05)	2.21	0.63	1.81	1.11	1.70	1.12	5.21	1.85

Table 3. . Effect of seed pretreatment with leaf extracts of bel and kalmegh (50g in 500 ml of water of each) followed by accelerated ageing treatment for 45 days on changes of fresh weight and dry weight of gram plants.

Data were recorded from 30 and 60 days old uniformly grown plants raised from the 0 and 45 days of accelerated ageing seeds

Treatments	Fresh weight (g)				Dry weight (g)			
	Plant age (days)							
	30		60		30		60	
	Accelerated ageing (days)							
	0	45	0	45	0	45	0	45
Control	26.0	09.5	298.9	10.0	5.7	0.98	92.4	1.9
Bel	31.7	10.8	365.7	19.3	7.6	1.9	118.3	2.5
Kalmegh	30.1	10.3	354.6	16.1	6.8	1.3	112.1	2.1
LSD (P=0.05)	2.55	0.72	26.85	2.08	1.05	0.60	7.26	0.70

Table 4. Effect of seed pretreatment with leaf extracts of bel and kalmegh (50g in 500 ml of water of each) followed by accelerated ageing treatment for 45 days on changes of chlorophyll and protein contents in leaves of gram plants.

Data were recorded from 30 and 60 days old uniformly grown plants raised from the 0 and 45 days of accelerated ageing seeds.

Treatments	Chlorophyll (mg/g fr. wt.)				Protein (mg/g fr. wt.)			
	Plant age (days)							
	30		60		30		60	
	Accelerated ageing (days)							
	0	45	0	45	0	45	0	45
Control	2.30	1.06	3.89	0.88	17.49	13.25	28.25	15.32
Bel	3.43	1.28	5.27	1.23	21.37	19.89	47.60	21.33
Kalmegh	3.36	1.17	5.11	1.16	20.22	17.51	45.52	20.63
LSD (P=0.05)	0.68	0.15	0.13	0.17	1.60	1.13	1.83	1.64

peroxidase enzymes (Table 5). The plant extracts-induced alleviation of the deleterious effects of ageing on the overall growth and metabolism of experimental plant thus indicates the retention of potential status of the experimental plants by leaf extracts used in this experiment.

Chlorophyll, protein, catalase and peroxidase are regarded as reliable indices of vigour status of plants. In this investigation, comparatively better plant health and higher metabolic status of plants, raised from the plant extracts-treated seeds, are indicative of invigouration of seeds under storage. And the invigourated seeds

Table 5. Effect of seed pretreatment with leaf extracts of bel and kalmegh (50g in 500 ml of water of each) followed by accelerated ageing treatment for 45 days on changes of catalase and peroxidase activities in leaves of gram plants.

Data were recorded from 30 and 60 days old uniformly grown plants raised from the 0 and 45 days of accelerated ageing seeds.

Treatments	Catalase (unit/h/g fr. wt.)				Peroxidase (unit/h/g fr. wt.)			
	Plant age (days)							
	30		60		30		60	
	Accelerated ageing (days)							
	0	45	0	45	0	45	0	45
Control	32.4	11.9	31.6	11.7	53.8	19.7	50.9	17.4
Bel	48.3	12.7	45.2	12.2	68.2	34.6	67.7	20.4
Kalmegh	43.4	12.3	42.0	12.0	66.1	28.5	64.6	19.7
LSD (P=0.05)	2.05	1.10	1.40	1.33	1.08	1.07	3.05	1.09

subsequently exhibited better field performance which was recorded in terms of plant growth and metabolism. Superior performance of plants raised from high vigour seeds is available in the literature (Rai, 2000). In this investigation, herbal-induced retention of seed viability, plant growth and metabolism clearly indicate the hardening or invigouration property of the pretreating agents. And such hardening effect on seed was reflected in plant growth and metabolism. In fact, the magnitude of the loss of the chlorophyll and protein (Table 4) as well as catalase and peroxidase (Table 5) activities were found to be significantly less in plants developed from seeds which underwent pretreatment with the leaf extracts of the bel and kalmegh plants.

Loss of some vital cellular components occurred during the process of seed deterioration are available in literature (Abdul-Baki and Anderson, 1972; Kole and Gupta 1982). Catalase (Abdul-Baki and Anderson 1972, Yadav *et al.* 2003) and peroxidase (Bhattacharjee and Choudhuri, 1986, Yadav *et al.*,2003) activities are generally used as very reliable indices for the evaluation of seed viability. High level of catalase activity in high vigour seeds have also been reported (Bhattacharjee *et al.*, 1999, Pati, 2007). So, from the present observations of higher metabolic status of the leaf extracts of bel (*Aegel marmelos*) and kalmegh (*Andrographis paniculata*) pretreated gram seeds, it seems quite apparent that the seed pretreating agents considerably hardened the seeds and such hardening is effected at the metabolic level which subsequently resulted in retention of seed vigour and consequent extension of seed viability with concomitant enhancement of plant potential.

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OBESITY STATUS OF COLLEGE STUDENTS IN TRIPURA (INDIA)

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Abstract ■ Across-sectional analysis of 83 healthy students aged ≤ 21 years was examined to assess overweight and obesity. For the study 83 subjects (44 female and 39 male) were randomly chosen from a collage in Tripura.. The body mass index (BMI), waist circumference (WC) and waist-hip ratio (WHR) were measured to assess overweight and obesity. The prevalence of obesity amongst the male and female students based on BMI alone were found to be nil but percentage of overweight was 12.8% in male and 18.2% in female students according to cut-off point decided by WHO for obesity and overweight. The waist circumference and WHR showed good conformity with WHO indices for overweight and obesity. The results based on waist circumference indicated that percentage of overweight and obese female students were 18.2% and 11.4% respectively but the percentage of overweight and obesity in male students were nil. On the other hand the percentage of overweight and obese was 18.2% and 52.2% respectively in female students and 23.1% and 2.6% respectively in male, when measured in terms of WHR. On comparison of the data obtained by measuring the degree of adiposity (obesity and overweight), based on the three parameters (BMI, WHR and WC) it was found that the correlation between BMI and WHR was significant. There was also a positive correlation between BMI and WC. The WHR and WC showed the maximum degree of correlation. It was concluded that The BMI should not be taken as the sole parameter for evaluating obesity.

Key words : BMI, Waist circumference, Waist-hip ratio, Obesity, Overweight

INTRODUCTION:

Despite the growing concern about adiposity-related problems among young, no universally accepted classification system for adolescent obesity exists. Although simple anthropometric measurements have been used as surrogate measurements of obesity and have more practical value in both clinical practice and for large-scale epidemiological studies. Body mass Index (BMI), which relates weight to height, is the widely used and simple measure of body size, and is frequently used to estimate the prevalence of obesity within a population (WHO, 1988; Colditz et al, 1995) and has also been recommended for screening overweight and obesity. Measurements of waist circumference and waist-hip ratio (WHR) have been reviewed as alternatives to BMI. Waist circumference has been shown to be the best simple measure of both intra-abdominal fat mass and total fat (Han, 1997; Lemieux ,1996). Obese individuals differ not only in the amount

of excess fat that they store, but also in the regional distribution of the fat within the body. The distribution of fat induced by the weight gain affects the risk associated with obesity, and the kind of disease that results. Obesity is perhaps the most prevalent outcome of malnutrition. It is a health hazard and a detriment to well being which is reflected in the increased morbidity and mortality. As a chronic disease, prevalent in both developed and developing countries, and affecting children as well as adults, it is now so common that it is replacing the more traditional public health concerns including undernutrition. It is one of the most significant contributors to ill health. The first adverse effects of obesity to emerge in population in transition are hypertension; hyperlipidoemia and glucose intolerance, while coronary heart disease and the long-term complications of diabetes, such as renal failure begin to emerge several years later (WHO, 2002).

The etiology of obesity is complex and the probable causes are genetic (Falkner, 1980). Recent studies have shown that the amount of abdominal fat was influenced by a genetic component accounting for 50-60 percent of the individual differences (WHO, 2000). Sedentary lifestyle particularly sedentary occupation and inactive recreation such as watching television promote it. Physical activity and physical fitness are important modifiers of mortality and morbidity related to overweight and obesity (WHO, 2003). There is a clear inverse relationship between socioeconomic status and obesity as well as between educational level and prevalence of overweight (WHO, 1995).

In the present study a comparison of degree of obesity has been done among the college going students of Tripura, a northeastern state of India on the basis of BMI, waist circumference and WHR measurements. Here BMI, waist circumference and WHR are compared as indices of obesity to ascertain their degree of correlation

METHODS AND MATERIALS:

Subjects:

For the study 83 students (male: 39 and female: 44) were randomly chosen from a college in Tripura state. The age range of the subjects was 19 years 21 years. Most of the selected subjects belonged to middle and lower middle income groups. All subjects volunteered for the study.

Anthropometric measurements:

Height: It was measured without shoes using an anthropometer. Each subject stood with heels, buttocks and shoulders resting lightly against the backing board so that the Frankfort plane (a line connecting the superior border of the external auditory meatus with the infra orbital rim) was horizontal (i.e. parallel to the floor).

Weight: was measured after removal of shoes and when wearing light clothing only, using a portable human weighing machine with an accuracy of 0.5 kg.

BMI: Body mass index was calculated as weight (Kg)/height (m) ^{2}.

The obesity status was determined following BMI guidelines suggested by World Health Organization (WHO, 1995). Those with a BMI of 25.0-29.9 Kg/ m^2 were classified as overweight, whilst those with a BMI $\geq$ 30.0 Kg/ m^2 were classified as obese.

Waist circumference: It was measured using a steel measuring tape, with measurements made halfway between the lower border of the ribs, and the iliac crest in a horizontal plane. Men with a waist circumference 94-101.9 cm and women with a waist circumference 80-87.9 cm were classified as obese (WHO, 1998).

Hip circumference: It was measured at the widest point over the buttocks using a steel measuring tape.

WHR: Waist–hip ratio was obtained by dividing

the waist circumference by the hip-circumference. Men with a WHR 0.90-0.99 and women with a WHR 0.80-0.84 were classified as overweight, whilst men with a WHR $\geq$ 1.00 and women with a WHR $\geq$ 0.85 were classified as obese (WHO, 1998; Deurenberg-Yap et al, 2000).

Analysis of data

Data are expressed as mean ± SD. Pearson correlation coefficients were used to examine the relations between BMI, waist circumference and waist-hip ratio in detecting both overweight and obesity as well as the degree of correlation among the above three parameters in accessing obesity. Scattered diagrams with trend lines are used to graphically represent the degree of correlation.

RESULTS:

The mean values of different anthropometric measures have been present in Table 1. The female students were significantly lighter ($p<0.001$) and shorter ($p<0.01$) than that of their male counterpart. The mean values of BMI and waist circumference were higher in female students than that of male students; the difference was not statistically significant. The waist circumference was also non-significantly higher in female students than that of male students.

The obesity of the subjects was determined by three criteria, e.g., BMI, waist circumference and WHR, using respective cut-off values. The prevalence of obesity and overweight has been given in Table-2.

The results revealed that 15.7% of the population in the present study was considered to be overweight but none was found to be obese while using BMI as the parameter. While using waist circumference 16.7% of the subjects were considered to be above normal, with approximately 6.0% of them fell into obesity category. It was noteworthy that no male student was either overweight or obese. When classification was based on WHR, 49.4%

Table 1: Anthropometric measures (Mean ± SD) of male and female college students in Tripura

Parameters	Male (n=39)	Female (n=44)
Age(y)	20.6 ± 0.6	20.1± 0.4
Weight (Kg)	56.6 ± 9.7	49.5± 9.5**
Height (m)	165.0 ± 5.2	150.4± 5.7***
BMI (Kg/m^2)	20.7 ± 3.2	21.8± 3.5
Waist circumference (in cm)	69.7 ± 8.5	73.1±10.9
Waist–hip ratio (WHR)	0.85 ± 0.1	0.85±0.1

**P<0.01 ***p<0.001 w. r. t. male

Table 2: Prevalence (%) of obesity and overweight by BMI, waist circumference and WHR among male and female students in Tripura

Category Obesity	Body mass index			Waist Circumference			Waist-hip ratio		
	M	F	TOTAL	M	F	TOTAL	M	F	TOTAL
Normal	87.2	81.8	84.3	100	70.5	84.3	74.4	29.5	50.6
Overweight	12.8	18.2	15.7	0	18.2	10.7	23.2	18.2	20.5
Obese	0	0	0	0	11.4	6.0	2.6	52.3	28.9
Total	100	100	100	100	100	100	100	100	100

of the subjects were above normal and 28.9 % of them fell in to obesity category. Differences between genders in the prevalence of obesity were observed for the three different measures. Amongst male subjects, obesity as defined by WHR accounted for only 2.6% of the total, compared with none for waist circumference and none for BMI (Table-2). In female subjects, however, the obese group defined by waist circumference was 11.4% and none by BMI; the prevalence of obesity defined by WHR was considerably higher (52.3%). Similarly, differences between genders in the prevalence of overweight were observed for the three different indices. Amongst male and female subjects, overweight as defined by WHR accounted for only 23.1% and 18.2% respectively, compared with zero and 18.18% in male and female students by waist circumference. When male and female students were compared by BMI it was found that the greater percentage of female students were overweight than that of their male counterpart. The correlation among three parameters for obesity has been presented in Table 3. BMI and WHR showed significant positive correlation in both male ($p<0.05$) and female ($p<0.05$) students. A significant positive correlation was also observed between BMI and waist circumference ($P< 0.001$ in males and $P<0.001$ in females). Again, waist circumference and waist-hip ratio showed significant positive correlation ($P<0.001$ in males and $r=0.863$, $P<0.001$ in females). Strong association was noted between waist circumference and waist–hip ratio as well as between BMI and waist circumference (Fig. 1).

Table-3: Correlation between BMI with WHR and waist circumference (WC) among male and female students in Tripura

	WHR				WC			
	Male		Female		Male		Female	
	r	p	r	p	r	p	r	p
BMI	0.403	0.011	0.298	0.050	0.753	0.000	0.662	0.000
WC	0.724	0.0	0.863	0.0	-	-	-	-

DISCUSSION:

The prevalence of obesity among the students might be due to their sedentary life style. The greater occurrence overweight and obesity in female students than that of male students might be related to the hormonal status as well as lesser physical activity. The higher amount of fat in the female might be due to their higher absolute level of subcutaneous fat as well as their lower absolute level of lean body mass. The difference in the occurrence of obesity in terms of WHR between male and female students might be due to the variations in distribution of fat in the body (Shaver, 1982).

The results indicated that the percentage of occurrence of overweight and obesity showed a marked difference between the cutoff value for BMI and WC or WHR. It appeared from the findings that the cutoff value for BMI for the student population of Tripura might be modified. Other investigators also had similar opinion regarding the modification of cutoff value of BMI for determining obesity. Janssen (2004) concluded that WC, and not BMI, explains obesity-related health risk. Thus, for a given WC value, overweight and obese persons and normal-weight persons have comparable health risks. A WHO working group was formed by the

WHO Expert Consultation (2004) and had undertaken a further review and assessment of available data on the relation between waist circumference and morbidity and the interaction between BMI, waist circumference, and health risk. Sing et al (2008) suggested that large scale studies be conducted to define BMI cut-off points for overweight and obese specific to various subsets of the Asian Indian population group. A BMI cut-off of $\geq$ 25 kg/m^2 for over-

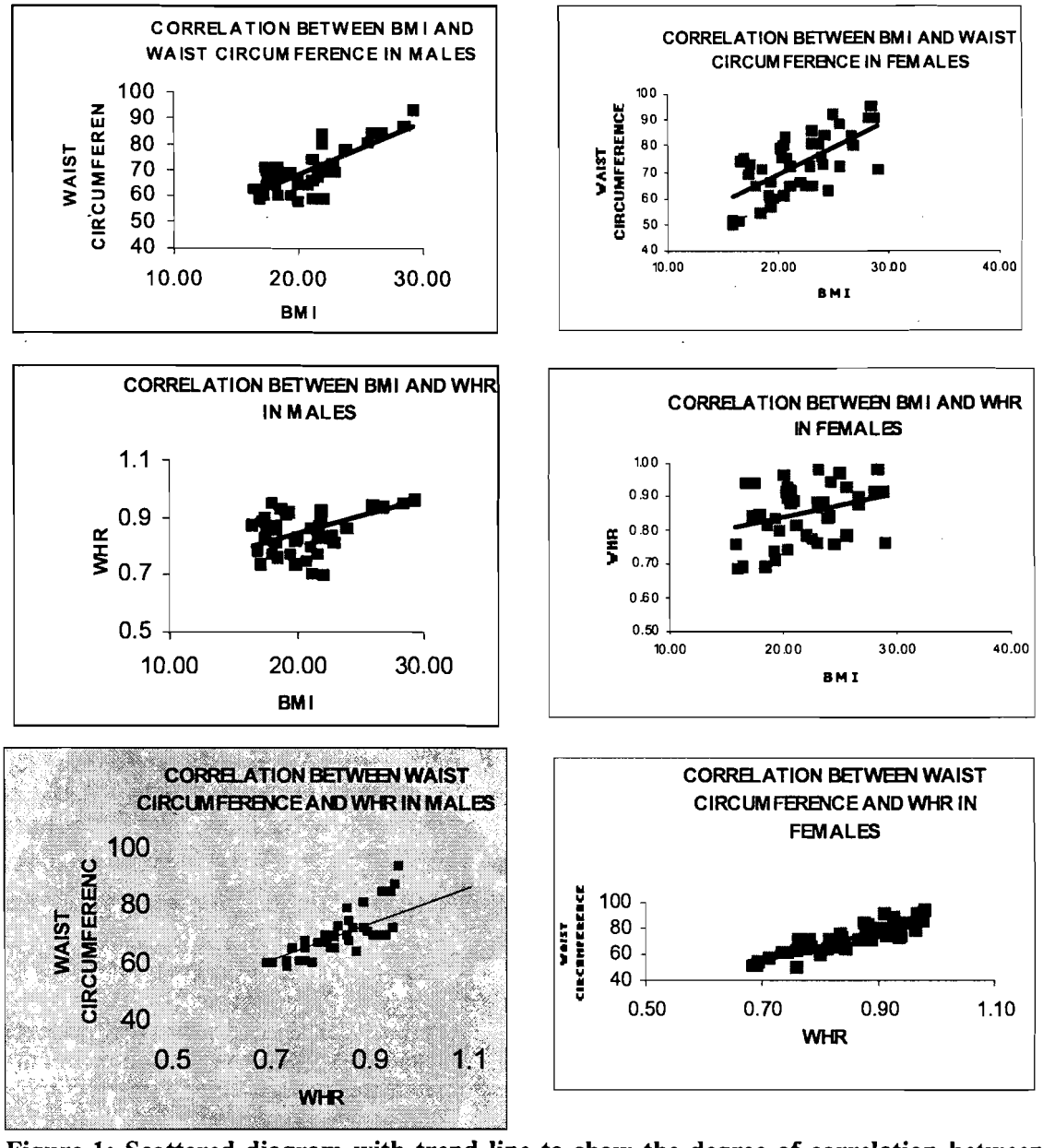

Figure-1: Scattered diagram with trend line to show the degree of correlation between the three parameters: BMI, WHR and waist circumference.

weight and 30 kg/m^2 for obesity has a very low sensitivity in our population. Hence due caution must be exercised when using these criteria in deciding on overweight/obesity.

If BMI alone is regarded as an index for assessing obesity in these students, the cut-off points for obesity for Asians by the WHO would need to be lowered, because the other measurements based on the two parameters WHR and waist circumference that are widely used as indices for obesity cannot be justified. So, we cannot use BMI as the sole index for assessing an obese, since this would have immense public heath implications mainly used as a predictor of metabolic abnormalities (non-insulin dependent diabetes), carotid atherosclerosis, cardiovascular diseases or risk factors, atherogenesis *etc.* It is a positive effort for determining adiposity in subjects of northeast state of India (Tripura).

So, there is a gap in the study of obesity by BMI based on Asian standards of WHO (WHO,1998) but if the BMI cut-off points for obesity would have reduced to 26 Kg/m^2 for Indians (Deurenberg-Yap et al, 2000) then we would be able to get a strong positive correlation between BMI and WHR. This study needs further evaluation. There are some residual factors that might play a role for such deviations. Further work is needed on in this aspect.

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FORAGE PATTERN OF MIGRATORY CULTURE OF *APIS MELLIFERA* DURING AUTUMN-WINTER IN BANKURA DISTRICT, WEST BENGAL

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Abstract ■ Forage pattern of *Apis mellifera* during Autumn-Winter in Belboni area of Bankura District, West Bengal has been worked out by pollen analysis. The overall forage spectrum includes altogether fourteen species of angiosperms. Quantitative analysis reveals that *Eucalyptus globulus* is the most important nectar source for the bee. *Acacia auriculiformis* and *Brassica nigra* constitute the primary sources of pollen grains.

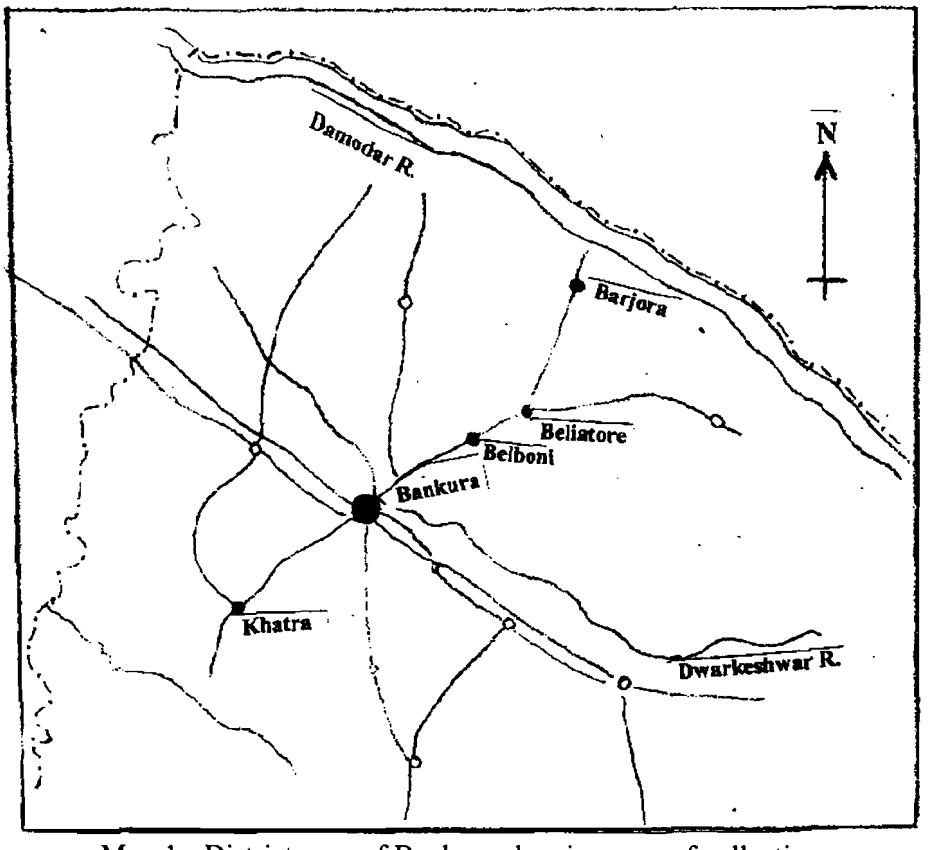
INTRODUCTION

The natural resources required by honeybees for their survival are water, nectar and pollen (Seedley, 1985). Water is used for cooling the hive. Nectar is the principal source of carbohydrate for the honeybees while pollen is the major source of nonliquid food, which contains proteins, fatty substances, minerals and vitamins. Melissopalynological study is one of the most suitable means in understanding the bee forage (Maurizio, 1951). Simultaneous analyses of honey samples and pollen pellets provide the details of the foraging pattern of a bee species in an area (Chakraborti, 1987; Kalpana and Ramanujam, 1989; Mondal and Mitra, 1980). The knowledge of bee-forage helps to assess the potentiality of a particular biozone together with the potentiality of the bee for improvement of crop yields in the area (Mukhopadhyay, *et al.* 2007).

Bankura district of West Bengal is well known for its 'Sal' (*Shorea robusta*) forest supported by lateritic soil. Otherwise, the district is agriculturally not very productive. During September to December 2005, a group of migratory beekeepers with their hives of *Apis mellifera* visited the Belboni area of the district (Map-1). To understand the foraging pattern of the bee species in this particular bio-zone, the present melissopalynological investigation was undertaken and also undertaken earlier by several workers (Deodikar, 1965; Chanda and ganguly, 1981; Chaubal and Kotmire, 1980).

MATERIAL AND METHODS

Pollen pellets were regularly collected during September to December 2005. They were collected mainly by the pollen baskets of the honeybees as pollen loads. Some pellets were also available from the pollen cells of the hive. Honey samples were available only in December, when the beekeepers used to



Map-1 : District map of Bankura showing area of collection

extract honey from the hives, usually once or sometimes twice a week. Altogether fifty-six pollen pellets and ten honey samples were analysed. Palynological preparations of pollen pellets and honey samples were made using Erdtman's acetolysis method (Erdtman, 1960; Faegri and Iversen, 1975). Microscopic examination was done under a Leica DMLB bright field trinocular microscope with 40X and 100X (oil immersion) apochromatic objectives. For qualitative analysis pollen grains were identified with the help of reference slides prepared from local flora. Quantitative estimations of representative taxa are based on a count of at least two hundred pollen grains for each sample. The data were processed as per methodologies recommended by International Commission for Bee Botany (1970) and Louveaux (1978).

RESULTS AND DISCUSSION

Pollen grains belonging to eleven species of flowering plants were identified in preparations from the samples of honey, thereby indicating the nectar forage spectrum of the bee. Those are *Acacia auriculiformis*, *Adhatoda zeylanica*, *Brassica nigra*, *Cocos nucifera*, *Eucalyptus globulus*, *Martynia annua*, *Neolamrkia cadamba*,*Psidium guajava*, *Tectona grandis*, *Tridax procumbens* and *Ziziphus mauritiana*. Pollen grains of ten species viz, *Acacia auriculiformis*, *Brassica nigra*, *Cocos nucifera*, *Eucalyptus globulus*, *Jatropha gossypifolia*, *Luffa acutangula*, *Neolamrkia cadamba*, *Poa gangetica*, *Psidium guajava* and *Tridax procumbens*

have been recovered from the samples of pollen pellets, which indicate the pollen forage spectrum of the bee. As revealed from qualitative analyses, altogether fourteen species of angiosperms constitute the overall forage spectrum of *A. mellifera* during Autumn-Winter in and around Belboni. Out of total fourteen species four are visited exclusively for nectar (N), three exclusively for pollen grains (P) and remaining seven for both nectar and pollen grains (N-P) [Table 1]. Based on quantitative analyses, all the honey samples are found to be unifloral showing overwhelming dominance of *Eucalyptus globulus* (42%) pollen grains (Fig. 1). In pollen pellets *Acacia auriculiformis* (38%), *Brassica nigra* (25%) and *Jatropha gossypifolia* (16%) were the most preponderant (Fig. 2).

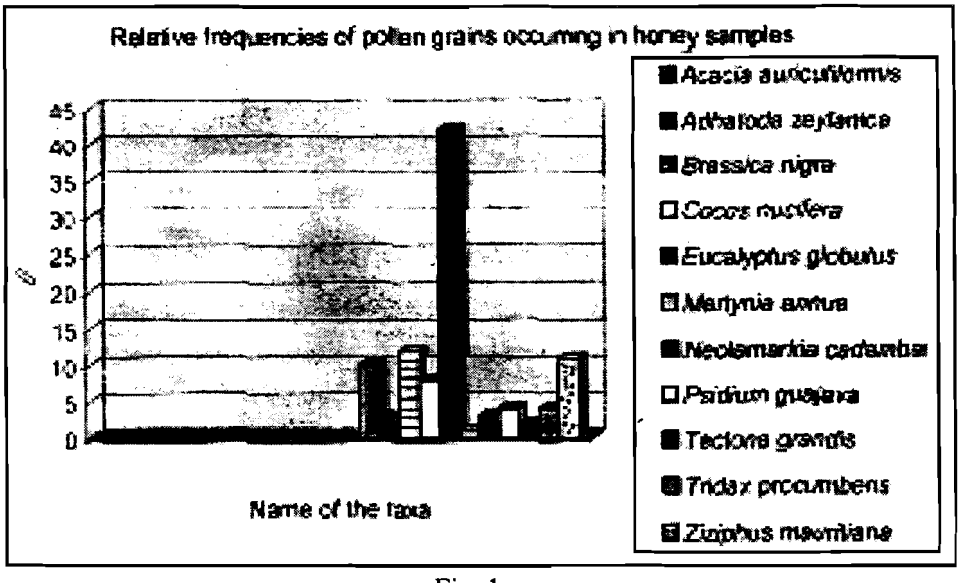


Fig. 1

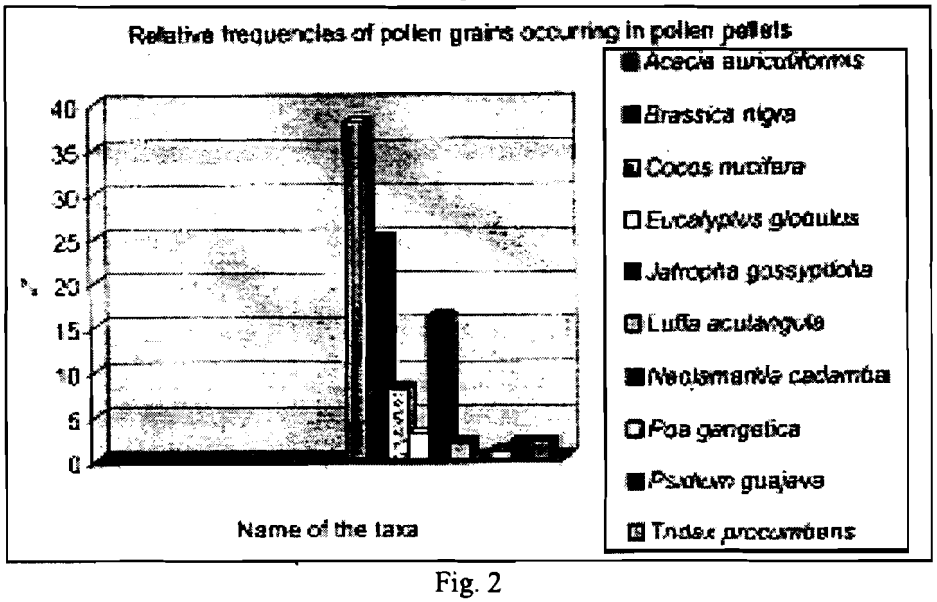


Fig. 2

Table - 1

Forage Pattern	Name of the Taxa
N-P	*Acacia auriculiformis, Brassica nigra, Cocos nucifera, Eucalyptus globulus, Neolamrkia cadamba, Psidium guajava, Tridax procumbens*
N	*Adhatoda zeylanica, Martynia annua, Tectona grandis, Ziziphus mauritiana*
P	*Jatropha gossypifolia, Luffa acutangula, Poa gangetica*

N-P: Nectar-Pollen, N: Nectar, P: Pollen

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ROLE OF CHROMIUM ON CERTAIN CHANGES OF NUCLEIC ACID AND PROTEIN SYNTHESIS IN EXPERIMENTAL RATS

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Abstract ■ The impact of chromium exposure was studied in lungs and heart of male Wistar rats (80-100 gm body weight). It was observed that treatment of rats with chromium (ip, at a dose of 0.8 mg/100 gm body weight / day) for a period of 28 days caused significant increase in chromium content in lungs and heart while lowering the body weight. It was also observed that there was a significant decrease in the DNA content of both the organs tested. Also, a significant decrease in RNA content was observed in lungs and heart. The lungs and heart showed significant decreases in total protein content in chromium treated animals. On the other hand, RNase and pronase activities were found to be significantly increased in both the tissues studied.

It is suggested that chromium exposure at the present dose and duration induces the changes of nucleic acid and protein synthesis in the form of depressive effects on nucleic acids (DNA & RNA) and protein contents as well as activities of RNase and pronase in lungs and heart tissues.

Keywords : Chromium, Nucleic Acids, Pronase, RNase.

INTRODUCTION

Chromium (Cr) is a widely used industrial chemical, finding applications in steel, alloy cast irons, chrome, paints, metal finishes, wood treatments, and it is known to cause allergic dermatitis as well as to have toxic and carcinogenic effects on humans and animals (Bagchi et al, 1995). Cr occurs in the workplace primarily in the valence states of Cr^{3+} and Cr^{6+}. Von Berg and Liu (1993) have summarized the acute toxicity, chronic toxicity, neurotoxicity, reproductive toxicity, genotoxicity, carcinogenicity, and environmental toxicity of Cr. Cr^{6+}or chromate, the biologically active form of environmental Cr, is taken up by cells and reduced intracellularly to reactive Cr^{5+} and Cr^{4+} species and then to stable Cr^{3+}. In the process, Cr induces oxidative DNA damage, DNA strand breaks, DNA-DNA and DNA-protein crosslinks, and mutations (DeFlora et al, 1990 ;

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Klein et al,1992). The genetic consequences of the various types of Cr complexes and the relative importance of oxidative DNA damage are unknown. Although much of the published research on the mechanisms of Cr-induced genotoxicity has dealt with the complex intracellular metabolism of Cr and the DNA damage produced by Cr^{4+} in whole cells (Standeven and Wetterhahn, 1989), it is well established that only the reduced forms of Cr, such as Cr^{3+} and Cr^{5+} form complexes with DNA and proteins. Since these complexes constitute the most persistent forms of intracellular Cr, the toxicology of Cr is of significance.

In view of that, the present study was intended to study the in vivo effects of chromium on nucleic acid and protein synthesis in terms of DNA, RNA and protein contents as well as the ribonucleolytic and proteolytic activities.

MATERIALS AND METHODS

A) Maintenance and Treatment of Animals

Male albino Wistar rats weighing 80 to 100 gm were used for the present investigation. The rats were fed with a diet containing protein, carbohydrate, fat, salt mixture and a vitamin mixture, as reported elsewhere (Chatterjee et al, 1976, 1984). Water was fed ad libitum. All rats were acclimated to this diet and the laboratory environment for 4 to 5 days. Twelve animals were distributed into two groups of equal average body weight. They were housed in cages and given a 12 hour light / dark cycle. The animals in one of the groups were injected intraperitonially with chromium in the form of CrO_3 at the dose of 800 μg per 100 gm body weight per day (20%LD_{50}) for a period of 28 days (Dey et al, 2003). The animals in the group serving as the control group received only the vehicle (0.9% NaCl). The animals in the control group were pair-fed with those of the Cr-treated group.

B) Tissue Collection

After the experimental period, the rats fasted overnight and were sacrificed by cervical dislocation. Lungs and Heart were immediately dissected out of the body, wiped and weighted. The tissues were then quickly stored at -20°C.

C) Biochemical Estimation

A weighed amount of each tissue was digested with acid mixture containing nitric acid, sulfuric acid and perchloric acid in the ratio of 6:1:1, over a regulated heater. After the digestion, the acid mixture was evaporated with occasional addition of triple distilled water and the colourless-odourless solution thus obtained was used for the estimation of chromium content. Measurement of chromium content of the acid free solution was carried out using Atomic Absorption Spectrometer. Four standard chromium solutions of 1.000 ppm, 3.000 ppm, 5.000 ppm and 10.000 ppm were used far all the estimations. The values of chromium contents of the measured tissues were computed from the standard curve of atomic absorbances of the solutions with known chromium concentrations as obtained from the measurement of atomic absorption.

DNA and RNA were isolated from homogenates in 0.25M sucrose by the method recommended by Munro and Fleck (1969). The DNA and RNA nucleotides were measured in the respective extracts by means of UV absorption, as employed by Nayak and Chatterjee (1998). Protein was estimated using Folin-Ciocalteau reagent and following the method describe by Lowry et al (1951), using bovine serum albumin as the standard. Ribonucleolytic activity was estimated on the basis of UV absorption of acid-soluble degradation products of RNA (Josefsson and Langerstedt, 1962). Broad-spectrum proteolytic activity was assayed spectrophotometrically using casein as substrate (Barman, 1974).

D) Statistical Analysis

The data were expressed as mean ± standard error. The significance in the differences between the means was evaluated by student's 't' test, and probability levels of 5% or less were considered to be statistically significant (Fisher and Yates, 1974).

RESULTS

Changes in the rat's body weight during the period of treatment have been depicted in Figure-1. It is observed that the body weight of Cr-treated rats significantly decreased as compared with that of the control group of rats. Upon exposure to Cr, lungs and heart tissues showed significant increases in Cr content (Table-1).

The results presented in Figure–2 & 3 demonstrates the alterations in DNA and RNA content in lungs and heart tissues in response to Cr exposure. The DNA content significantly decreased by 29.9% in lungs and 24.77% in heart due to Cr-treatment. But the RNA content significantly decreased by 28.89% in lungs and 17.87% in heart due to Cr exposure. On the other hand, the protein content in lungs and heart showed significantly reduced by 33.64% and 35.05% respectively in response to Cr exposure (Figure-4).

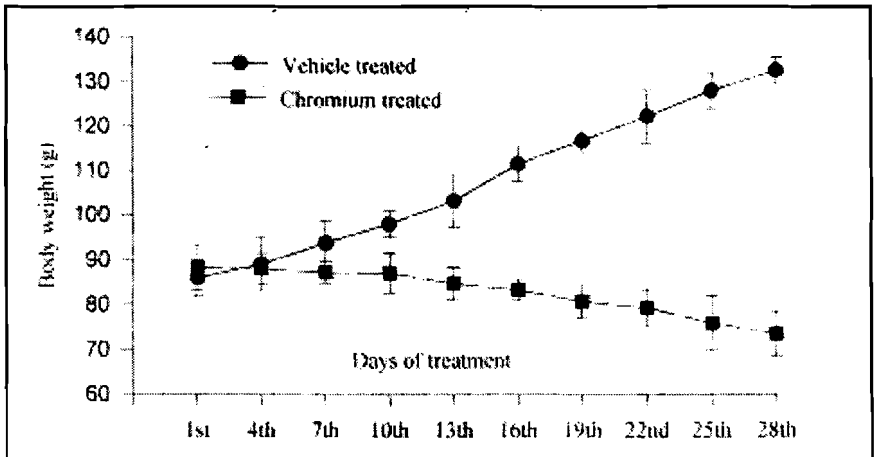

Fig. 1. Changes in body weight of rats during the period of treatment.

The ribonucleolytic (RNase) and proteolytic (Pronase) activities were observed to be significantly increased in all the organ studied in response to Cr treatment (Figure-5 & 6). The RNase activity increased in lungs and heart

Table-1 : Chromium content of organs after chromium treatment.

Chromium content (μg / gm tissue)		
Groups of animal	Lungs	Heart
Control	0.57 ± 0.04	0.38 ± 0.02
Chromium-treated	3.12 ± 0.16*	2.46 ± 0.11*

The values are the means of six no bservations ± SEM

* Indicates significant difference between two groups ($p<0.05$)

by 19.54% and 18.02% respectively due to Cr exposure in relation to control. On the other hand, it was found that the pronase activity increased in lungs and heart by 30.13% and 30.48% respectively due to Cr treatment.

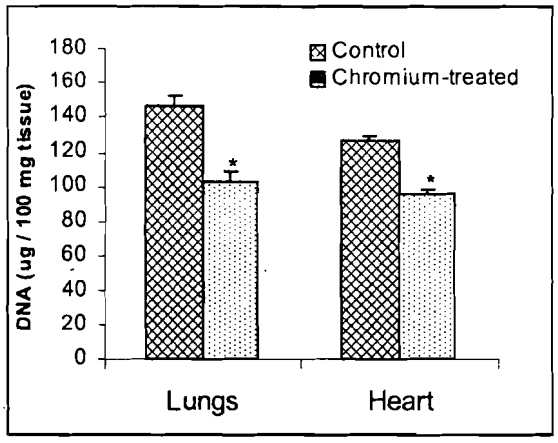

Figure-2 : Effect of chromium treatment on DNA content of lungs and heart tissues.
The values are means of six observations ± SEM
* Indicates significant difference between two groups ($p<0.05$).

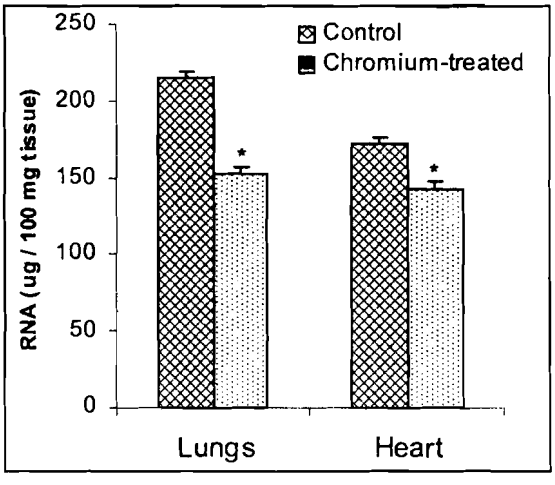

Figure-3 : Effect of chromium treatment on RNA content of lungs and heart tissues.
The values are means of six observations ± SEM
* Indicates significant difference between two groups ($p<0.05$).

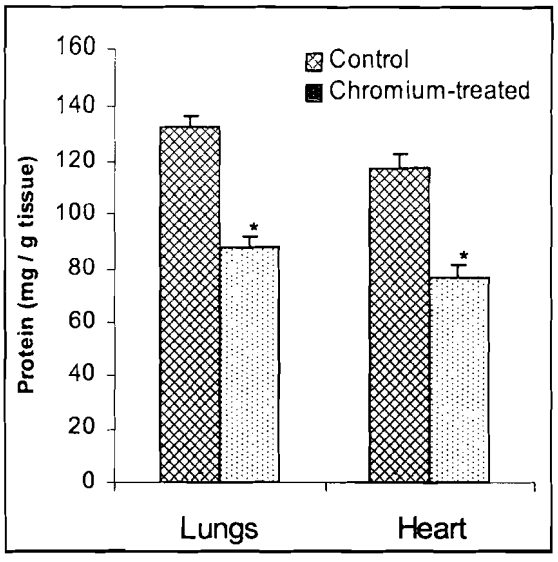

Figure-4 : Effect of chromium treatment on Protein content of lungs and heart tissues.
The values are means of six observations ± SEM
* Indicates significant difference between two groups ($p<0.05$).

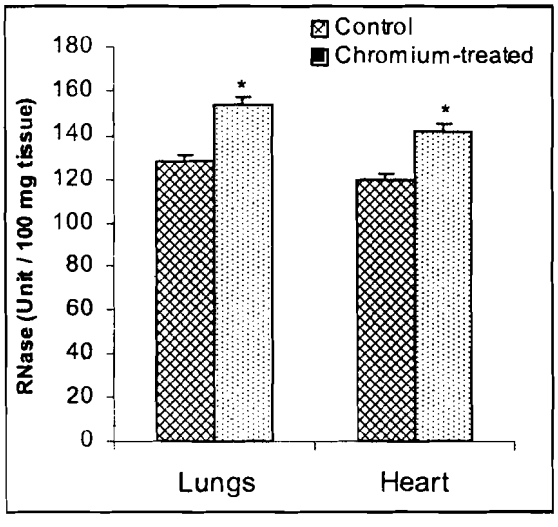

Figure-5 : Effect of chromium treatment on RNase activity of lungs and heart tissues.
The values are means of six observations ± SEM
* Indicates significant difference between two groups ($p<0.05$).

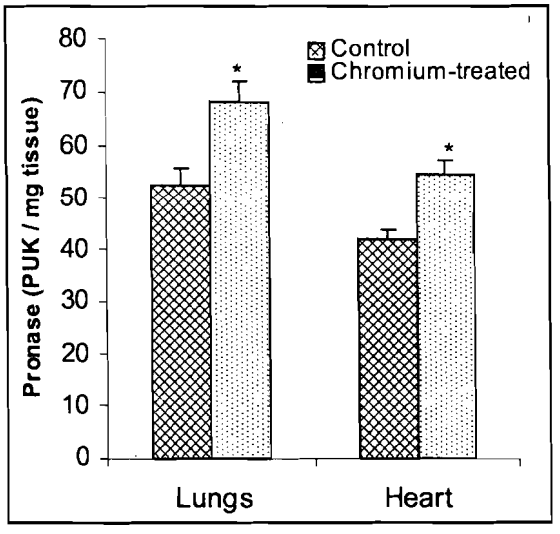

Figure-6 : Effect of chromium treatment on Pronase activity of lungs and heart tissues.
The values are means of six observations ± SEM
* Indicates significant difference between two groups ($p<0.05$).

DISCUSSION

Cr^{6+} compounds have been shown to exert toxic and carcinogenic effects in human (Anttila, 1990). Two cases of bronchogenic occupational carcinoma reported in Germany in 1932 were attributed to Cr exposure, and chromate was acknowledged as an agent causing lung cancer in Germany in 1936 (WHO, 1988). An increase in the incidence of lung cancer by Cr compounds was reported in workers as early as 1943 (IARC, 1990).

In this present study, it appears that weight gain decreased in Cr-exposed rats and Cr content increased in lungs and heart tissues (Figure-1 & Table-1). This impact on body weight may be due to the direct effect of Cr and not due to reduced food intake, as the control rats were pair-fed with the Cr-treated rats.

In this study, it was observed that DNA content decreased significantly in lungs and heart in response to Cr exposure (Figure-2), which corroborates earlier findings (Connett and Wetterhahn, 1983). Cr^{6+} has been demonstrated to induce a variety of DNA lesions such as single-strand breaks (Liu et al, 1997). Since it has been reported that Cr^{6+} do not react with isolated DNA (Tsapakos and Wetterhahn,1983), the reduction of Cr^{6+} by cellular reductants has been thought to be important step in the mechanism of Cr^{6+}-induced DNA damage (Liu et al, 1995). The Cr^{5+}-induced DNA damage disrupts the normal functioning of DNA in critical cellular processes, including transcription and replication (Wetterhahn and Hamilton, 1989). Manning et al (1994) observed that Cr-DNA adducts formed on a synthetic DNA template in vitro inhibited the progression of a DNA polymerase in a dose-dependent manner. Rapid inhibition of DNA replication by chromate has previously been reported that the Cr^{6+}-induced DNA damage is strongly dependent on the Cr^{5+} intermediates (Ayar et al, 1990). Thus, Cr interacts with DNA to form Cr-DNA adducts, thereby making them susceptible to attack by DNase in various organs and consequently perhaps also causing a reduction in DNA content.

Like the response shown by DNA, the RNA content of tissues was also found to be decreased significantly (Figure-3). Okada et al, (1983) reported that Cr^{6+} administration did not enhance but rather inhibited RNA synthesis in mouse liver. Cr^{6+} not only inhibits DNA and RNA synthesis, but also affects the uptake of exogenous nucleosides, thus modifying the labelling pattern of nucleic acids in treated cells (Bianchi, 1982). Levis et al, (1978) also reported that dichromate induces a sudden blockage of DNA replication, whereas RNA and protein synthesis are secondarily inhibited. Suppression of total RNA and mRNA synthesis correlated with the presence of DNA-Cr adducts (Ueno et al, 1989). Thus, the decrease in RNA content may be the result of decreased DNA content in both tissues after Cr exposure.

In the event of suppression of total RNA synthesis and increased ribonucleolytic activity (acid RNase) due to activation of enzyme activity in both the tested tissues (Figure-5), the cause of the decreased cellular RNA concentration in response to Cr treatment needs to be ascertained by further studies. However, it was demonstrated on several occasions that the activity of ribonuclease influences the cellular concentration of RNA (Ghosh et al, 1992 ; Thakur et al, 1992). It is therefore possible that the increased cellular concentrations of RNA might result from the inhibition of alkaline RNase by Cr. This, however, requires confirmation by study of the alkaline RNase activity.

Total protein content of the studied tissues showed significantly decreased in response to Cr exposure (Figure-4). It has been reported that Cr^{6+} dimishes the total protein, and that corresponds to the increases in leakage of intracellular enzymes (Susa et al, 1989). Thus, the decreased DNA and RNA levels may be done of the factors responsible for the decrease in protein levels in both the tested tissues. The increased proteolytic enzyme activity due to activation of enzyme activity for Cr exposure in lungs and heart tissues (Figure-6) may also be responsible for the observed changes in total protein content of both the studied tissues.

These findings suggest that Cr exposure has an important role to changes the nucleic acid and protein synthesis in terms of the alteration of DNA, RNA and protein contents as well as RNase and pronase activities in lungs and heart tissues.

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STUDIES ON THE DISSIPATION KINETICS AND RESIDUAL CONTENT OF OXYDEMETON METHYL IN FINISHED TEA AND TEA LIQUOR UNDER NORTHEASTERN INDIAN CLIMATIC CONDITIONS

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Abstract ■ A two-season pesticide residue trials were conducted in tea under northeastern Indian climatic conditions with oxydemeton methyl a stomach and contact action insecticide and acts as choline esterase inhibitor. The initial deposit of this compound in finished tea was ranged between 0.0737 to 0.1321μg g^{-1} following application @ 250 and 500 g a.i. ha^{-1} respectively. The half-life of the compound was ranged between 1.297 to 1.722 days irrespective of treatments in made tea. However, in tea liquor the residue of oxydemeton methyl was ranged between 0.0281 to 0.0032 μg mL^{-1} and went below the detectable range at 3rd day after application irrespective of application rates. The Post Harvest Indices are 9.105d and 8.898d and 11.515d and 11.769d for the treatments T_1 and T_2 respectively for the two consecutive seasons

***Keywords :** Metasystox; Tea; Residue; Dissipation; Post Harvest Index.*

INTRODUCTION

Various cultivars of tea (*Camelia sinencis* L.) are grown throughout the northeast India and constitute one of the major supply sources of tea product worldwide. India is the highest producer of tea in the world. The European Council issued in June, 1999 new standards for tea safety i.e. Maximal Residual Level (MRLs) of pesticides for imported tea products (Zhuang, 2000). The new standards have been effective since July, 2000 and strictly limited export of tea products. For instance, the current limits for fenvalerate and dicofol are <0.1 mg kg^{-1}, very much lower than the previous levels in tea products to increase safety, thus a new strategy is needed for tea pest control (Feng, *et al.*, 2004). Indian tea in recent past is facing a lot of tough competition with Kenya, Sri Lanka and Bangladesh in respect of tea export as well as in controlling red spider mite. The Indian Tea Board and Commerce Ministry are urgently developing mechanism for monitoring and

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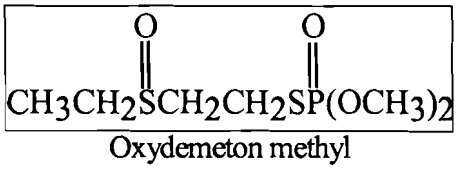
lowering the residue level.

Oxydemeton methyl [O, O-dimethyl S-2-(ethylsulfinyl) ethyl phosphorothioate] is a widely used systemic insecticide with stomach and contact action and acts as choline esterase inhibitor. Oxydemeton methyl shows selective toxicity for aphids, sawflies, suckers and other sucking insects on fruit, vegetables, vines, ornamentals etc. Oxydemeton methyl is reported to control a wide spectrum of sucking pests like *Bemisia tabaci* (Singh and Sirohi, 1997), aphids like *Pentalonia nigronervosa* (Behura and Mallik, 1995), *Myzuz persicae* (Karimullah and Paracha, 1995), thrips like *Haplothrips ganglabaueri* and *Fulmekiola serrata* (Bal *et al.*, 1995), red spider mite like *Tetranychus urticae* (Cho *et al.*, 1993) etc. Parker *et al.*, 1999 found metasystox as one of the most target specific insecticides for *Pseudatomoscelis seriatus* in cotton, with least disruptive effect on the beneficial arthopods (predators). The Indian Tea Association has proposed a more selective use of chemicals for specific pests. The chemical structure of Oxydemeton methyl is shown below.



Oxydemeton methyl

Tea is attacked by a variety of pests, which multiply rapidly and cultivators apply a combination of pesticides over a larger area to combat these pests. Red spider mite is one of the major pests in tea cultivation especially in northeastern region of India. There are a number of miticides available, out of which dicofol, ethion, fenvalerate etc causing residual problems in finished tea. So a vacuum has been created in the use of acaricides to combat pest problems in tea plantations.

Based on the above information, a two seasons field studies with oxydemeton methyl (metasystox) were conducted in northeast India for determination of the dissipation pattern as well as the residue level in finished tea and tea liquor.

MATERIALS AND METHODS

A two seasons [1st season, September 2003; 2nd season, March 2004] field experiment on tea (variety TV1) was conducted at Kamalpur Tea Estate, Darjeeling, West Bengal, India. Metasystox 25 EC was applied on tea bushes twice at an interval of 15 days as high volume spray (400 L ha^{-1}) by a knapsack sprayer. It was applied @ 250.0 g a.i. ha^{-1} (recommended application rate, i.e., T_1) and 500.0 g a.i. ha^{-1} (twice the recommended application rate, i.e., T_2). Untreated control (T_3) was simultaneously maintained. Each treatment including control was replicated thrice in a randomized block design (RBD). The number of bushes per treatment was 100 and spacing between the bushes was regular double hedge type. Green tea leaves (two leaves and a bud, 1 kg) were plucked randomly from each treatment plots replication wise at different time intervals [0 (2 h after last spraying), 1, 2, 3, 5,7 and 10 days]. The green leaves were processed for finished CTC tea (100 g) at Kamalpur Tea Estate factory following standard manufacturing methods.

Since sulfoxides are known to decompose during GLC, the peak obtained after its injection was assigned to sulphide impurities or its thermal degradation product unsaturated demeton–S-methyl. Oxydemeton methyl therefore can not be estimated as such by GLC. It is estimated after conversion to its sulfone, which is not thermally labile. A simplified method for estimating extractable residue of oxydemeton methyl as its sulfone from a crop matrix (mustard) was developed by Mukherjee and Gopal (1993). The insecticide was oxidized with meta-chloroperbenzoic acid to yield its

sulfone. Analytical study was carried out using GLC coupled with a thirmionic specific detector.

Metasystox was quantified using Gas liquid chromatography (Model 1000 Chemito) equipped with Thirm Ionisation Detector (T I D) coupled with Chemito 5000 Data Processor. For recovery studies, finished tea samples were fortified with acetone solution of metasystox to obtain concentrations corresponding to different doses. The samples were immediately extracted three times with 100 mL of acetone on an electric blender. The extracts were combined and evaporated to dryness in rotary vacuum evaporator with the water bath temperature adjusted to 40 ^{O}C. Metasystox was partitioned in CH_2Cl_2 (100+50+50 mL) and the combined organic extract was evaporated in rotary vacuum evaporator. The concentrated extract was then subjected to adsorption chromatography over florisil (60-120 mesh) with 2.0 cm layer of anhydrous sodium sulphate on the top. The eluate was again concentrated in a rotary vacuum evaporator and oxydemeton methyl was converted to its sulphone by oxidation with m-chloroperbenzoic acid (MCPBA) solution. The volume was made upto 10.0 mL with distilled hexane for final analysis by GLC.

N_2 (Flow rate: N_2 – 50, H_2 – 30 and Air – 30 mL min^{-1}) was used as carrier gas. Initial column (OV 101) temperature was set at 160^{o}C for 1 min and raised to 200^{o}C by increasing 10^{o}C min^{-1}, maintained at this temperature for 4 min and finally raised to 220^{o}C by increasing 10^{o}C min^{-1}. Detector and Injector temperatures were set at 230 and 200^{o}C respectively. The average recovery was 90-92% whereas the limit of detection of the method was 0.005 mg kg^{-1}. Thus the quantification of residues in the treated samples was carried out as per the recovery study.

The liquor was prepared by adding 10 g of finished CTC tea in 200 mL of boiling water and was stirred for 5 min with spoon and then filtered. The same analytical procedure as adopted in finished tea was followed for the tea liquor after extraction of the compound by liquid-liquid partition with dichloromethane.

RESULTS AND DISCUSSIONS

Data regarding the initial deposits, percent dissipation, half-life values and regression equations of oxydemeton methyl residues in finished tea and tea liquor following the application at recommended and double the recommended doses have been presented in Tables 1 - 3.

Table 1. Persistence of oxydemeton methyl in finished tea for consecutive two seasons at the recommended application rate

Season	DAT	Residue in ppm (*M ± S.D)	Dissipation (%)	Regression equation [Half life (days)]
	0	0.0881 ± 0.0011		
	1	0.0526 ± 0.0014	40.295	
I	2	0.0394 ± 0.0003	55.278	Y= 1.9521 – 0.2269X
	3	0.0142 ± 0.0009	83.882	[1.327]
	5	0.0071 ± 0.0017	91.941	
	7	*BDL		

Season	DAT	Residue in ppm (*M ± S.D)	Dissipation (%)	Regression equation [Half life (days)]
II	0	0.0737±0.0018		Y= 1.911 – 0.2321X [1.297]
	1	0.0589±0.0007	20.081	
	2	0.0311±0.0013	57.802	
	3	0.0118±0.0005	83.989	
	5	0.0063±0.0009	91.452	
	7	0.0037±0.203		
		BDL		

* mean of three replications
BDL = Below Detectable Limit

The initial deposit of oxydemeton methyl in finished tea after two hours of spraying ranged between 1.58 - 1.77 and 3.26 - 3.46 mg kg^{-1} irrespective of the seasons for the treatments T_1 and T_2 respectively. The loss of residues over a period of time showed a steady dissipation from 61.58 – 84.36 % within 3 days. The residue level fell below detectable limit on the 7th and 10th day for T_1 and T_2 respectively.

The dissipation of oxydemeton methyl in finished tea followed first order reaction kinetics in both the application rates as a straight line was obtained in each case when log values of the residue was plotted against different time intervals. From this study it appears that the rate of dissipation is independent of initial deposit and the half-life ($t_{½}$) of oxydemeton methyl ranged between 1.22 – 2.15 days irrespective of the seasons and application rates. From the experimental results the

Table 2. Persistence of oxydemeton methyl in finished tea for consecutive two seasons at twice the recommended application rate

Season	DAT	Residue in ppm (*M ± S.D)	Dissipation (%)	Regression equation [Half life (days)]
I	0	0.1228 ± 0.0014		Y= 2.1675 – 0.1789X [1.683]
	1	0.1046 ± 0.0060	14.821	
	2	0.0847 ± 0.0003	31.026	
	3	0.0451 ± 0.0012	63.274	
	5	0.0129 ± 0.0002	89.495	
	7	0.0096 ± 0.0011	92.182	
	10	*BDL		
II	0	0.1321 ± 0.0017		Y= 2.126 – 0.1748X [1.722]
	1	0.0965 ± 0.0008	26.949	
	2	0.0698 ± 0.0012	47.161	
	3	0.0329 ± 0.0015	75.095	
	5	0.0148 ± 0.0004	88.796	
	7	0.0094 ± 0.0013	92.884	
	10	BDL		

* mean of three replications
BDL = Below Detectable Limit

proposed waiting period was found to be range between 9.83 days to13.32 days irrespective of rate of applications and seasons under experiments. The MRL value of Metasystox in various crops is 0.01 mg/kg established by Australian Pesticides and Veterinary Medicine Authority.

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Table 3. Residue of oxydemeton methyl in tea liquor at recommended and twice the recommended application rate

Treatment	Concentration remaining ($\mu g\ mL^{-1}$) $\pm$ SD on day			
	0	1	2	3
Season I				
T_1	0.0281 $\pm$ 0.003	0.0084 $\pm$ 0.019	BDL	BDL
T_2	0.0413 $\pm$ 0.004	0.0171 $\pm$ 0.001	0.0054 $\pm$ 0.009	BDL
Season II				
T_1	0.0363 $\pm$ 0.007	0.0115 $\pm$ 0.016	0.0032 $\pm$ 0.002	BDL
T_2	0.0482 $\pm$ 0.021	0.0197 $\pm$ 0.006	0.0082 $\pm$ 0.002	BDL

BDL = Below Detectable Limit

Oxydemeton methyl residues in tea liquor from zero (0) day samples were found between 0.028 - 0.036 and 0.041 - 0.048 mg L^{-1} irrespective of the seasons for the treatments T_1 and T_2 respectively which in the 1st day sample were in the range of 0.009 – 0.011 and 0.017 – 0.019 mg L^{-1} and the residue in tea liquor went below the detectable range after 3rd day of last application.

For the untreated control, no residues of oxydemeton methyl were detected irrespective of the seasons. The half-lives of oxydemeton methyl in finished tea were found short and would be of no concern for contamination both in the food chain and the environment.

The MRL value of oxydemeton methyl has not yet been established in any substances including tea by WHO/FAO/JMPR/GOI. It might be stated that oxydemeton methyl may not pose any residual toxicity problem in tea as no residue was detected after 10th day.

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DEMOGRAPHY OF THREE COMMUNITIES OF DARJEELING DISTRICT IN WEST BENGAL— AN OVERVIEW

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Abstract ■ A systematic micro-level demographic study with special emphasis on sex-ratio (female - male ratio or FMR) has been done among the three communities, viz. Dhimal, Rajbanshi and Mech of Darjeeling District in West Bengal. These three endogamous groups in a particular ecological zone of Terai region of the foot-hills of Himalayan Mountain differ in socio-economic, cultural and other attributes of ways of life. However, they have some degree of common ethnohistory, though very remote. Results show that each of these communities has own demographic identity with respect to sex-ratio, fertility, mortality, literacy rate and other indicators. Dhimals show highest values of sex-ratio in most of the age-groups, crude death rate and infant mortality rate compared to the two other ethnic groups. On the other hand, Rajbanshis exhibit highest annual crude birth rate, crude birth rate, general fertility rate, child-woman ratio, percentage of persons (0-4 years), percentage of old-age population and least crude death rate and infant mortality rate among these three communities. The Rajbanshi and Mech do not differ in literacy rate, which is much lower as recorded among the Dhimals.

Keywords : Dhimal, Rajbanshi, Mech, Demography, Sex-ratio

INTRODUCTION

Demography has its focus on empirically collected data on human populations and their quantitative and statistical analysis with a view to identify and measure as precisely as possible the factors that underlie population changes. In the case of tribes such factors may assume an added significance in view of their supposed cohesive structure and collective representations. The tribes of India comprise approximately 8% of the total population of the country, having probably the largest number of tribal communities in the world (Topal and Samal, 2001). Demographic data on different tribal populations are indeed needed in order to estimate the biological, social and economic status of the tribals of India and thereby to take appropriate measures towards their development. Sex-ratio is one of the most significant demographic indicators that helps in estimating the nature of population structure; it also reveals the trends of gender-bias at any age

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or age-group(s). Sex-ratio further indicates the role and status of women in a society and their involvement in different economic pursuits. In measuring gross fertility, and relative mortality rates of the two sexes at different ages (Datta Banik, 2006, 2007, 2008) sex-ratio proves quite handy. Agnihotri (2000) examined the disaggregation of sex-ratio (FMR) patterns and causes for low and declining proportions of females in India's population. It may be worth examining whether the same trend prevails in the tribal populations as well, given the understanding that in a tribal society, women enjoy a relatively higher status compared to the non-tribals.

Darjeeling is the northern most district of the state of West Bengal in eastern India. Geographically the district can be divided into two broad divisions, the hills and the plains. The foot-hills of Darjeeling Himalayas, which comes under the Siliguri subdivision, is known as Terai. The population of Darjeeling is extremely heterogeneous. The majority of the people in the hills are of Mongoloid origin, belonging chiefly to various Nepalese castes, that also includes a large number of Lepchas, Bhotias and Tibetans. Dhimal, Mech, Koch, Toto, Garo, Chakma, Bhutia, Lepcha, Rabha and Limbu are some of the Mongoloid tribes of North Bengal. Dhimals are Tibeto-Burman language speaking Indo-Mongoloid tribe or 'Kiratas' of North Bengal like the Meches (Basu, 1922).

The word Mech is a corrupt form of 'Mlecch' or people having low status. *Yogini Tantra* calls the Mlecchas or Kavach (Grierson, 1903). Meches had come from Morong region of the country of Kichoks in Nepal. Some of the Meches of Assam also call themselves Rajbangsis. Ethnohistorically, Dhimal, Limbu and Toto originated from this stock in the bordering areas of India and Nepal in West Bengal, along the valley of river Mechi. Dhimal represents a small community in North Bengal. Evidence of anthropological research on this community is not available excepting a few reports (Datta Banik *et al.*, 2005, 2007, 2008). Dhimals live beside the river Mechi in the bordering areas of India and Nepal. The total population of the Dhimals in India is below one thousand. Unofficial records count 908 (done by the Dhimal people themselves in 2005). Since Census of India 1941, separate enumeration for Dhimals had been execluded. It was 1060 as recorded by Maitra (2001). Dhimals have their own unique language, dress preferences and culture. In spite of nurturing an indigenous culture and folk traditions over the centuries, Dhimals have not been enlisted in the Scheduled Tribe (ST) category. They are enlisted in Other Backward Class or OBC category in West Bengal. Rajbanshis have been transformed into Hindu caste of North Bengal, who were originally Koch. They have now been claiming to be an outlying branch of the Kshatriya. They also belong to Mongoloid ethnicity.

MATERIALS AND METHODS

This present paper is based on the results of a micro-demographic investigation carried out among the Dhimal, Rajbanshi and Mech communities in Darjeeling district of West Bengal during June-July 2008. The area of study remained limited to the region of rural parts of this district under Naxalbari and Matigara blocks in 17 villages where the people of these three communities live in separate villages. Altogether 450 families has been surveyed among these three communities (Dhimal- 152; Mech - 169; Rajbanshi- 129). Total 2556 individuals (Male- 1352; Female- 1204) from three communities, Dhimal (Male- 438 ; Female- 423); Mech (Male- 539 ; Female- 460) and Rajbanshi (Male- 375; Female- 321) took part in this study as subjects. The villages are located around 45 – 50 kilometers from Siliguri town, which is

approximately 580 kilometers from Kolkata, the provincial capital of West Bengal.

To measure the socio-demographic status of three communities; Dhimal, Rajbanshi, and Mech, some standard demographic methods and techniques were adopted (Ram Kumar, 1986; Kammeyer and Gian, 1986; Heer and david, 1987) - Female-Male Ratio (FMR), Age Dependency Ratio, Child-Woman Ratio, Percent of Persons (0 - 4), Percent of Old-age Population, Percent of Persons under 15 years of age, Work Participation Rate, Mean Household Size, Literacy Rate, General Fertility Rate (GFR), Annual Crude Birth Rate (ACBR), Fertility Projection, Crude Literacy Rate, Crude Death Rate, Infant Mortality Rate, Crude Birth Rate and Masculinity Proportion (MP).

The entire procedure of data collection had two primary elements: collection of data (facts) and a systematic analysis. The data included both textual, i.e. derived from written documents as well as contextual, i.e. the data collected from the field. The methods of data collection included direct extensive and intensive observations. Direct observation was done by sampling and questionnaire techniques. The random sampling has been done in the present context. In questionnaire, the poll procedure was done both in the form of structured as well as unstructured interviews. The open and closed ended questionnaires were adopted for this survey. Apart from these, case studies and genealogies were taken to record data according to the prevailing situations faced during the field work.

RESULTS AND DISCUSSION

The FMR (all ages) of three communities, Dhimal, Rajbanshi, and Mech show a value of 965.75, 856.0 and 853.43 respectively. The FMR_{04} of Dhimal community is quite high (1548.38), compared to the two other communities, namely, Rajbanshi and Mech who have FMR_{04} 785.71 and 934.78 respectively (Table 1). FMR_{59} in these three communities recorded were 915.25 (Dhimal), 864.86 (Rajbanshi), and 966.10 (Mech). Out of these three communities, only Dhimal show a decline of FMR_{59} from FMR_{04}. The decline of FMR_{59} from FMR_{04} indicates preferences of male child in the Dhimal families, which apprehends excess neonatal girl child mortality. The $FMR_{(09),(04),(10\text{-}19)}$ in cases of Dhimal and Rajbanshi do not show much variation but in case of Mech it shows some differences. In case of Rajbanshi, no such remarkable change in FMR is found among them during the last 15 years, which is evident from FMR_{04} and $FMR_{10\text{-}19}$, but the Mech community shows some remarkable changes in last 15 years. Dhimal community also shows significant variation of FMR in last 15 years. The higher sex-ratio among the Dhimal community is probably indicative of better maternal health. Indeed, the participation of female population in the total working population is considerably higher in the case of Dhimal than in the rest of the two communities, which is revealed from the $FMR_{15\text{-}64}$.

Table 1 : Demographic variables expressed through calculation of rates among the three communities of Darjeeling District in West Bengal

Sl. No.	Variables	Dhimal	Rajbanshi	Mech
1.	FMR (ALL AGES)	965.75	856.0	853.43
2.	FMR04	1548.38	785.71	934.78
3.	FMR 59	915.25	864.86	966.10
4.	FMR 09	1050.00	822.78	952.38
5.	FMR 0-14	1092.19	916.66	930.23
6.	FMR 10-19	990.47	797.87	795.77
7.	FMR 15 – 64	909.40	818.54	806.09
8.	ACBR	13.93	17.24	13.01
9	GFR	57.69	77.28	55.79
10	Fertility Projection	11.99	11.99	12.99
11	Crude Birth Rate	13.94	17.24	13.01
12	Crude Death Rate	17.42	4.31	0.00
13	Infant Mortality Rate	2583.33	1384.61	2083.33

Annual Crude Birth Rate (ACBR) of Rajbanshi (17.24) is the highest, followed by those of Dhimal (13.93) and Mech (13.013). Similarly, the General Fertility Rate (GFR) in case of Rajbanshi (72.28) is higher compared to Dhimal (57.69) and Mech (55.79). The number of births projected for the year with the help of Fertility Projection in case of Mech (12.99) is higher, followed by both Rajbanshi and Dhimal with the same value 11.99. The Crude Birth Rate of the Rajbanshi (17.24) is highest among the three communities. The Crude Death Rate of the Dhimal (17.42) shows the highest value, followed by Rajbanshi (4.31) and Mech (0.00) according to the last calendar year. The Infant Mortality Rate among these three communities shows remarkable variation : Dhimal (2583.33), Mech (2083.33) and Rajbanshi (1384.61) (Table 1).

Table 2: Demographic variables based on calculation of ratio among the three communities of Darjeeling District in West Bengal

Sl. No.	Variables	Dhimal	Rajbanshi	Mech
1.	Child – Woman Ratio	0.379	0.451	0.381
2.	Age Dependency Ratio	0.571	0.543	0.518
3.	% Of Persons (0 – 4)	9.175	10.34	8.90
4.	% Of Old-Age Population	2.09	2.15	1.50
5.	% Of Persons (0 –14)	34.26	32.61	33.23
6.	Masculinity Proportion	50.37	53.33	53.95

The Child –Women Ratio among Dhimal, Rajbanshi, and Mech are not much different revealing values of 0.379, 0.451, and 0.381 respectively. Clearly, all the communities are suffering from serious limitations due to high infant and child mortality. The Age Dependency Ratio of Dhimal community (0.571) is the highest, followed by Rajbanshi (0.543) and Mech (0.518). Rajbanshis have recorded a higher percentage of children in the age-group 0 – 4 years (10.34%), compared to Dhimal (9.175%) and Mech (8.9%) communities. But the percentage of persons in the extended category (0 – 14 years), shows a higher value among the Dhimal (34.26%) than Mech (33.23%) and Rajbanshi (32.61%). When the three communities are compared with regard to work participation rate, it is noted that the Mech community exhibits (65.26) higher value than the rates worked out among the Rajbanshi (64.79) and Dhimal (63.64) communities. Similarly, the percentage of old age people is slightly more among the Rajbanshi community (2.15%) than Dhimal (2.09%) and Mech (1.5%). The masculinity proportion of these three communities shows no remarkable variation between these communities. The highest value among the three communities is 53.95, which belongs to the Mech followed by Rajbanshi (53.33) and Dhimal (50.87) (Table 2).

Table 3: Demographic variables based on socio-economic factors among the three communities of Darjeeling District in West Bengal

Sl. No.	Variables		Dhimal	Rajbanshi	Mech
1.	Work Participation Rate		63.64	64.79	65.26
2.	Literary Rate	Total	71.73	78.48	78.49
		Male	83.16	87.50	85.03
		Female	59.45	67.97	37.26
3.	Mean Household Size		5.66	5.39	5.91

The Mean Household Size of these three communities exhibits a higher value in case of Mech (5.91), followed by Dhimal (5.66) and Rajbanshi (5.39). The Literacy Rates among three communities reveal not much difference. Similarly, male populations of these three communities show no distinction but the female populations show remarkable variations. Female populations among the Mech community show very low literacy rate (37.26) (Table 3).

SUMMARY AND CONCLUSION

Micro-level demographic studies have their limitations. At the micro-level, demographic variables do not always give a correct index in terms of demographic structure and growth of populations. Still, these have certain advantages in terms of their revelation of the specificity of the situation. The survival of a small community cannot always be explained in terms of demographic considerations, rules and laws. Social conditioning factors and economic determinants play a vital role in shaping the demographic structure of small community. Furthermore, all small communities do not reveal the same trend as because each

community is the product of specific socio-cultural and economic conditioning factors. The present study with its focus on comparing three tribal communities of North Bengal, namely, Dhimal, Mech and Rajbanshi, bear testimony to that.

Of the three tribal communities under study, Mech form the largest sample with a population 999 (Male – 539, Female – 460) and Dhimal forming the second largest sample (Male – 438, Female – 423, Total – 861) have been striving hard for a separate tribal identity. Rajbanshi constitute the smallest sample of 696 individuals (Male – 375, Female – 321). The Census of India 2001 shows a total population of Mech in West Bengal as 35996 (Male – 18,148, Female – 17,848) and in the Darjeeling District it is 2,159 (Male – 1,136, Female – 1,023). The villages surveyed belong to a particular area on the Southeastern bank of Mechi River. The communities under study are economically dependent on each other and the economic ties very often cut across village level relationships. Intra-village and inter-village socio-cultural ties, marriage and kinship, religion and rituals, festivals and common political programmes also bind them together. Of the three communities, the Rajbanshis have wider distribution in North Bengal and even in Assam. They are the numerically dominant Scheduled Caste of Darjeeling district. In Assam, they are included in the Scheduled Tribe category. It is difficult to take them as a closely integrated group from the demographic point of view. However, at the social and cultural level, they still manifest certain commonalities, which are responsible for integrating them socially and culturally. Understandably, this may also have some demographic implications. Each community represents a specific situation, which is partly their own creation and partly the creation of others. It must be admitted that no community today can represent a situation exclusively in terms of its own specific features. There has been much interaction and interdependence between communities, which get reflected in the demographic data and the results obtained from those.

Population Pyramid of Dhimal, Mech, Rajbanshi communities reveals almost the same feature with males outnumbering the females. Yet, at the specific level there are some differences. The sex-ratio of the Dhimal is the highest (965.75), compared to Rajbanshi (856.0) and Mech (853.43). This is rather confusing in the sense that the Dhimals are the most disadvantaged, as reflected in the Infant Mortality Rate, which stands at 2583.33 as against 1384.61 among the Rajbanshi and 2083.33 among the Mech. They have a lower Literacy Rate (71.73) as against 78.48 of the Rajbanshi and 78.49 of the Mech. They also have a higher value of Crude Death Rate (17.42) compared to 4.31 of the Rajbanshi. Similarly, the Age Dependency Ratio shows a higher value among the Dhimal than those observed among the Rajbanshi and Mech communities. It may be tentatively suggested that the Dhimal are yet to attain a demographically stable position of a group in view of their overt and covert relationship with the Dhimal population of Nepal. The present population of Dhimal, as it is revealed in the study, is representative of a selective sampling effect. It, however, does not mean that he Dhimals enjoy a better economic status or health status. Malaria is rampant in the Dhimal villages and almost every member of the Dhimal community had a history of Malaria at one time or the other. Other infectious diseases are also common.

FMR values of all ages do not show a consistent pattern of community variation. Still, in the case of Dhimal the FMR values of all ages and values ranging from 0-4, 5-9, 0-9, 0-14, 10-19, 15-64 show slightly higher values,

compared to the two other communities. These may be consistent with a higher Sex Ratio among the Dhimal, which again may be attributed to a relatively lesser number of female deaths. Datta Banik (2008), while examining the demography of Santals in West Bengal and Jharkhand, lays stress on better life-conditions of the Santals in comparison with other tribal groups in this region, which get reflected in the demographic profile. In the present case, it is difficult to suggest a better life-condition for a community that may contribute to better demographic standard. Economically all the communities stand almost at the same level.

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PHYTOTOXIC EFFECTS OF TWO EXOTIC WEEDS ON GERMINATION AND GROWTH OF MUNG BEAN

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Abstract ■ Phytotoxins released into the environment by one plant can inhibit the growth of other. This kind of interaction is known as allelopathy. While the significance of allelopathy is well known in weed-weed interaction, little is known on the allelopathic effect of weeds on crops. Keeping this in mind, the present study is an attempt to screen the leaf leachates of two weeds *i.e., Eupatorium odoratum* L. and *Lantana camara* L. for their phytotoxicity on germination behaviour of mung bean seeds. They reduced the percentage of germination and TTC stainability along with extended T_{50} values of mung bean seeds. The leaf leachates showed pronounced inhibition of root length and shoot length of 30-days old plants and the effect was fully concentration dependant. Thus, from the overall result it can be concluded that various inhibitors present in *E. odoratum* and *L. camara* can impart strong inhibitory effect on mung bean. These results suggest that the leaves of *E. odoratum* and *L. camara* possess phytotoxic or allelopathic chemicals which potentially rendered the inhibitory action on mung bean seeds.

INTRODUCTION

Exotic and invasive plants are major economic problem and threat to ecological diversity throughout the world (Callaway 2002). Many invasive species are uncommon in their native range, but become very abundant in their new habitats (Louda *et al.*, 1990). Nowadays, exotic plants are big threats to biodiversity everywhere. These species exert their harmful effects either directly on the growth, development and survival of native species or indirectly by altering the natural functions of the environment. Amongst these mechanisms involved, allelopathic interference has been shown to be involved in exotic invasion. Some exotic plants have allelopathic potential by releasing allelochemicals to their surroundings that have either deleterious or even deadly effects on others plants in the vicinity. They also influence the other plants communities by releasing phytotoxins as leachates, exudates and volatiles and decomposition products (Rice, 1984). Allelochemicals affect the seed germination, seedling growth and all functions

of plant life (viz., photosynthesis, respiration, nutrient uptake, transpiration and resistance) and also influence the synthesis of proteins mediated by DNA and RNA (Grodszinsky, 1989; Saxena *et al.*, 2003). On the other hand, phytotoxic substances may act in many biological processes, such as the suppressing the mineral uptake by plants, inhibiting cell elongation and cell division, as well as retarding the photosynthesis, respiration and enzymatic activities, resulting in the retardation of plant growth. They may also interfere with the action of the growth promoting substances e.g., gibberellins or auxins (Chou, 1980). Exotic and invasive plants species usually suppress the native species and disturb the structure and functioning of native communities, leading to the reduction in biodiversity and huge economic losses. Although much studies in this direction are being done elsewhere, in India this aspect has attracted very little attention. Considering the above in mind, the aim of the present study is to screen out the allelopathic (inhibitory) effect of two exotic weeds *E. odoratum* and *L. camara*. which have become invasive and forms monospecific thickets in roadsides, forest margins and crop field edges in West Bengal (Bhakat and Maiti, 2003 **a** and **b**). In fact, allelopathic inhibition exerted by a species in natural environments turns it aggressive which could influence the community structure and composition leading to loss/ displacement of biodiversity.

E. odoratum contains allelochemicals like ceryl alcohol, eupatol, lupeol and β-amyrin, salvigenin, isosakuranetin, isosakuranetin mono-methyl ether, 4,5-dihydroxy-3,7-dimethoxy flavone and odoratin and *p*-anisic acid (Ambika, 2002). *Lantana* leaves contain allelochemicals like phenolic compounds, mono- and sesquiterpenes, triterpenes, triterpenoides, quinines, essential oils, flavonoids, biocides, juvenile hormones and growth hormones (Raghavan, 1976). These allelochemicals interfere with various physiological and biochemical processes of seed germination, root elongation and plant growth, and metabolic activities of many species (Bhakat *et al.* 2006; Maiti *et al.* 2008). To test this, *E. odoratum* and *L. camara* are selected as donor weeds and mung bean as the target species.

Thus, in this investigation, experiments were designed to determine the phytotoxic potential of different concentrations of leaf leachates of *E. odoratum* and *L. camara* and to analyse the correlative changes of germination behaviour and growth of mung bean.

MATERIALS AND METHODS

Experiments of the present investigation were carried out with fully viable healthy seeds of mung bean (*Vigna radiata* cv. K851) procured from Midnapore. Healthy leaves were collected from actively growing populations of *Eupatorium odoratum* L. (Asteraceae) and *Lantana camara* L. (Verbenaceae) for sources of allelochemicals.

Leaf leachates: Shed dried 500 g leaf samples of *E. odoratum* and *L. camara* each were kept immersed in 300 ml double distilled water in 1000 ml beaker and kept at room temperature (27°C) for 48 h. Thereafter it was stirred manually for two min and filtered through Whatman No. 1 filter paper to prepare aqueous leachate and the leachate was decanted in a separate beaker. The total volume of the leachate was then made up to 500 ml using double distilled water and this was taken as the 1:1 (w/v) proportion of leaf leachate. From this stock solution another concentration grade in the proportion of 1:2 and 1:3 (w/v) were prepared using double distilled water. Thus, three concentration grades (1:1, 1:2 and 1:3) of leaf leachates were prepared.

Mung bean seeds were surface sterilized with 0.1% $HgCl_2$ solution for 90 sec. The seed lots

were then separately presoaked in the three concentrations of leaf leachates of *E. odoratum* and *L. camara* for 24 h and then thoroughly surface-washed with tap water followed by distilled water. Physiological tests were then performed using the pretreated seeds. Data on seed germination percentage, T_{50} value, TTC stainabilty, speed of germination, were recorded.

Germination was recorded 7 days after seed soaking following ISTA rules (1976). The time required for 50% seeds germination (T_{50}) was determined as per the method of Coolbear *et al.* (1984). Speed of seed germination was recorded by analysing germination (%) of seed lots of each treatment at 12-h intervals in laboratory up to 120 h after seed soaking as per ISTA (1996). To analyse TTC stainability, 100 dehusked seeds were allowed to imbibe in 0.5% TTC (2, 3, 5-triphenyl tetrazolium chloride) solution (w/v) in petri dishes for 24 h in dark. The percentage of TTC-stained (red coloured) seeds were calculated from the total number of seeds (Halder 1981).

The field experiment was conducted at the Research Laboratory of Vidyasagar University Farm, Midnapore during the years 2006 and 2007. Data on root length and shoot length of 30-days old plants were recorded.

All the data in this investigation were statistically analysed at the treatment and replication levels (Panse and Sukhatme 1967). In table LSD (least significant difference) values (at 5% level) were incorporated. In figures SEM (standard error of means) values were presented.

RESULTS

Effect on germinability, T_{50} value and TTC stainability (Table-1): The leachates of *E. odoratum* and *L. camara* strongly retarded the percentage seed germination on mung bean seeds. The result showed that the leaf leachates significantly decreased the rate of germination of the pretreated seed samples as compared to control. The effect of *E. odoratum* was found more inhibitory than *L. camara*. Concomitantly, in treated seeds time (h) required for 50% germination was noted very high than control. Mung bean seeds treated with leaf leachates of all concentrations showed differential TTC stainability. This reduction was substantially increased in treated seeds and this decreasing trend was highly concentration-dependent. The inhibitory effect was found to be much more significant at later observation periods and the degree of inhibitory effect was fully concentration-dependent.

Table 1. Effect of seed pretreatment with leaf leachates of *E. odoratum* and *L. camara* on percentage of germination (%), time (h) for 50% germination (T_{50}) and TTC stainability (%) of mung bean seeds.

Treatment	Germination (%)	T_{50}	TTC stainability (%)
Control	100.00	15.85	100.00
***E. odoratum* (1:1)**	42.68	NA	44.27
***E. odoratum* (1:2)**	68.27	64.25	72.85
***E. odoratum* (1:3)**	86.00	46.98	92.05
***L. camara* (1:1)**	67.25	81.75	68.75
***L. camara* (1:2)**	81.25	46.68	87.00
***L. camara* (1:3)**	93.20	33.45	96.76
LSD=(P=0.05)	4.41	2.05	4.50

Effect on speed of germination (Table-2): The result shows that in control samples the percentage of germination of seeds increased with the advancement of the germination period as recorded from 12 to 120 h of seed soaking. However, leaf leachates of both the weed species rendered inhibition on seed germination during the observation periods recorded at 12 h intervals. Germination speed was significantly slowed down in all the treatments. Here also, *E. odoratum* leaf leachates exerted much more injurious effect than *L. camara*, and the effect was found drastic with respect to retardation of the speed of germination.

inhibitory effect on the seedling growth. The phytotoxic effect was also observed in root length and the suppression of growth was more pronounced with leaf leachates. During germination the radicle becomes brownish, eventually whole radicle becomes dark brown in colour leading to death of the germinating seeds during the experimental periods.

DISCUSSION

The allelopathic effects of different concentrations of aqueous leaf leachates from leaves of *E. odoratum* and *L. camara* were inhibitory to all parameters *viz.*, seed germination

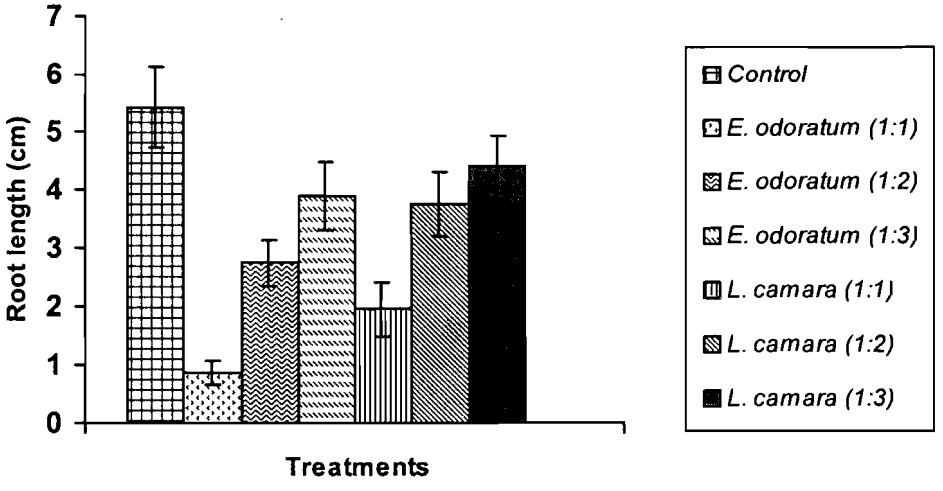

Figure-1. Effect of leaf leachates of *E. odoratum* and *L. camara* on the changes in root length in leaves of 30-days old mung bean plants.

Effect on root length and shoot length (Figure-1 & 2): Data clearly revealed that the length of the roots and shoots of the seedlings were significantly reduced in the seedlings which were raised from seeds pretreated with leaf leachates of each concentration. The seedling growth recorded in terms of length of roots and shoots was decreased upon increasing the concentrations of leaf leachates. Further, higher concentrations of leaf leachates rendered more

to metabolism of mung bean seeds (Tables 1-2, Figures 1-2). Results revealed that leaf leachates of *E. odoratum* and *L. camara* caused significant inhibition on the germination behaviour of mung bean seeds (Table 1). This study clearly showed the suppressive effect of *E. odoratum* and *L. camara* leaf leachates on the germination of mung bean seeds.

Data further showed that after the treatment of the seeds with leaf leachates percentage of

Table 2. Effect of seed pretreatment with leaf leachates of *E. odoratum* and *L. camara* on speed of germination of mung bean seeds.

	Speed of germination (12 hours interval)									
Treatments	**12**	**24**	**36**	**48**	**60**	**72**	**84**	**96**	**108**	**120**
Control	43.72	69.30	84.25	97.00	100.00	100.00	100.00	100.00	100.00	100.00
***E. odoratum* (1:1)**	00.00	16.21	22.92	31.25	35.86	38.85	42.68	42.68	42.68	42.68
***E. odoratum* (1:2)**	12.75	27.95	36.70	46.20	49.25	58.51	68.27	68.27	68.27	68.27
***E. odoratum* (1:3)**	25.85	33.52	45.48	51.25	60.67	69.45	74.46	86.00	86.00	86.00
***L. camara* (1:1)**	09.29	31.37	38.25	42.85	47.86	49.95	63.35	67.25	67.25	67.25
***L. camara* (1:2)**	25.86	39.25	47.31	54.25	69.75	71.49	76.22	81.25	81.25	81.25
***L. camara* (1:3)**	34.59	47.00	56.68	67.80	76.45	83.00	88.49	93.20	93.20	93.20
LSD=(P=0.05)	01.33	02.89	03.01	03.15	03.67	04.10	04.39	04.41	04.41	04.41

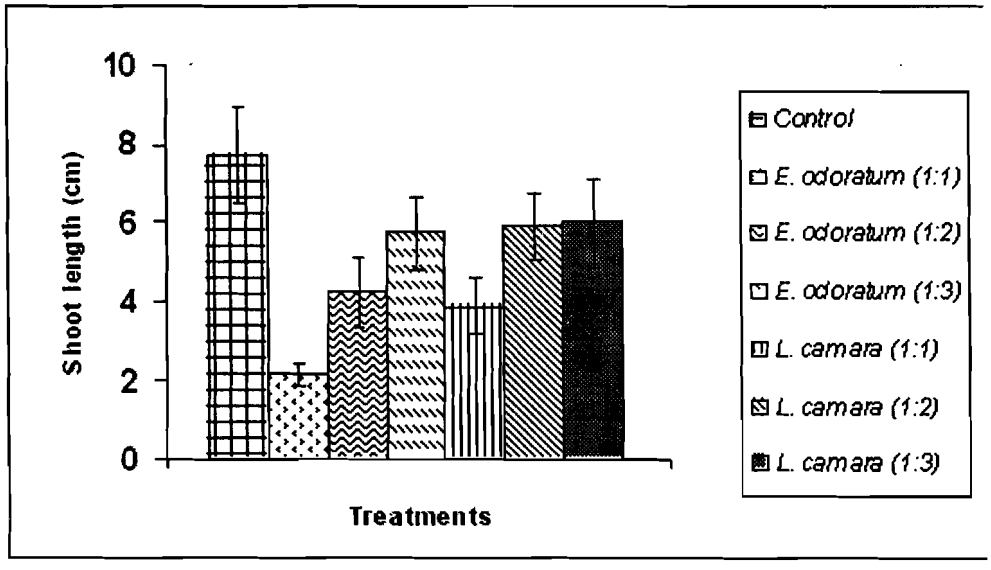

Figure-2. Effect of leaf leachates of *E. odoratum* and *L. camara* on the changes in shoot length in leaves of 30-days old mung bean plants.

seed germination and TTC stainability were decreased, whereas the time required for 50% germination (T_{50}) of the seeds was increased. All concentrations of *E. odoratum* and *L. camara* leaf leachates reduced germinability and caused slower rate of germination which are considered to be the important visible and reliable indices for the evaluation of allelopathic effect. Similar types of observations were also noted in *Casuarina equisitifolia*, *Ipomoea pes-caprae* (Bhattacharjee *et al.*, 2003), *Eupatorium odoratum* (Bhakat *et.al.*, 2006), *Lantana camara* (Maiti *et al.*, 2008) leachates. Maximum inhibition was observed in mung bean seeds when pretreated with 1:1 concentration of plant leachates. This indicates that inhibitory effects of the leaf leachates were concentration-dependent. The germination

potency of pretreated seeds of mung bean with leaf leachates can also be determined from the percentage of TTC staining (Table 1). Results showed that the TTC stainability declined steadily with increased concentrations of the plant leachates. The results are in agreement with those found by Bhattacharjee *et al.* (2003), Bhakat *et al.* (2006) and Maiti *et al.* (2008).

It is evident from the present study that the speed of germination (Table 2) was adversely affected in the seeds pretreated with the test leaf leachates. Reports exist in the literature that impairment of seed germination and seedling vigour might be due to imbalance in metabolism and metabolite transport, regulated by various enzyme activities from seeds (Padhy *et al.*, 2000). Leaf leachates mediated damage of cell membrane structure might be the other factors that augment phytotoxicity to pretreated seeds. The allelopathic potential of *E. odoratum* and *L. camara* can further be substantiated from plant growth and metabolism of mung bean plants. The plant growth performance includes root length (Figure 1) and shoot length (Figure 2).

The modified physiological processes inhibited and delayed the germination as well as growth of mung bean under the influence of allelochemicals present in leaf leachates. This may restrict the spread of obnoxious weed. These studies indicate the potential to use the allelopathic species to suppress the growth of other weeds. The aqueous leaf leachates of *E. odoratum* and *L. camara* have shown strong inhibitory effects on mung bean in this study, whether they can be used as herbicide to control other invasive weed is an interesting issue which needs further study.

This study thus concludes that by virtue of strong inhibitory effect, both the invasive and exotic weeds, *E. odoratum* and *L. camara*, have the potential to interrupt regeneration processes of mung bean and other species by decreasing germination, reducing early growth rates, metabolism and selectively increasing mortality. Therefore, as the density of *E. odoratum* and *L. camara* in natural and agricultural ecosystems increases, the species richness and the crop productivity are likely to be decreased. Thus in the near future it would reduce the availability of forest areas and natural resources as well as agricultural products on which people depend. This is a serious concern for biodiversity conservation and human society. Keeping the above in mind, the inhibitory and growth suppressing allelopathic property of *E. odoratum* and *L. camara* should be treated as a potential threat to plant diversity, both in natural and man-made ecosystems. Therefore, this work calls to advocate for the proper management of *E. odoratum* and *L. camara* and other the exotic invasive weeds showing similar behaviour.

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PROTEIN DEFICIENCY IN THE DIET CAUSED IMMUNE IMPAIRMENT AFTER *Staphylococcus aureus* INFECTION IN MICE

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Abstract ■ The association of poor host nutritional status with increased susceptibility to infectious diseases has long been thought to be related to the host immune response. The association between malnutrition with consequential enhancement in the susceptibility to infection is currently under investigation. Our objective was to study the effect of protein deficient diet on the alteration in immune response with special reference to pathogenic *S. aureus* infection in mice. Gradual increased numbers of *S. aureus* were found in the blood, spleen and lymph node of protein deprived mice indicating reduced clearance of bacteria due to protein deficiency. The titer was obtained at lowest antibody concentration (1:128) in the protein enriched diet fed mice after *S. aureus* infection, whereas serum from mice after pre-exposure of protein deficient diet followed by *S. aureus* infection, showed early agglutination (1: 2). However, the exact mechanism by which deficiency of protein in the diet leading to prolonged *S .aureus* infection or inhibits cell functions as well as alters antibody titer in serum could be clarified from further experiments in our ongoing study.

Key words: antibody production, bacterial infection, cell function, immune response, protein energy malnutrition,

INTRODUCTION

Interest in the influence of undernutrition on the susceptibility to infection emerged long ago with the observation that malnutrition appeared to lead to increased infection in some instances and increased resistance to infection in others (Scrimshaw 1975 and 1997). Malnutrition is known to induce a state of immunodeficiency and a predisposition to death from infectious diseases (Chandra 1996). Attention to nutritional status, therefore, is relevant to all aspects of infectious disease from infection, through the progression of the disease and recovery, and on to subsequent infections (Gordon 2008). Protein energy malnutrition (PEM) is associated with a significant impairment of cell mediated immunity, phagocyte function; complement system, secretary immunoglobulin-A, and cytokine production (Chandra 1997). It is recognized that malnutrition and infection are

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the two major obstacles for health, development and survival worldwide and, poverty and ignorance are the most important contributing factors (Chandra 1996). It is well known that malnourished individuals are more susceptible to infection and thus considered as immunodeficient (Scrimshaw et al 1995). Several studies dealing with malnutrition and infection in human have demonstrated defects in cell mediated immunity in malnourished subjects (Wolowczuk et al 2008). The cyclical relationship between poor nutrition, increased susceptibility to infectious disease leading to immunological dysfunction and metabolic responses that further alter nutritional status was reported (Gerald 2003). Interaction of malnutrition and immune impairment with specific reference to immunity against parasite has been established (Hughes 2006). Current concepts and controversies surrounding the complex influences of malnutrition on infection and immunity have been elaborated (Ulrich 2007). Studies also focused on the specific dietary influences on the maturation of immune system (Keush 1998). The strong association between malnutrition and infection has been established through epidemiological studies conducted in several countries.

Protein calorie malnutrition (PCM), as one of the global health problems, arises during protein and / or energy deficit due to disease and nutritional inadequacy. It has been shown that PCM elicited oxidative stress with the activation of the phase II detoxifying gene expression, which was reversed by cysteine supplementation (Lee 2002). The emergence of new infectious diseases and old diseases with new pathogenic properties is a burgeoning worldwide problem. Severe acute respiratory syndrome (SAARS) and acquired immune deficiency syndrome (AIDS) are just two of the most widely reported recent emerging infectious diseases. It has been reviewed recently that host nutritional status can influence not only the host response to the pathogens, but can also influence the genetic make-up of the viral genome. This latter finding markedly changes our concept of host-pathogen interactions and creates a new paradigm for the study of such phenomena (Beck 2004).

The objective was to study the effect of protein deficient diet on the alteration in immune response with special reference to bacterial infection in mice. We reported here that deficiency of protein in the diet causes compromised immune function including decreased production of antibody, reduced intracellular capacity to clear bacteria from blood and tissue.

MATERIALS AND METHODS

Bacterial Strain:

Pathogenic *Staphylococcus aureus* (CMC-524) was obtained from Calcutta Medical College, Kolkata and was maintained in our laboratory.

Animals:

Male Swiss albino mice, 4 weeks of age with body weight 8 ± 2 gm were purchased from local registered animal suppliers to our department. Upon arrival, mice were randomized into plastic cages with filter bonnets and saw dust bedding, followed by a one-week quarantine period with normal diet. Mice were housed 8 per cage supplemented with different diet and water *ad libitum* as mentioned below. Animal holding rooms were maintained at 21 to 24°C and 40-60% humidity with a 12-hour light dark cycle. Animals were divided into 3 groups containing 8 mice in each group; a) normal lab diet fed, b) protein enriched diet fed, c) protein deficient diet fed.

Composition of diet:

Each mouse was fed 1.5 g of diet per 10 g body weight per day. The compositions of different diets as recommended for mice, by the

National Center for Laboratory Animal Sciences, National Institute of Nutrition, India was as follows: -

Normal-lab-diet	Protein-enriched-diet (g/kg of diet)	Protein-deficient diet
Wheat starch	casein – 263g	54% starch
Sattu	sucrose – 498g	26.6% glucose
Milk powder	starch – 195g	7.4% vegetable oil
Salt	*salt-mixture –35g	*0.6% salt-mixture
Vitamin-mixture	vitamin -mixture –10g	0.6% cellulose

*Salt-mixture : 34% $CaCO_3$, 36.8% KH_2PO_4, 19% NaCl, 2.85% $FeSO_4$, 0.25% $MnSO_4$, 0.15% $MgSO_4$, 0.03% KI, 0.03% $CuSO_4$.

All experiments used mice as accredited by the Institutional Animal Ethical Committee

Preparation of viable bacteria (*S. aureus* CMC 524) for *in vivo* infection:

To obtain bacteria in the mid logarithmic phase 100µl of an overnight culture made in nutrient broth was added to 10 ml of nutrient broth and incubated for 2-5 hr at 37°C with orbital shaking. The bacteria were washed in 10mM Phosphate buffer (pH 7.4) and their concentration estimated by spectrophotometry at A_{620} on the basis of the relationship (Yao 1997) :

A_{620} 0.2 = 5 x 10^7cells per ml, prior inoculation to mice.

Infection to mice:

After 3 weeks of diet supplementation normal-lab-diet, protein-enriched-diet, and protein-deficient-diet fed mice were injected (i.v.) via the tail vein with viable *S. aureus* (CMC 524) (10^7 CFU/mouse) cells in 0.1 ml saline (Yao 1997). Control mice received only sterile saline. At different time intervals after infection (0, 3, 5 days post-infection) the mice were sacrificed under ether anesthesia and their blood (with heparin) and tissues (spleen, lymph node in PBS) were collected aseptically.

In vivo clearance of bacterial load from blood and spleen, lymph node:

The blood from each infected mice was plated on nutrient agar. Spleen, lymph node, were excised, weighed, homogenized, diluted in saline and plated on nutrient agar (Yao et al 1997). Results were expressed as the number of bacterial CFU/100µl of blood and per 100 mg of each tissue.

Preparation of attenuated (heat killed) *S. aureus* antigen:

S. aureus CMC 524 strain (one loopful) was inoculated in 250 ml nutrient broth and was incubated at 37°C in a shaker, for 24 hr. After overnight cultures, the cells were centrifuged at 5000 rpm for 10 min. The supernatant was discarded and the pellet was dissolved in 0.9% saline. The suspension was further centrifuged for 10 min, the supernatant was decanted and finally the pellet was resuspended in 0.9% saline, and cell numbers were adjusted spectrophotometrically as before and autoclaved at 15 lbs/square inch pressure for 15 min.. This was the final heat killed (or attenuated) antigen solution.

Estimation of antibody titer by bacterial agglutination:

Antibodies directed against surface antigen can be detected by bacterial agglutination reaction (Kabat 1967). Serum which is suspected to be infected with a given bacteria was serially diluted (two fold) in a series of tubes to which attenuated bacteria was added. The last tube showing visible agglutination will reflect the serum antibody titer which can also serve as a useful semi-quantitative measure of the concentration of agglutinating antibodies.

Statistical Analysis:

One-way model one ANOVA was performed between the different groups. Levels of $P<0.05$ was considered significant

RESULTS

Bacterial density (CFU/100il blood) in blood of infected mice:

The bacterial density in blood of infected animals was increased 3 and 5 days after infection but no bacteria were found in blood 0 day after infection indicating that bacteria were not cleared from the blood during protein deficiency ($p<0.05$) (Fig.- 1).

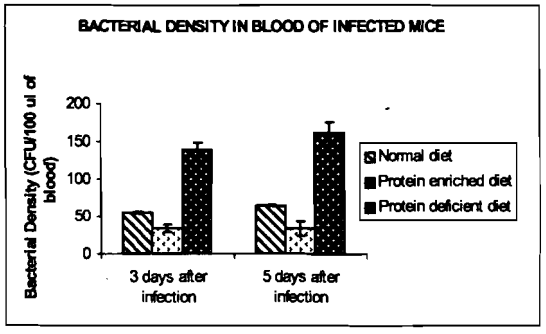

Fig.1 Bacterial density (CFU/100il blood) in blood of infected mice

Bacterial density (CFU/100 mg of spleen) in spleen of infected mice:

The bacterial density in spleen of infected animals was increased 3 and 5 days after infection but no bacteria were found in spleen 0 day after infection indicating that bacteria burden were increased in the lymphoid tissue during deficiency of protein in the diet ($p<0.05$) (Fig.-2).

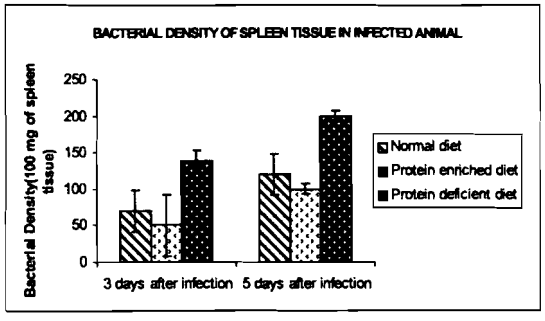

Fig.2 Bacterial density in spleen of infected mice

Bacterial density (CFU/100 mg of lymph node) in lymph node in infected mice:

The bacterial density in lymph nodes of infected animals was increased 3 and 5 days after infection but no bacteria were found in lymph nodes 0 day after infection indicating that bacterial load were increased in the lymphoid tissue during protein deficiency ($p<0.05$) (Fig.-3).

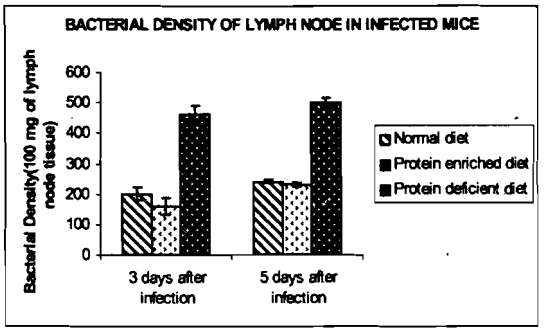

Fig.3 Bacterial density in lymph node of infected mice

Antibody titer of serum in infected mice:

In *S. aureus* infected serum, agglutination titer was obtained at lowest antibody concentration (1:128) whereas, serum from protein deficient diet fed mice followed by *S. aureus* infection showed early agglutination (1:32) Following bacterial infection the concentration of antibody in serum raised in all mice but the concentration of antibody in the protein deprived mice raised in much lesser degree than the protein enriched diet fed mice (Table-1)

Table 1: Antibody titer of serum after *S. aureus* infection in different diet supplement mice

Dietary supplementation	Zero days after infection	3 days after infection	5 days after infection
Normal-lab-diet fed mice	1:16	1:16	1:32
Protein-enriched-diet fed mice	1:64	1:128	1:128
Protein deficient diet fed mice	1:4	1:4	1:2

DISCUSSION

Host nutritional deficiency would lead to an impaired immune response. This impairment in immune function would result in increased vulnerability to infectious disease. Such a situation leaves unanswered, the question of whether any specific dietary deficiency (such as protein) may act preferentially to limit precursor cells, cell division after successful antigenic stimulation or the synthesis of antibody, all of these events require protein synthesis. Therefore, attention to nutritional status of the host is relevant to all aspects of infectious disease from bacterial infection, through the course of disease and recovery and on subsequent infections. Here we reported that protein deficient diet causes compromised immune function including decreased production of antibody, reduced intracellular capacity to clear bacterial burden from blood and tissue. Although mice fed with protein deficient diet for 3 weeks showed higher than control, this weight gain in mice even after protein deprivation may be due to normal ageing of the mice The serum protein level in all mice infected with *S. aureus* gradually decreases with the progress of days after infection indicating loss of tissue protein due to bacterial infection. The serum protein level even in the protein enriched diet fed mice decreases as the mice utilizes body stored protein for production of antibody that is required for defense against the bacterial infection (unpublished observation). Protein diet helps in the clearance of bacteria from the blood since less number of bacteria remains in the blood of protein enriched diet fed mice compare to the normal diet fed mice. Subsequently, the gradual increment in the number of *S. aureus* in the blood protein deprived mice indicating reduced clearance of bacteria due to protein deficiency. Diminished rate of bacterial clearance from the spleen and lymph node of protein deprived mice reveals that bacteria may survive intracellularly for a prolonged period indicating susceptibility to severe opportunistic infectious diseases. Therefore it can be suggested that protein deficient diet fed mice are more prone to infections as they cannot phagocytose efficiently and as a result cannot clear the invading microorganism from spleen and lymph node which may lead to diseased state upon bacterial invasion during protein deficiency.

Bacterial agglutination finds frequent clinical use in diagnosis usually when the patient is well in the road to recovery. A substantial rise in the titre of specific agglutinating antibodies during the time the patient's illness is a good evidence of the identity of the pathogen. When the serum of protein enriched diet fed mice against *S. aureus* was added to bacterial cells for agglutination, the titre was obtained at lowest antibody conc. (1:128) whereas serum from mice after pre-exposure of protein deficient diet followed by *S. aureus* immunization, showed early agglutination (1: 2) or required more antibody to agglutinate the same amount of antigen. The major requirements are that the antibodies be multivalent and that the particles be able to approach each other to be cross-linked by the antibodies. Change in the titre upon pre-exposed protein deficient diet followed by *S. aureus* clearly indicates that protein deprivation may somehow alter the functions of immune cells and it reflects in defective antibody production. These findings indicate that diverse mechanisms exist for the processing of these antigens and protein deficiency may be a useful tool to study this diversity. However, the underlying mechanisms by which protein deficient diet supplementation and prolonged *S. aureus* infection inhibits cell functions as well as alters serum agglutination titre remain still unclear.

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JOINT FOREST MANAGEMENT IN SOUTH-WEST BENGAL : MICRO-LEVEL REALITIES

Amit Kumar Kisku

Abstract ■ West Bengal is a pioneer state to implement the joint forest management programme in India. The programme has influenced the daily life activities of the tribals and other non-tribal communities who are living in the forest or forest fringes. Since its implementation, various assessments have been made and research works on the programme have been undertaken by the researchers, academicians and social scientists. However, very few of them have been able to focus on the ground realities which is revealed from the reactions of those forest living tribals who have to come to terms with the joint forest management programme. This paper, based on an empirical study in a uniethnic Santal village and its adjoining tribal and non-tribal villages of south West Bengal, attempts to throw light on inter and intra tribal interactions in relation to forest management.

Key words- Joint Forest Management, tribals and forest protection committee

INTRODUCTION

By the term 'forest', we generally mean a complex ecological setting in which trees are the dominant form, which provide numerous amenities in addition to being a source of wood product to human population. To a large extent the life-supporting activities of a forest based community are dependent on it. Since long, the tribals and non-tribal communities living in the vicinity of forests, have developed a symbiotic relationship with it. In India around 573 tribal communities constituting 8.08 percent of the total population occupy 15 percent of the total geographical area of the country (Census of India 2001). About 60.04 percent of the forest cover of the country, and 63 percent of the dense forest, lie in 187 tribal districts. These communities had been using the forests for thousands of years in extracting the various forest materials essential for their daily household use, which include leaves, food, fodder, flesh, honey, woods for building houses, fuel wood, woods for making furniture and agricultural implements. In this context, these

people have often been viewed as destroyers of forests through over-utilisation and overgrazing (Saxena 1997). With the advent of British colonial rule, the relationship of these people with the forest started to change and in post-colonial period further changes have taken place. The net result of these changes have witnessed severe curtailment in the use and control over the forest resources by the tribals. Under this general historical background, various State Governments as well as the Central Government have formulated and made attempts to implement different forest protection policies. The recent one is the Joint Forest Management programme (hereafter JFM), which has received a wide popularity in different parts of India. It has also become successful in attracting huge funding from the international donors (Joshi 98/99). But at the village or community level the programme cannot be said to attain the desired results. The programme is not functioning 'as it should be' and the reactions towards JFM from the tribals and non-tribal communities, those who are living in the vicinity of the forests, are also varied (Roy Burman 1997). Being well aware about all the realities, the planners, researchers, academicians and social scientists have worked on and assessed the programme and its impact on the forest living communities. As Krishnaswami (2005) has opined, the Indian State is snatching away the forest rights of the tribals in the name of conservation and protection as the British did. Madhu Sarin (2005) argues "while the constitution provides the state the right to protect environment and wildlife, it also binds the state to safeguard the resource rights, cultural traditions and well being of its vulnerable tribal communities." This case study has also revealed that the policy makers should realize the actual role of the state in safeguarding the rights of the tribals. Moreover, not many researchers, academicians, conservationists and development planners have been able to reveal the ground realities marked by interaction of the forest living communities with the forest management programme producing adverse reactions from the former.

JOINT FOREST MANAGEMENT PROGRAMME IN INDIA AND WEST BENGAL

West Bengal is a pioneer state in India which has made an attempt to integrate the environmental consideration with the social and economic welfare of the people in the form of a policy called Joint Forest Management (WBFDC 2006). The JFM programme was formalized in 1989 in West Bengal (Joshi 98/ 99) and revised in 1990. This revised resolution has provided the opportunity for the majority of the villagers to join the Government Forest Departments to form Forest Protection Committees (Roy 1998). As the product of one of the most successful programmes in West Bengal till date, 3892 forest protection committees have been engaged in protecting 604,334.00 ha. of forest cover (2001 assessment of GoWB). India has adopted the national Joint Forest Management resolution in June 1990, and now 16 out of 21 states have passed JFM resolutions (Joshi 98/99), which are protecting 17,331,955.12 ha. forest cover in India with 84,632 JFM groups (2001 assessment of GoWB).

EXPERIMENT OF JFM AT ARABARI

In 1970, an Indian Forest Service official Dr. A. K. Banerjee had been appointed as chief silviculturist at Arabari, 30 km. to the north of Midnapore town, South West Bengal. At that time the local villagers were constantly disrupting the silvicultural experiment on sal, teak, eucalyptus and other timber species by cutting fuelwood and grazing their cattle on the experimental plots. Dr. Banerjee attempted to

offer the villagers a comprehensive employment programme to absorb them in plantation work. Due to limited budget and employment opportunities, he later revised the programme promising them a 25 percent share of the sal timber and right to all minor forest products including leaves, medicinal plants, fiber and fodder grasses, mushrooms and fruits. In 1972, the Forest Protection Committees (hereafter FPC) were born for the first time. Dr. Banerjee was able to motivate 3,607 people consisting 618 families from 11 revenue villages around the Arabari Forest to form FPC (Roy 2000).

By the early 1980s, a growing number of communities and villagers began to take over public forestlands without the Forest Department's permission. But side by side the local and panchayat leaders throughout the state were informed that forest department was committed to involve communities meaningfully in forest management. And the leaders lost no time in sending this message to the communities concerned. In a situation like that, West Bengal Government came out with the Joint Forest Management Policy in 1990.

THE STUDY AREA AND METHODOLOGY

The broad objective of this study was to observe the ground realities concerning adoption of JFM and the views and reactions of the villagers who have come under the Joint Forest Management (JFM) programme, of South West Bengal. With this objective in mind, I chose my native village where I was born and brought up. The duration for the collection of the data was six months (November2003-April2004) although some additional observations made before and after that period have also been incorporated in the final report. The village named Keshia (J.L. No. 738), which is situated 3 km. away from Jhargram town, is under Sapdhara Gram Panchayat of Jhargram Block, Paschim Medinipur District, West Bengal was my study village. The Forest Protection Committee of the village was formed under Pukuria Beat, Jhargram Range, West Midnapore Forest Division. Keshia is a uniethnic village and is totally inhabited by the Santal people numbering 289 distributed in 58 households. The Forest Protection Committee was named as Keshia Banraksha Committee, which was registered in 1996. With a member strength of 62, it is engaged in protecting 28.34 ha. forest cover, which is about 300m. away from the village. The forest, locally known as Tengia Bir, is composed of uniform sal trees, with some teak and other species.

The documentary data have been collected from the record book of Pukuria Beat Office, Jhargram Range Office, West Midnapore Divisional Forest Office, Block Development Office of Jhargram and also from the Office of the Sapdhara Gram Panchayat. Some qualitative data have also been collected from the offices by frequent visits to these places. But the empirical data were mainly collected from the village Keshia through participant observation. The methods, which have been employed in the study area to collect data on different aspects of this study, are census and social survey, open ended interviews, participant observation, case study, panel discussion and oral history.

FORMATION OF FOREST PROTECTION COMMITTEE AT KESHIA

In 1982, the then Pukuria Beat Officer Ajit Kumar Patra, called a meeting in collaboration with the DFO of West Midnapore Forest Division and Range Officer of Jhargram Range Office at Keshia village. They invited the Pradhan and Upa-pradhan of Sapdhara Gram Panchayat and villagers from Keshia, Dhabadhabin, Nakat and Tengia villages. The officers discussed with the villagers the

importance of forest, the prevailing condition and the urgency for protection of the surrounding forests.Then the villagers, with the help of local leaders like Manoranjan Mahato, Ganesh Mahato, Dhiren Kisku, and Nalinikanta Murmu formed four forest protection committees namely, Keshia FPC, Nakat FPC, Dhabadhabin FPC, and Tengia FPC, for the protection of the Tengia Forest.

But the relationship between the forest department and the villagers was far from cordial. Manoranjan Mahato (65), one of the local leaders, informed that during 1980s, when the foresters used to come to any village for conducting a meeting, the people would flee from the village. People used to think that the foresters were coming to arrest them. And the foresters at that time had interaction only with the local leaders, who also played a conducive role for the functioning of the FPCs at a later period.

After the massive degradation of forests in 1994, the Beat Officer Pranab Malladev and Keshia FPC members sat in a meeting in the same year. The officer made them aware that, as per Govt. order, if the villagers could protect the forest for ten years, then during the felling, the members would be entitled to get 25% share of the net income from the final harvest. The villagers would also have the right to collect dry firewood, NTFPs, such as sal leaves, medicinal plants, vegetable food, etc. After that, the villagers submitted an application to register their FPC. There were three hamlets in the village and an executive committee was formed taking representatives from each hamlet and the FPC was registered in 1996.

PROBLEMS ENCOUNTERED BY THE FOREST PROTECTION COMMITTEE OF KESHIA VILLAGE

The problems regarding the protection of demarcated forest area by the Keshia Forest Protection Committee has been identified in course of our discussion with the villagers described in the following order.

The people of the village Keshia are heavily dependent on agriculture (Table 1). When they are engaged in agricultural activities they do not get enough time to go for regular patrolling to the forest or to attend meetings. Just after the formation of the Keshia FPC, a list was prepared for patrolling the forest and to restrict illegal felling on a routine basis. It was prepared in such a manner that everyday 8 members used to go for patrolling and every FPC member had to undertake the job once in a week. Bhagbat Hansda (45) informed us that there were two minute books of the FPC at the initial stage of the formation of the committee, one for recording the resolutions and the other for routine wise distribution of individuals to be engaged in forest patrolling by the FPC members. But both of them have been lost. The meetings are now held very rarely and in most of the cases the forest department staff simply collect the signatures of the members from

Table 1. Occupational Diversity of the People of Keshia

Composition	Cultivation	Agricultural labour	Non-agricultural labour	Service	Business	Total
Male	105(38.18)	44(16.00)	63(22.91)	16(5.82)	6(2.18)	275
Female	107(48.20)	65(29.28)	25(11.26)		1(0.45)	222
Grand Total	212	109	88	16	7	497

*Figures in the parentheses indicate percentage out of column total

their home. The Pukuria Beat Officer P. K. Giri explained that most of the villagers are agricultural labourers or daily labourers, and they go to the agricultural field early in the morning. As the people are not available for participating in meetings, patrolling could not be done regularly.

There is a communication gap between the FPC members and the forest department. When the FPC was formed, it was decided to conduct at least one meeting of the FPC in a month. But meetings could not be held regularly. There remained a communication gap between them and for the executive committee members "this communication gap created loss of interest among FPC members" According to the Keshia FPC executive committee members, the forest department staff do not come regularly to conduct meetings although they are supposed to hold at least one meeting in a month. Beat Officer of Pukuria defended their action by saying that that there were only 6 departmental staff looking after 33 FPCs in the Pukuria Beat distributed over a large area and it became very difficult for the staff members to cover the area and hold meetings regularly. When the study was conducted, no FPC meeting was held at a stretch for four months, although the signatures of few individuals were collected to show that the meetings were held as per schedule.

During fieldwork, forest officials reported that in the demarcated area of Tengia forests of FPCs other than Keshia, illegal felling was frequent. Only in the demarcated area of Keshia FPC it was not common, although not totally absent. The staff reported that the villagers of Keshia were relatively more united than other neighbouring villages as regards forest protection. However, the villagers of Keshia alleged that the people of adjoining villages showed vindictive attitude towards them and they very often tried to cause harm to the forest protected by the Keshia FPC. The Tengia forest is protected by 5 forest protection committees, namely, Keshia FPC, Dhabadhabin FPC, Nakat FPC, Tengia FPC, and Satyadi FPC and each of them has its own demarcated area for protection. The demarcated area of forest under the Keshia FPC is probably the best protected and for that the credit goes to the FPC members. Koilas Murmu (30), maintains that the villagers from other villages usually frequent their area for cutting wood, as they have already harmed their forests by illegal felling. And most of the time they have a clash with the Keshia FPC members when they are caught red-handed while cutting the trees illegally. Very often the FPC members siege their axes, but one or two days later they again sneaked into the forest. It was revealed that the FPC members have engaged in conflict with the other neighbouring villagers in their effort to protect their own demarcated area of forests. For the females, the situation is far from comforting. They are caught between the two contradictory positions of the males. On the one hand the male heads of the family restrict them from collecting firewood from forests, while on the other they keep them engaged in household activities. This sometime creates conflicting situations. Kalachand Murmu (40), a member of the Keshia FPC but not a member of executive committee, even goes to the forest alone for patrolling and very often he has to come to open conflict with the women of Keshia as well as neighbouring villages. He found women to be the most frequent forest product collectors as they collected sal leaves, mushrooms, sal sticks for brushes, etc., besides firewood. But they also carry large branches of the sal trees for their cattle and very often they cut down the big trees. It becomes very difficult for the male FPC members to restrict these females who belong to the same village, although they restrict females coming from outside from cutting down the trees. To the

females patrolling the forest is a male affair and they do not like to get themselves engaged in it. The interests of the two sexes are rather different.

Even Panchayat and Panchayat Samity members nowadays show a lukewarm interest in forest protection. During the formation of FPC at Keshia village the then Pradhan of Sapdhara Gram Panchayat Binoy Mahato, and Upa-pradhan Manoranjon Mahato played a significant role to encourage the people of the village to form the FPC. But they now care little for forest protection.

Encroachment into the FPC protected forest area by the people of neighbouring villages is also a question of great concern for the FPC of Keshia. A part of the forest of around 5 ha. has been encroached upon by few villagers of the neighbouring Dhabadhabin and Dharampur villages. The Keshia FPC members had brought the fact to the notice of the forest department and also requested them to take a plantation programme in the encroached areas. But their reply was that unless the area was 10 ha. As specified in the rule, the plantation programme could not be undertaken.

The local leaders who performed important roles at the initial stage when JFM was introduced seemed to be less active these days. The people like Dhiren Kisku (55), Sambhu Hansda (58), Manoranjan Mahato (60), who were the influential figures of the area and who took initiatives to form village forest protection committees during the formative period maintaining a good rapport with the forest department as representatives of the villagers no longer show any interest in forest protection related matters. As Dhiren Kisku states, 'I have a business and a family too. If I engage myself in these matters then both my family and business will be affected.'

Lack of interest of the FPC members is also responsible for the poor functioning of the Keshia FPC. Kunal Murmu (25) found forest protection an unprofitable venture. According to him, 'the FPC members after protecting the forest for ten to twelve years will get 25% share of the net income which will be hardly Rs. 500-1000/- per head.' Motilal Hansda (46) observes that the final felling has not yet been done in the *Tengia Bir* (the forest under the protection of Keshia FPC) although the FPC has been protecting the forest for more than ten years. He is of the opinion that unless the people make some money out of the forest, they will hardly find any interest in its protection. We did not find any villager who showed interest to adopt forest-related trades through the selling of forest products to the Forest Department which has the provision for purchasing forest products at a reasonable price from the FPC.

CONCLUSION

This case study is a typical example of the problems found and ground realities encountered by the villagers under the joint forest management programme in general and forest protection committees in particular in the villages of south West Bengal. Central and the state Governments have tightened their laws and formed stringent policies time and again without paying attention to the questions of maintenance of livelihood of the tribals and other forest living communities. At present, people can protect the forests as FPC members but cannot use the resources in the way they have been using it since long. On the other hand, villages distant from a particular forest area may be using the forests which they are not protecting. Under the JFM, new lines of demarcation within the traditional resource base have been created, new sets of roles and responsibilities have also been imposed on villagers from the Forest Department. This has resulted in intra and inter village as well as inter-community conflicts over the use of forest resources.

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