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MOLECULAR ASPECTS OF UROLITHIASIS

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Abstract

Stones in kidney have plagued the civilizations for thousands of years and Hippocrates in 400 BC had also referred to kidney stones in his oath. Up to 15% of people experience stones during their lives. Kidney stones are hard, solid pellets formed in the urinary tract that cause one of the most painful ailments. These are hardened mineral deposits which originate as microscopic particles and develop into stones over time.

The etiology of kidney stone formation varies with inborn errors of metabolism, congenital urological abnormalities, diet etc. Although the disease appears to run in families, it is unclear whether this is associated with inherited physiological factors or is merely the result of shared family dietary preferences. In the past several years, we have begun to decipher the genetic basis of hereditary kidney diseases and related dietary factors which interact to inhibit or promote stone formation. Most kidney stones pass out of the body without any surgery but stones having other complications may require specific treatments. By understanding the etiology and hereditary causes of kidney stones we can find the most effective treatment for it.

Key words: calculi, kidney stones, inhibitors, promoters.

INTRODUCTION

Urolithiasis is the medical term used to describe stones occurring in the urinary tract. There are about six types of renal stones majority of which are composed of calcium oxalate and calcium phosphate which occur preferably in men while magnesium ammonium phosphate stones largely occur in the bladder, predominantly in women. The principal causative factor for the formation of calcium salt stone is attributed to the supersaturation of urine with precipitating salts. Risk indications of kidney stones are family history, chronic dehydration or little fluid intake. A number of other conditions can contribute to the causation of kidney stones. These include urinary tract blockage, urinary infections that recur, bowel disease, and certain inherited disorders. Most kidney stones pass out of the body without any intervention by a physician. Cases that cause lasting symptoms or other complications may be treated by various techniques, including extra-corporeal shock wave lithotripsy, laser destruction of the stone with ureteroscopy and percutaneous destruction of large kidney stones but excessive use of these may cause discomfort also. Although various body fluids in vertebrates are supersaturated with regard to various stone forming constituents yet there are so few cases of pathological mineralization. This selective mineralization process could be attributed to certain biomolecules present in body fluids which by acting either as inhibitors or promoters of mineralization control this process.

Types of kidney stones

There are six major types of crystalline substances involved in kidney stone formation and these have been characterized. These are calcium oxalate (monohydrate-Whewellite, dihydrate-Weddelite), calcium phosphate (Brushite, Apatite, Whitelockite, Octacalcium-phosphate), magnesium ammonium phosphate (Struvite, Newberryite), purine and their salts (uric acid, uric acid dihydrate, urate monohydrate, ammonium acid urate, xanthine), cysteine and others such as drug related matrix (Mandel, 1996).

Organic phase. Morphologically, the organic matrix exists as either amorphous or fibrous forms. The organic matrix is believed to be mucoprotein in nature and has been designated as uromucoid (Keutal and King, 1964). The matrix is found to be formed of 64% protein, 9.6% non-amino sugars, 5% glycosamine, 10% bound water and traces of lipids with organic ash as remainder. The proteins are generally characterized by high glutamic acid and aspartic acid contents and the frequent occurrence of gamma-carboxy glutamic acid. In matrices of CaP crystals, the principal proteins were Tamm-Horsfall protein followed by albumin, prothrombin-related proteins and osteopontin. However, when crystals were induced in the urine of stone formers, albumin was the major component of the organic matrix of both CaOx and CaP crystals

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(Atmani and Khan, 2002). Morever, 9 of the 13 proteins were found in all types of stones: human serum albumin (HSA), alpha 1-acid glycoprotein (alpha 1-GP), alpha 1-microglobulin (alpha 1-M), immunoglobulins (Igs), apolipoprotein A1 (apo-A1), transferrin (Tr), alpha 1-antitrypsin (alpha 1-T), retinol-binding protein (RBP) and renal lithostathine (RL). The beta 2-microglobulin (beta 2-M) was present only in calcium oxalate and uric acid stones (Dussol *et al*, 1995). An *in vitro* study shows that the canine renal distal tubular cell line "Madin-Darby canine kidney (MDCK)" forms calcium phosphate microliths during a long-term culture which is found to contain osteopontin (OPN) and calprotectin (CPT) (Sakakura *et al,* 1999). In another study an extracellular protein, produced from *Pseudomonas fluorescens* strain D with molecular mass of 41.5 kDa was partially purified from stones (Daskalova *et al* 1998). The organic matrix of calcium stones is also found to contain significantly more acidic and complexed phospholipids than uric acid and struvite stones (Khan *et al* 2002). Osteopontin was undetectable in calcium oxalate monohydrate (COM) extracts, but clearly visible in calcium oxalate dihydrate (COD). Prothrombin fragment 1 was abundant in COM but present in COD in lesser amounts than osteopontin (Ryall *et al.,* 2005).

Inhibitors of renal stones

According to the basic laws of chemistry, kidneys should be full of kidney stones. Although the urine is supersaturated with calcium and oxalate, the basic components of kidney stones, only three to five percent of people in the Western Hemisphere form them. Most people pass microscopic calcium oxalate crystals with their urine before they can grow into dangerous masses. It is the natural fate of supersaturated solutions to grow crystals. Something must be working to prevent this in the kidneys and these are inhibitory biomolecules. These inhibitors are also present in soft tissues like tendons, aorta etc. An *in vitro* study demonstrated that flexor tendons of rabbit contain an acidic polypeptide which inhibits the mineralization (Tandon,1997). In another study the inhibitory activity of the aorta extract was found to be primarily due to the presence of three biomolecules having molecular weights of 66, 45, and 27-29 kDa. These inhibitory biomolecules loosely associated with aorta may be involved in the control of calcification associated with arteriosclerosis (Tandon, 1998). The crystallization inhibitors help in avoiding or delaying calculi development (Costa, 2005).An ELISA based assay system showed that a potent inhibitor having molecular weight between 14.2 and 16.2 kDa was primarily responsible for the differences observed in the urinary inhibitory activity between normal persons and kidney stone patients. This assay system can be used to screen human beings for potential stone formers (Moghadam, 2003). Among the most prominent inhibitors is citrate, which, by forming a soluble complex with calcium, reduces the amount of calcium available to form an insoluble complex with oxalate (Chow,2004). High molecular weight inhibitors have also been investigated which inhibit one or more phases of stone formation *in vitro*. These include several urinary proteins viz. Tamm-Horsfall protein, uropontin, prothrombin F1 peptide (Ryall, 2005), uronic acid rich protein (Bikunin), nephrocalcin etc and glycosamino-glycans (Borges, 2005) viz. chondroitin sulfate and heparan sulfate. The bone matrix protein like Osteocalcin, Osteonectin and Gla protein are also known to inhibit stone formation process. An anti-inflammatory protein called calgranulin, plays a key role in the prevention of kidney stones. Calgranulin present, even in minute amounts stops the growth of calcium oxalate crystals, which is the major component of kidney stones. It is made up of two distinct subunits, which are often defective in stone-formers (Pillay, 1998). Another potent inhibitor is inter-alpha-trypsin trimer which could contribute to the differences in CaOx urolithiasis between sexes (Ricchiuti, 2002). Recently, it is investigated that some oxalate binding protein like, histone H(1B) (27.5kDa), nuclear membrane protein (68 kDa) and nuclear pore complex protein (205 kDa) present in nucleus were having the oxalate binding properties. The oxalate binding proteins were thought to modulate the crystallisation process in a hyperoxaluric condition similar to calcium specific binding protein modulators (Selvam, 2003).

It has been observed that cations as well as anions inhibit *in vitro* mineralizaton. Some of these are Mg^{2+} (Gupta,2004), Mn^{2+}, Zn^{2+}, Cu^{2+}, (Ozgurtas, 2004) Co^{2+}, Cd^{2+}, Sr^{2+}, Fe^{2} ,HCO_3^{-1},$P_2O_7^{4-}$, F^{-1}, CrO_4^{2-}, SrO_4^{2-}, $C_6H_5O_7^{3-}$. Studies showed that these inhibitors act by getting adsorbed on the surface of microcrystalline calcium salts, thereby increasing the activation barrier to the recrystallisation process. Similarly amino acids have also been shown to increase the solubility of calcium and magnesium phosphates.

Epidemiology of kidney stones: its causes and distribution

The epidemiology of renal stones includes urinary and preurinary risk factors.

Urinary factors: These factors can be: concentration of ions in urine, volume of urine, pH of urine, enzyme concentration and level of various stone formation inhibitors in urine.

a. **Effect of pH:** Uric acid stones occur especially in patients with very low urine pH (below pH 5.0) and in those with hyperuricosuria (Sakhaee, 2002). In some patients this very low urine pH is caused by a defect in renal ammonia secretion that results in less buffering of secreted hydrogen ion and lower urine pH. Sakhaee *et al.* (2002) suggested that the very low urine pH is in some way related to insulin resistance.

b. **Concentration of salts in urine:** The key process in the development of kidney stones is supersaturation. Salts such as calcium oxalate, uric acid, cystine, or xanthine can become extremely concentrated under certain circumstances. If the volume of urine is significantly reduced; or if abnormally high amounts of crystal-forming salts are present, they precipitate out and form crystals. An *in vitro* study has shown that oxalate - either in crystalline or in soluble form - triggers a spectrum of responses in renal cells that favour stone formation, including alterations in membrane surface properties that promote crystal attachment and alterations in cell viability that provide debris for crystal nucleation. Activation of cytosolic PLA2 appears to play an important role in oxalate stones, triggering a signaling cascade that generates several lipid mediators by (arachidonic acid, AA; lysophosphatidyl choline, Lyso-PC; ceramide) acting as key intracellular targets (mitochondria, nucleus). The net effect is increased production of reactive oxygen molecules (that in turn affect other cellular processes), an increase in cell death and an induction of a number of genes in surviving cells, some of which may promote proliferation or may promote secretion of urinary macromolecules that serve to modulate crystal formation (Jonassen *et al.*, 2004). Further it was found that calcium oxalate monohydrate gets precipitated at membrane lipid rafts (Benitez *et al.*, 2005).

c. **Volume of urine:** Increasing urinary volume is an important tool in the prevention of calcium renal stones. Urine dilution considerably reduces crystallization phenomena induced *in vitro* by an oxalate load in both calcium stone-formers and normal subjects (Guerra, 2005).

d. **Enzymes presence in urine:** The initial step in the pathogenesis of urolithiasis must be the precipitation of an organic matrix of mucoproteins. An important factor in this process may be the activity and/or concentration of the urinary enzyme, urokinase, which would affect the level of urinary mucoproteins. A decrease has been observed in urinary urokinase concentration of renal stone patients which, once again, underlines the possible involvement of urokinase in renal stone formation (du Toit, 1997). Increased excretion of urinary enzymes like LDH, alkaline phosphatase, gamma-GT and beta glucuronidase—in calculogenic rats indicates membranuria and damage to proximal tubules during stone formation (Subha, 1993).

Preurinary factors: The preurinary factors could be both intrinsic and extrinsic. Intrinsic factors include the heredity, age and sex. Extrinsic factors, on which the incidence of renal stones occurrence depends are geographical distribution, climatic factors and dietary factors which include water intake.

a. **Age and Sex:** The inhibition of calcium oxalate crystal growth is influenced by a complex combination of gender and age. With age, the vigorous ability to inhibit crystallization is reduced. Men are at higher risk for kidney stones then women. The daily excretion of magnesium and zinc was higher in men than in women, which was attributed to the higher body weights of the men. Urinary citrate and magnesium excretion were lower, and glycosaminoglycan and zinc excretion were higher in stone formers than in controls. The citrate:creatinine excretion ratio was significantly higher in women than men. The higher citrate excretion in women may explain the lower incidence of calcium stones in women (Trinchieri, 1992). Another known reason attributing to the higher incidence of kidney stones in men than women is alpha-trypsin trimer. It was shown that the inter-alpha-trypsin trimer is a calcium oxalate (CaOx) crystallization inhibitor which is a function of age and sex-hormone status in males and females. In males, a decrease in I alpha TI-trimer was associated with the onset of adulthood and entry into the 'stone-forming years'. Females did not show this decrease, and neither sex showed an increase in I alpha TI-trimer in the above 60 year age-group (Ricchiuti, 2002).

of forming stones differs in various parts of the world: 1-5% in Asia, 5-9% in Europe, 13% in North America, 20% in Saudi Arabia. The composition of stones and their location in the urinary tract, bladder or kidneys may also significantly differ in different countries. Stones in the upper urinary tract appear to be related to the life-style, being more frequent among affluent people, living in developed countries, with high animal protein consumption. Bladder stones are nowadays mainly seen in the Third World, on account of very poor socio-economic conditions. A high frequency of stone formation among hypertensive patients has been reported, and among those with high body mass as well (Ramello, 2000). In India two high incidence stone belts have been found to occur. The first belt starts from Amritsar in North and while passing through Delhi and Agra ends up in U.P. The other belt which starts from Jamnagar in west coast extends inwards towards Jabalpur in central India. Very low incidence areas have been in West Bengal and coastal areas of Maharashtra, Karnatka, Kerala, Tamil Nadu, Andhara predesh (Tandon,1999).

c. **Climatic Factors:** While determining the geographic variability in rates of kidney stones in the United States, it was found that ambient temperature and sunlight levels are important risk factors for stones and the differences in exposure to temperature and sunlight and beverages may contribute to geographic variability (Soucie,1996). Reasons for higher incidence in summers could be an increased conversion of vitamin D_3 to its active metabolites resulting in increased calcium absorption from intestine and decrease in urine production due to loss of water as sweat causes supersaturation of urine with stone constituents.

d. **Dietary factors:** Diet plays an important role in the pathogenesis of kidney stones. Because the metabolism of many dietary factors, may change with age, the relation between diet and kidney stones may be different in older persons. Uncertainty also remains about the association between many dietary factors, such as vitamin C, magnesium, and animal protein, and the risk of kidney stone formation. For men aged less than 60 yr, relative risk for stone formation in the highest quintile of dietary calcium as compared with the lowest quintile was 0.69. By contrast, there was no association between dietary calcium and stone formation in men aged 60 yr or older. The relative risk for men who consumed 1000 mg or greater of vitamin C and magnesium per day compared with those who consumed less than the recommended dietary allowance was 1.41 and 0.71 respectively. This shows that magnesium intake decreases and total vitamin C intake seems to increase the risk of symptomatic nephrolithiasis (Taylor, 2004). Currently, the recommended upper limit for ascorbic acid intake is 2000 mg/d. However, because it is endogenously converted to oxalate and appears to increase the absorption of dietary oxalate, supplementation may increase the risk of kidney stones. The 1000 mg ascorbate twice each day increased urinary oxalate and Tiselius Risk Index (TRI) for calcium oxalate kidney stones in 40% of participants, both stoneformers and non-stoneformers given oxalate and ascorbate for 24 hr (Massey, 2005). Vitamins have been associated with the kidney stones disease. The potential benefits of dietary or supplemental calcium and vitamin D in reducing the risk of recurrence kidney stones have been documented (Moyad, 2003). It was also suggested that idiopathic renal stone genesis could be generated by vitamin A deficiency (Sakly, 2003). Vitamin K deficiency has also been associated with stones of renal origin. Vitamin K has been known to promote the formation of gamma carboxy-glutamic acid which has high affinity for calcium. A reduced carboxylase activity was observed in the urolithic patients, this suggests its important role in the course of renal calcium oxalate urolithiasis (Chen, 2003). Recently it was shown that the "stone clinic effect" which encourages a high intake of fluid and recommended diet, significantly decreased urinary supersaturation for calcium oxalate and the formation of new kidney stones in 80% of patients during first year of follow-up (Carvalho,2004).

e. **Hereditary factors**

Nephrolithiasis is a complex phenotype that is influenced by both genetic and environmental factors. There are several rare, heritable causes of nephrolithiasis that result in the onset of oxalate stone disease early in childhood and frequently lead to renal failure. A study done by Goldfarb *et al.* (2005) showed that kidney stones are genetically linked. Their study done on dizygotic and monozygotic twins showed the heritability of the risk for stones was 56%. Many instances have also showed that nephrolithiasis disproportionately affects white patients. Whites have a higher prevalence of hypercalciuria compared with nonwhites (Maloney, 2005). Previous studies suggest a familial incidence in a subset of persons who have recurrent urinary tract stone disease. Identification

and characterization of families of recurrent stone formers is essential for the identification of unique genetic, environmental and metabolic factors that predispose individuals to recurrent calcium oxalate stone formation. As oxaluria and calciuria have a prominent role in calcium stone formation, any genes that influence their excretion can be considered prime candidates in calcium nephrolithiasis. Among the determinants of urine calcium salt saturation, the rate of urine oxalate excretion is an important risk factor for the development of renal calcium lithiasis. Genetic studies have identified a small group of individuals with known inherited metabolic disorders who develop recurrent calcium oxalate stones at a very early age. Recently a new suggestive gene locus (NPL1) for autosomal dominant nephrolithiasis, has been discovered. It is localized on chromosome 9q33.2-q34.2. The responsible gene will provide new insights into the molecular basis of nephrolithiasis (Wolf, 2005).

There exist many inhibitors in our urine which prevent stone formation. These inhibitors are encoded by genes, so a congenital defect can increase the risk of stone formation. Such proteinaceous inhibitors are bikunin, a glycoprotein crystal adhesion inhibitor, heparin, a recently found anti-inflammatory protein called calgranulin, made up of two distinct subunits, and many more. The genes encoding them are often defective in stone-formers.

The majority of urinary tract stones are composed of calcium oxalate. The genetic contribution to development of this more prevalent, calcium oxalate stone diseases of adult can be due to increased calcium and oxalate absorption. Since vitamin D_3 plays a central role in calcium metabolism in the intestine, kidneys and bone, and its plasma levels are usually increased in stone patients (Weisinger, 1996), a genetic disorder in its pathway was a most attractive hypothesis to explain this trait; the candidate genes contributing to hypercalciuria and, hence, calcium stone formation, could involve some steps of the vitamin D pathway, or anomalies in its receptors, or in its synthesis or activation. Formation of kidney stones is hypothesized to be associated with the vitamin D receptor gene (VDR). On evaluating the association between calcium stone disease and the polymorphism VDR gene in a North Indian population, it was found that the VDR FokI polymorphism may be a good candidate as a marker for calcium oxalate-stone disease (Bid, 2005).

Oxalate is transported into the cells through chloride bicarbonate exchanger band 3 protein 'AE1'. Oxalate competes with chloride for the same transporter (Cousin, 1976). Oxalate is also found to be a substrate of sulfate transport system due to its negative charge and structure. Oxalate is transported across the mitochondrial membrane by a phosphate linked, carrier-mediated system similar to or identical to the dicarboxylate transporter (Strzelecki and Menon, 1986). The affinity of oxalate to these transporters is much less as compared to their respective substrate but due to some mutations in their respective genes an increase may occur in their transportation.

APRT (Adenine phosphoribosyl transferase) deficiency, is known to cause dihydroxyadenine (2, 8-DHA) urolithiasis. APRT catalyses the synthesis of AMP from adenine and 5'-phosphoribosyl-1-pyrophosphate in the presence of Mg^{2+}. In APRT deficiency adenine is oxidized to 2, 8-dihydroxyadenine (2, 8-DHA) by xanthine dehydrogenase (XDH). This defect is inherited as a recessive autosomal trait. The gene is located on chromosome 16q24. Caucasian species predominantly show this defect (Simmonds, 2003).

The genes responsible for several uncommon but important kidney stone diseases have been cloned, including those for cystinuria, primary hyperoxaluria, hereditary distal renal tubular acidosis, X-linked nephrolithiasis (Dent's disease), and hereditary hypomagnesemia-hypercalciuria. Each of these diseases is inherited as a single mendelian trait and has clinical features that distinguish it from other causes of kidney stones. Primary Hyperoxaluria is inherited as an autosomal recessive trait and is caused by a mutation in the gene for alanine:glyoxalate aminotransferase. Inactivation or impairment of this enzyme's activity increases the risk of calcium oxalate stones and nephrocalcinosis leading to renal failure. Cystinuria is an inherited autosomal recessive trait that impairs renal reabsorption of cystine. It is of two types viz type I and non-I type. Type I disease is caused by a mutation in the solute carrier family 3 gene, *SLC3A1* encoding heavy subunit (rBAT) of the heterodimeric transporter. Cystinuria non-I type is caused by mutations in the SLC7A9 gene. Cystinuria is a heterogenous disorder at the molecular level as a patient is a compound heterozygote for one SLC3A1 and one SLC7A9 mutation (Skopkova, 2005). The inherited autosomal

dominant form of distal renal tubular acidosis (dRTA) is caused by mutations in a gene for the basolateral anion exchanger (AE1) responsible for bicarbonate transport. AE1 mutations can result in both recessive and dominant dRTA, possibly depending on the position of the amino acid change in the protein (Yenchitsomanus, 2003). The first molecular defect associated with hypercalciuric stone formation was in the voltage-gated chloride channel protein, CIC-5. The product of a gene on the X chromosome, CIC-5 is predominantly expressed in the kidney, primarily in the subapical endosomes of proximal tubule cells. In Dent's disease, defects in the CIC-5 channel inhibit chloride entry into the endosomes. This prevents the acidification needed for post-endocytotic degradation of low-molecular-weight proteins (Devuyst,2004).The gene responsible for hereditary hypomagnesemia-hypercalciuria, a syndrome characterized by magnesium and calcium wasting in the urine, nephrolithiasis, nephrocalcinosis, and muscle weakness, encodes a protein, paracellin-1, that may function either as a component or a regulator of a cation channel for paracellular reabsorption of magnesium and calcium in the loop of Henle and distal tubule. Homozygous mutations of PCLN-1 results in a selective defect of paracellular Mg and Ca reabsorption in the thick accending loop (Blanchard, 2001). Up to 40% of patients with idiopathic hypercalciuria have a family history of kidney stones. Patients have excessive intestinal calcium absorption, or renal calcium leak, some may also have secondary hyperparathyroidism. This shows involvement of genes such as those for the calcium-sensing receptor (Pidasheva, 2004) renal sodium-phosphate cotransporter, vitamin D-receptor, renal 1-alpha-hydroxylase (vitamin D-activating enzyme), or factors affecting bone mineralization.

Bacteria: as stone stimulator and inhibitor

Several species of bacteria were found to form an intracellular crystalline material when grown in urine obtained from a subject with a history of infrequent renal calculi formation. The following species: *Proteus mirabilis, Proteus rettgeri, Providencia stuartii, Enterobacter aerogenes, Enterobacter cloacae, Escherichia coli*, and *Candida albicans* formed crystals of hydroxyapatite. *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Proteus vulgaris* produced crystals of calcite -II. Several of these bacteria have been isolated from the kidneys of patients with renal caculi indicating that microorganisms may be involved in the nucleation process during calculogenesis (Keefe, 1977).

The nanobacteria are a recently characterized group of extremely small bacteria capable of precipitating calcium salts implicated in the pathogenesis of urinary calculi (Kajander and Çiftçioglu, 1998). These are very small, Gram negative bacteria and range in diameter from 50 to 500 nanometers. They belong to the Proteobacteria group and are about 1/20 the size of most of their larger cousins in this group. These bacteria are unique by developing a needle-shaped calcium apatite cell wall, forming an enclosure around the organism. Under certain growth conditions, including the acidity and mineral concentrations found in urine, the microbes somehow induce precipitation of dissolved calcium, phosphates, and other minerals into carbonate apatite, a major component of many kidney stones. Urease-negative bacteria or those with weak urease activity may also be involved in the formation of struvite renal calculi although urease-positive bacteria seem to make a greater contribution (Rivadeneyra, 1999). When urine is infected with urease-producing bacteria, urea is hydrolyzed to carbon dioxide and ammonia. A urine pH above 8.0 indicates such an infection, usually involving *Proteus, Klebsiella,* enterococci, or *Pseudomonas* species, but not *Escherichia coli*. This change in the chemistry of the urinary tract favours the precipitation of magnesium ammonium phosphate stones. These stones, called struvite, often form when the urinary tract is functionally or anatomically obstructed. In a significant number of the patients with infection and containing magnesium ammonium phosphate stones, no urease-producing microorganism could be cultured, rather *Escherichia coli* was frequently cultured from the stones in these patients. This suggests the possibility that *E. coli* might be involved in stone formation (Bratell, 1990).Certain other bacteria may have a more positive effect. In man and animals oxalate is a non-essential toxic end product of metabolism and is excreted unchanged, since there are no known naturally occurring enzymes in vertebrates capable of degrading oxalate. There are few bacteria in the body which can degrade oxalate. *Oxalobacter,* an intestinal bacteria is believed to help prevent the most common type of kidney stone (Duncan, 2002). Oxalate is detoxified (catabolized) via the action of two enzymatic proteins, formyl coenzyme A transferase (encoded by the *frc* gene) (Baetz, 1990) and oxalyl coenzyme A decarboxylase (encoded by the *oxc* gene) (Baetz, 1989; Lung, 1994) contained in the cytosol of *Oxalobacter formigene. O. formigenes* is a useful bacterium that people pick up from the environment from about 1 to 3 years of age, but is susceptible to antibiotics, dietary changes, health

conditions and unknown environmental factors. People who make lot of stones lack the *O. formigenes* bacteria necessary to degrade oxalate (Sidhu, 1997). A limited number of aerobic bacteria, which are able to utilize oxalate as sole carbon and energy source are *Methylobacterium extorquens, Pseudomonas oxalaticus, Ammoniiphilus oxalaticus and Ammoniiphilus oxalativorans* (Zatsev, 1998). If it can be definitively shown that oxalate absorption and bacterial titers are correlated, measures to increase *O. formigenes* growth in the intestinal lumen might prove to be a novel strategy for preventing calcium oxalate stone formation. Absence of intestinal *O. formigenes* could represent a pathogenic factor in calcium oxalate urolithiasis when antibiotics are prescribed generously, since antibiotics decrease the level of *O. formigenes* in the gut (Mittal, 2005). Dietary supplementation with a beneficial bacterium that plays a key role in breaking down oxalate linked to kidney stone formation could someday prevent stone disease in people. A recently found oxalate degrading bacteria *Providencia rettgeri* have been isolated from human stool. The mechanism of oxalate degradation by *P. rettgeri* appears to be similar to that of *Oxalobacter formigenes*. This is the first report of a facultative oxalate-degrading organism that is one of the *Enterobacteriaceae* (Hokama, 2005).

Treatments

Kidney stones that do not occur as a result of a genetic or metabolic disorder are considered to be a diet-related condition. Proper nutrition can support healthy kidney function and may discourage stone formation, and natural therapies may help ease the pain and spasm that accompanies stone passage. The list of treatments mentioned in various sources for Kidney stones includes: drinking lots of water, Herbal Medicine, Surgical Treatment.

Drinking of water: water intake is useful in preventing stone disease (Meschi *et al.*, 2004). Drinking lots of water (two and a half to three pints per day) and staying physically active are often enough to move a stone out of the body. With increased water intake the kidney stones, of small size and appropriate shape can be passed out with urine. Increased water intake between meals to prevent renal stone recurrence should preferably be achieved with a relatively low calcium water and calcium-rich mineral waters should be avoided (Coen *et al.*, 2001).

Herbal Medicine: Herbs are used to ease the discomfort associated with stone passage. The herbs used are usually diuretic. Some of the common herbs used are, Bearberry (*Arctostaphylos uva-ursi*), Cleavers (*Galium aparine*), Corn silk (*Zea mays*), Crampbark (*Viburnum opulus*), Kava kava *(Piper methysticum*), Khella (*Ammi visnagi*), Seven barks (*Hydrangea aborescens*), Stone root (*Collinsonia Canadensis*).

Surgical treatment: Surgery is the only the option when the stone has a size or shape that will prevent its passage and is blocking the flow of urine or when it is causing damage to the kidney or another part of the urinary tract. Recovery time is longest with open surgery. Today, treatment for these stones is greatly improved and many options do not require major surgery and the recovery time is also reduced. Such treatments include, Ureteroscopic Stone Removal, Percutaneous Nephrolithotomy and Extracorporeal Shockwave Lithotripsy.

Ureteroscopic: It is usually needed for mid- and lower-ureter stones. No incision is made in this procedure. Instead, the surgeon passes a small fiberoptic instrument called a ureteroscope through the urethra and bladder into the ureter. The surgeon then locates the stone and either removes it with a cage-like device or shatters it with a special instrument that produces a form of shock wave. A small tube or stent may be left in the ureter for a few days to help the lining of the ureter heal. Before fiber optics made ureteroscopy possible, physicians used a similar "blind basket" extraction method. But this outdated technique should not be used because it may damage the ureters. Simultaneous combined use of flexible ureteroscopy and percutaneous nephrolithotomy to reduce the number of access tracts in the management of complex renal calculi (Marguet, 2005).

Extracorporeal shock wave lithotripsy (ESWL): Extracorporeal shock wave lithotripsy was first applied successfully in a patient with gallbladder stones in January 1985 and revolutionized for treatment of stones throughout the urinary tract. More than 1 million patients are treated annually with ESWL in USA alone. In recent years extracorporeal shock wave therapy is also used in veterinary medicine especially in equine orthopedics (Braun, 2005). ESWL produces high pressure shock waves which pass through the body. When the wave encounters the calculus, the pressure causes the stone to be stressed, then fractured and eventually disintegrated. A fluoroscopic x-ray system is used to direct the focus of the waves precisely on the stone. A lithotriptor is a medical device used in the non-invasive treatment of kidney stones (urinary calculosis) and gallstones. It works best with stone between 4 mm and 2 cm in diameter located in the kidney. Lithotripsy offers many advantages over kidney stone removal through surgery, with

lithotripsy, there is a sizable reduction in complications and pain. The trauma, inconvenience and pain of a surgical incision are avoided. Post-treatment complications are minimized and recuperation time after treatment is greatly reduced. Recuperation with lithotripsy usually takes only a few days compared to the average of three to six weeks following surgery. Lithotripsy is of two types, shock wave and laser. A shock wave is transmitted through the patient's skin and passes harmlessly through the patient's soft tissue. The shock wave is passed through the kidney and strikes the stone. At the stone boundary, energy is lost and this causes small cracks to form on the edge of the stone. Laser lithotripsy disintegrates the kidney stones using a pulsed laser. An endoscope (small tube) is inserted in the urinary tract next to the stone. With the endoscope in place, the laser fiber is inserted to touch the stone. The laser uses short rapid pulses of energy to break up the kidney stone into smaller particles allowing the body to flush the stone naturally. Sometimes, when kidney and urinary tract stone fragments are being passed, urine flow from the kidney can be blocked. If this causes severe pain or blockage of the kidney, a tube called stent may be placed through the back and into the kidney to keep the kidney drained until all the fragments pass out.

Lateral effects: The Extracorporeal shock waves lithotripsy is fundamental in the treatment of lithiasis. However there are evidences that it can produce renal damage (Cancho, 2005). High-energy shock waves (HESW) when applied to rat did not inhibit the animals growth but caused transitory histological lesion in spleen (proliferative changes in the red pulp) and in liver (cloudy swelling of hepatocytes) (Nardi, 2004). Shockwaves can enhance metastasis from tumors and this effect is attributable to cavitation (Miller, 2004). The effects of ESWL, on patients undergoing renal stone treatment have been studied using activities of glucose-6-phosphate dehydrogenase (G-6-PDH), superoxide dismutase and catalase and levels of malondialdehyde in the erythrocyte haemolysate. This study revealed that erythrocyte lipid peroxidation might be induced and antioxidative defence mechanism may be transiently impaired by ESWL (Aksoy, 2005). A case study also shows an unusual complication like rupture of the kidney after extracorporeal shock wave lithotripsy (May, 2004).

Prevention of damage caused by ESWL

A novel antioxidant agent caffeic acid phenethyl ester prevents shock wave-induced renal tubular oxidative stress and prevent the damage (Ozguner, 2005). Other drugs like remifentanil or fentanyl are used during extra-corporeal shock-wave lithotripsy (Cortinez, 2005). Mannitol may serve as a protective function by decreasing the amount of renal injury caused by ESWL for renal calculous disease (Ogiste, 2003).

Percutaneous Nephrolithotomy: Sometimes a procedure called percutaneous nephrolithotomy is recommended to remove a stone. This treatment is often used when the stone is quite large or in a location that does not allow effective use of ESWL. In this procedure, the surgeon makes a tiny incision in the back and creates a tunnel directly into the kidney. Using an instrument called a nephroscope, the surgeon locates and removes the stone. For large stones, some type of energy probe (ultrasonic or electrohydraulic) may be needed to break the stone into small pieces. One advantage of percutaneous nephrolithotomy over ESWL is that the surgeon removes the stone fragments instead of relying on their natural passage from the kidney.

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TIBETAN IMMIGRANTS IN INDIA : A STUDY OF FAMILY SYSTEM IN CONTINUITY AND CHANGE

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Abstract

The research paper essentially deals with the continuity and change in Tibetan Family system of an immigrant group of Tibetans. The migration of this immigrant group was because of political upheavals in Tibet on account of Chinese claim on this Geographical area. Since 1959 these immigrants are residing in various settlements in different parts of the country. Their approach to life and propensity to preserve the Tibetan tradition and culture is natural. A firsthand filledwork was carried out among the Tibetans-in-exile in the towns of Dharamsala and Kullu in Himachal Pradesh.

Key Words : Tibetans, continuity, immigration, culture change.

INTRODUCTION

Tibet was known for centuries for its inaccessibility. It was one of the most remote as well as most mysterious countries in the world. Geography and climate, in substantial measures, contributed to the mystery in which Tibet lay shrouded. People from different part of the world from time to time explored and informed the mysterious land of world and its people. The Tibetans called their country with a respectful term, *Po* and called themselves *Bod-pa*, which means "people living in the *Bod* region." As early as the 7th centuary A.D. some Tibetans called themselves *Bod*. It is believed that the name *Bod* is derived from the name *Bon*, an animist religion followed by many of the Tibetans at that time (Furan and Wenqing, 1984).

Tibetans can be categorised into three group's viz., Khampa, Amdo and Bodpo. This is basically a geo-demographic division. It may further be observed that though there are no appreciable racial differences among these distantly living population groups, there are some remarkable cultural differences among them which have also formed some stereotypes. Along the eastern border, on the both sides of the Sino-Tibetan frontier, there is the war- like people known as Khampas. The people of central Tibet look down upon the Khampas as the group having an inferior culture. Another group living in the north-west regions adjoining China is known as the Amdo. The Amdo people are reputed to be very clever businessmen and traders. In the central region, this includes the capital region of Lhasa, live the Bodpo people. The Bodpos proliferate even to the regions further west and are reputed to be pick of cultural sophistication in Tibet (Saklani, 1984:57).

Initially Tibetans that fled to India from Tibet were the ones who were most likely to be imprisoned by the Chinese then. These were the families of the present and past Dalai Lamas, former government official and military commanders and troops (French 1991: 190). Thereafter, Tibetans from all parts of the former Tibetan society escaped from their country.

Since 1959, a total of 54 refugee settlements have been established in India, Nepal and Bhutan, 26 of which are agricultural, 17 are agro-industrial and 11 are handicraft-based (Tibetan Bulletin, 1995). The Council of Tibetan Administration (CTA) claims that of the 1,20,000 refugees living in South Asia today almost 70,000 live in these settlements, while another 50,000 live in scattered communities in India and Nepal and four and half thousand live outside South Asia (ibid., 89). The report estimates that thirteen per cent of the total population is dependent on handicrafts, mostly carpet-weaving, which, it notes, provides a valuable source of secondary income for many more refugees; another twenty-nine per cent of the population engages in sweater-selling and other petty trading; and the remaining 30 per cent of the population is engaged in government service or work in hotels, restaurants or shops.

Palakshappa (1978) in his study, "Tibetans in India", described the Tibetan social structure and their culture in India. Saklani (1984) studied an in-depth study of Tibetans in India in his book, 'The uprooted Tibetans in India'. The uprooted Tibetans in India is a study of unprecedented movement of a whole culture under traumatic circumstances and consequential social

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change among the Tibetan refugees in India. Arakeri (1998) in 'Tibetan refugees settled in South India', studied the land and culture of Tibet, problems faced by the refugees while they were on flight, in transit camps and finally in the rehabilitation settlements.

OBJECTIVES OF THE PRESENT STUDY

1) To analyse the various changes in their family structure and other social aspects of Tibetan community after their migration into this area.
2) To analyse the interplay of demographic, technological, economic, political and cultural factors in the Tibetan society.
3) To analyse the impact of non-Tibetan communities on the migrated Tibetans on different aspects of social life.
4) To analyse the different forces that have accelerated the pace of social changes in this community.

RESEARCH METHODOLGY

Nature and sources of data: This study is primarily based on primary empirical data although it has relied on secondary data also for certain other general information regarding uprooted Tibetans in studied area.

Primary data: In terms of aims and objectives of the study we needed information all the earlier movements of Tibetan people, their occupational history, their traditional social system, causes of movements and so on. Such information was not readily available in any of the secondary sources of information. As such it was necessary for us to generate such primary data from the field by contacting these people personally. Accordingly the primary data of this study was collected by means of an intensive field investigation carried out at a micro-level.

Secondary data: However, data on certain other (general) aspects of study was readily available in some secondary sources. Thus for secondary sources of data the following literature was consulted and utilized in the study. These are census reports, Tibetan demographic survey, literature on Tibetan history, religion, culture and its social system, records of the Tibetan administration, Tibetan monthly journals, Tibetan magazines, Information on Tibetan refugees in the foreign registration records, historical documents on Indo-Tibetan relation, printed books, booklets, published articles by research and other scholars and anthropological journals. Accordingly, the information needed for particulars of their community, was gathered from the secondary sources.

Universe of the present study: In the study of continuity and change I have selected two locations i.e.McLeod Ganj (Dharamsala) in Kangra district and different Tibetan settlements in district Kullu. According to Tibetan Demographic Survey (TDS) 1998, there are 86149 Tibetan settlements in India. Tibetan migrants mostly populate these Tibetan settlements in Himachal Pradesh. The Tibetan population in above said pockets is 8694 and1934 respectively.

After having decided about the field or the universe of study, it was necessary for me to decide upon the actual method of doing fieldwork. Following the method of simple random sampling carried out the actual process of selecting the migrant families. The researcher selected a total sample of 200 and 103 Tibetan families from Dharamsala and district Kullu respectively, so the total sample was 303 families.

Tools of data collection: The objective of the study motivated the investigator to conduct an empirical investigation based on fieldwork. Traditional anthropological techniques, namely observation, interviews were conducted as primary tools of data collection. Also these were supported by schedules (partly structured and partly unstructured) for collecting the general opinion and attitudes on various variables related to the theme of the present study. To know more about these uprooted people, case studies of these families were also conducted.

Data analysis: For quantitative data analysis appropriate statistical tests (e.g. percentage, central tendency) were resorted to. Also different forms of graphical representation (e.g. graph, bar diagram etc.) were used to depict data for better understandings.

THE FIELDWORK

A culture is mired in meanings, all so well entangled which to naked eye appears like a plain text. An ethnographic description is like a prism: It refracts the culture and first 'impregnates' it with meanings. In the second step, through various ways (methodology), the ethnographer tries to uncover this meaning, and finally, interprets this meaning. In this work I have endeavored to be a humble researcher of the Tibetan refugees in India. I have utilised a host of methods in understanding the problem. Through observations, informal interactions, interviews, I have inquired into the meanings and values Tibetans and those surrounding them, attach to various aspects of their life. My interest in the community rose since my school time because I had many Tibetan friends in Kullu, we used to play together and sometimes I used to visit their houses

and the monastery in Manali with them. At Manali there is a big Buddhist monastery and I had, with my Tibetan friends, attended their fairs and festivals especially *Losar* (New Year celebration). Also I used to take fancy to many items being sold in the adjacent Tibetan market. This initial interest was further strengthened in the light of suitability of the community for my research problem I had proposed to study.

A scientific investigation requires a most cordial relation with the informants. The place of research, dialects, and culture everything may be known to begin with, both to the researcher, as well as to the informants. Frequently in the field the informants were hesitant to unfold the facts to a stranger, even when he claimed himself to be a researcher and promised anonymity and confidentiality. Besides innumerable barriers such as shyness, ego, fear, apprehension, had to be conquered and eliminated. The language problem for communication was negligible which was sorted out with the help of a suitable interpreter. To overcome the difficulty of initial introduction to these people and to collect the necessary information in depth, introductory letters were also collected from the concerned university in Chandigarh and from concerned Tibetan authorities.

The fieldwork for the present study was conducted at McLeod Gang (Dharamsala) and various Tibetan settlements in district Kullu (Manali, Patlikuhl and 15 Mile Camp) in Himachal Pradesh. The fieldwork in Kullu district was conducted from September 2000 to December 2001 and in Dharamsala from February 2002 to September 2002. The Tibetans settled in Kullu and Mcleod Ganj had different sets of problems. In district Kullu some of the Tibetan youths were known to me since long time because myself belong to Kullu Township. On the other hand the Tibetan settlement in McLeod Ganj was totally new for me.

During investigation general instructions to be followed by researcher maintaining informal social relation with the informants. The informants of the present study are those who have been migrated from their homeland and are struggling to survive in a foreign environment, which is everything new for them. In the beginning I had to struggle to mix up with Tibetans. It is because Tibetans are known for not interacting with outsiders. They thought that if the facts are disclosed to outsider it could create some problems to them. The main reason of not disclosing the facts is that Chinese could get this information's. At Kullu one of the old aged respondents asked me whether I was a Chinese spy. I convinced him, after much effort that I am a University student and wish to know about them and the difficulties and problems faced by their community. These Tibetans thought that their identity could create problem for their relatives in Tibet. So it was observed that Tibetans always try to keep secretes about their facts. First time in September 2000 when I met Tibetan welfare officer Miss Tsamchee Chakhang at Dhobi (Kullu) she directly refused to give any information about Tibetans. But later on I told her that I am a local resident of Kullu and is pursuing doing research work on the Tibetans in exile. After my repeated visits and requests she finally agreed to help me as much as possible. Later on she even provided much important information about Tibetans in Kullu town and the surrounding areas.

In the beginning during my fieldwork the language for communication was a problem. Very few members of older generation of Tibetans know Hindi or English; they were used to speak in their own language only. To overcome this problem one of my Tibetan friend, Mr. Tenzin accompanied me during my data collection. He is a Graduate from, Panjab University, Chandigarh and was known to me for a long time. Also during the fieldwork some times. I stayed for two to three days with a family, to whom I was a welcome guest, only to get maximum information. On several occasions of a family would offer me to have a meal with them. My respondents had often served their traditional (salted) tea. What I observed, while taking meals with them, that, their menu was much like the Indian food. Rice and *Chapati* with some vegetable curry. One day when I was conducting my fieldwork at Patlikuhl, which is about 30 km. from Kullu, I met one of local Tibetan families where some members of the family were drinking *chhang* (Tibetan wine). I sat with them and started informal conversation with them. Suddenly one man offered me to have *chhang* when I politely refused to drink it; they felt unhappy and were reluctant to respond positively to my queries. I was in a dilemma and thought that unless I join them in their drink session or have a sip of the drink, my purpose of visiting them would be defeated. So, I accepted their offer and took a few sips of *chhang*, which I found to be sour in taste. That act of mine really helped me in seeking favourable response from the group.

Sometimes direct conversation with the respondent, either in Hindi or in Tibetan language, made the discussion mere congenial. A knowledge of Preliminary Tibetan languages in a tradition a way and accelerated the confidence building process and familiarity with the respondents.

Yet the fieldwork in McLeod Ganj (Dharamsala) presented

a different set of problems. Before initiating the data collection at Mc Leod Ganj I met Mr. Norzang, the then president of Tibetan Students Association, Chandigarh, and also other Tibetan students in Chandigarh. As most of these Tibetan Students stay in sector 15, of Chandigarh, I was able to meet them almost daily. Of particular help were those students who belonged to McLeod Ganj. In my interaction with these Tibetan students my aim was to elicit as much information on the Tibetan settlements people of McLeod Ganj as possible through informal discussions. My fieldwork in McLeod Ganj (Dharamsala) was greatly helped by Mr. Norzang who gave me references and addresses of persons whom I could contact and seek their local help. Subsequently I met most of them and found their cooperation as great help.

During early days of my fieldwork I visited various important places like library of Tibetan works and Achieves, central Institute of Higher Tibetan Studies, Tibetan Medical Center (*Men-Tsu-khang),* Department of Information and International Relations (DIIR), Planning council, Namgyal Monastery and Nechung monastery. After visiting these offices and meeting those Tibetans who are working in these offices I gathered much relevant information relating to my research problem. During these visits I made good relationship with the librarian of Tibetan works and Archives Mr. Pema Yeshi, Dr. Kunchuk of Planning Council and Miss. Decchen of Tibetans Children's Village, upper Mcleod Ganj who is a computer instructor there.

By visiting McLeod Ganj and surrounding areas where the Tibetan settlements are located several times I tried to make my presence familiar to the inhabitants of the area so that I am not treated with suspicion and treated as an intruder. On the very first day of formal data collection I met one of the Tibetan shopkeeper whose reference was given to me by Mr. Norzang. Initially, he refused to talk to me. On my repeated request and appeal, he finally agreed on one condition nowhere should I disclose his or his address in my thesis.

What I observed during fieldwork filed work in McLeod Ganj is that Tibetans are educated and well mannered than in Kullu. Here people were aware of those who come here to study or research work. On the other hand in Kullu area people were least aware of all these things. Second thing I have observed that Tibetans respond well to foreigner researcher than Indian. In my case I was also facing the same problem but later on visiting them many times it was solved.

According to Tibetan Demographic Survey, 1998, Tibetan population in India is 85, 147, out of which 48,005 are males and 37,142 are females. Based on Indian regional classification, the Tibetan settlements are grouped into five principal regions, with 28.84% , the northern region recorded the largest share of the Tibetan refugee population, followed by 27.92% in the south, 18.26% in the east, 9.83% in the central India and least in the west.

In Dharamsala, the total population of Tibetans is 8694, out of which 4711 are males and 3983 are females. On the other hand, in district Kullu, total population of Tibetans are 1934, out of which 988 are males and 946 are females.

The major focus on Tibetan refugee's religious and national sentiments is Dharamsala, the present residence of His Holiness the Dalai Lama and the seat of many institutions serving the interest and cohesion of the thousands of Tibetans dispersed over the Indian subcontinent. While Lhasa is nostalgic memory for the older generation and a vague dream for those who left Tibet as children, Dharamsala is a centre of pilgrimage within the reach of most Tibetans living in India and Nepal. The main Tibetan settlement in Dharamsala is located in the township of McLeod Ganj.

It has been observed in Dharamsala that most of the people i.e. 63% are engaged in petty business like readymade garment shops, hotels and restaurants, tourist guides and small gift shops etc, 4% are in monasteries, 3.50% in nunnery, 2.23% in other residential schools, and about of 26.38% are in Tibetan Children's Village (TCV).

It has been observed in district Kullu that most the people i.e. 91.67% are engaged in petty business like readymade garment shops, hotels and restaurants, as tourists' guides and small gift shops etc, 1.65% are in monasteries, nun in nunnery and other residential schools, and about of 6.67% are in TCV.

The Family Structure in Tibet

A Tibetan family, based on kinship structure, can be classified into two major types- (1) elementary or nuclear family (2) extended or joint family. In Tibet by the necessity of the economic co-operation, joint families were more advantageous and were more in practice. Tibetans, who lived as peasants, nomads and traders, had many functions to perform simultaneously. Different occupations in the family, such as peasantry, nomadism, trade, made the male members to go away from their main household for longer period of time. During their

absence it required others to maintain their family and it was their own brothers who could do it. This factor necessitated them to bring into existence the marriage of all brothers to a single wife to hold the family jointly in economic co-operation. The same is known as fraternal polyandry. This was seen among all the classes in Tibetan society. The possible reasons for all the classes can be noted as below. The nomads had a dualistic system of life, *i.e.*, they came back to their homes only during winter season, and during other seasons they were away looking after their flock of sheep and herds of cattle. Another factor, which relates generally to all the classes and mainly to the peasants, was that they held land from their nobles and had an obligation to pay heavy taxes in terms of services and cash needed by their immediate master. It was necessary for them not only to maintain a joint family but also the practice of polyandry so that the family and its property are inherited through a single line. The unity of the family was also sought to be maintained. Among the nobles, who were more interested in keeping the family property intact so as to stand high in socio-economic and political position, maintained joint families, and elementary families were not preferred. Landlords or nobles, holding big estates and large number of serfs attached to them, tried to retain them intact by maintaining a single line of inheritance of property. Breaking-up of family property, therefore, was strictly forbidden among the Tibetans. In the cases where polyandry was not practiced many other methods were adopted to keep the family inheritance in a single line: wherein the eldest son inherited the entire property and also married to continue the family pedigree so as to inherit the family and its property in the next generation. Among other sons of the family a few of them joined the monastery, accepting celibacy and a few others married matrilocally to other families (*Mag-pa)* and became members of those families.

In those families where a male heir is not present to continue pedigree, accepted *mag-pa* in marriage for the eldest daughter of the family and others went to the same *mag-pa* in polygynous union and the family pedigree was continued or if they liked to marry outside they were allowed to, and were married off at any time, even after the dissolution of their *mag-pa* marriage. In a *mag-pa* household the woman holding matrilocal residence after marriage also holds the matrilineal succession and matriarchal authority over the property of the family which she inherits. The other type of family through the patrilocal residence, matrilineal succession and patriarchal authority, was much more in practice and was termed as *bag-ma* or *na-ma*. Value was always therefore attached t the continuation of the family name and family property without fail or breaks either through matrilineal or patrilineal descent. In *mag-pa* institution, all the rights of authority were with the woman, had the option to take some more husbands into the union forming non-fraternal polyandry.

Thus these two forms of residences–matrilocal and patrilocal, with their preferred type of marriages in the system formed the authority and succession through their respective system. This helped the family to bring stability and hold the family in elementary or extended form depending on the necessary of situation (Arakeri, 1998:112-113).

The Tibetan concept of social life is best expressed through the fundamental human aims and obligations, according to which an individual owes three debts: to gods, to sages and to ancestors. He is required to pay them off by responsible living in society, through devotion to worship and rituals, learning and teaching as well as through founding a family and raising children. If he fails, he is denied salvation and gets interminably bound to the cycle of transmigration. This comes very close to the traditional Hindu postulates of life-values. On describing the structure of Tibetan society, Stein pinpoints its basic complexity and also its secret of stability. He remarks " One feature of some morphology sufficiently common to be significant may be expressed in a formula, equally applicable to the family group and to the structure of political power. It is co-existence of two principles, which are both inter-dependent and antagonistic: egalitarian joint ownership and hierarchy (1972:94). This accord–discord relationship of these two principles is illustrated in the family system, and its two basic organising principles, i.e. the rules governing marriage and those governing succession and inheritance. However, the relationship embraces a much wider area. In Tibet the family and the structure of political power seeping deep into social system were so harmoniously balanced that from it came a unique (almost eternal) stability in the social system.

Continuity and Change in Tibetan Family Structure: The Present Scenario

After studying in detail the traditional family structure of the Tibetan society, the emphasis is now turned towards continuity and change in the family system in exile. In this respect the approach is very selective because it is pointless to focus on those features of the family systems which could have been transplanted here from there i.e. from the 'native' social context. The family, which is

most important part of all societies, assumes added significance in the Tibetan refugees context because of the uniquely important place it has had always occupied in their traditional society. Before outlining the changes that have found in Tibetan family system, it would be appropriate to describe how and from which region of Tibet these refugees came from. The movement of population from Tibet was by and large because of political interference of Chinese rule. All those respondents who have migrated from their homeland said that the main reason of their flight was essentially the political interference of Chinese rule. During the flight refugees left their native land, most of them did not move out as families but as individuals, or as parts of a family and with their community members. During the flight to India some of the respondents lost their kith and kin either kiled by Chinese army or by natural calamities. The passage to India was very hard and tough.

Many families had been reduced to the status of broken families since their members were lost. Widows or those, who lost their spouse in flight., joined the original noble family to which they belonged. In some cases the family grew out of new combinations of separated spouses and broken families, and the human problem would have been tremendous but for the freedom granted by the Tibetan tradition to forge new ties and bonds. During flight many separated from their families.

These separated men and women remarried and established new families with new partners. In certain cases after being separated by the force of circumstances, they searched desperately for their old partners. Thus during flight to India due to certain insecurity, uncertainty and necessity to lean on each other a few co-travelers became life-partners, without any formal marriage ceremony.

Form and size of family: In Tibet joint family system was mostly adopted by all the people. Tables 1 and 2 shows the change in the family structure after migration.

Table 1: Change in family structure (Dharamsala)

	Before migration		After migration	
Family structure	No.	%	No.	%
Joint	172	86	36	18
Nuclear	28	14	164	82

Table 2: Change in family structure (District Kullu)

	Before migration		After migration	
Family structure	No.	%.	No.	%
Joint	90	92.7	25	25.7
Nuclear	13	13.39	72	74.15

In Dharamsala, it is observed that 86% of families were of joint families (in Tibet before migration) and 28% of

Table 3: Attitude towards the institution of Joint Family (Dharamsala)

	As far as possible one must try to live in a joint family				A joint family helps in the economic development of the family				A joint family hinders the development of a child's personality			
	No.		Total		No.		Total		No.		Total	
	M*	F*		%	M*	F8		%	M*	F*		%
Agree	65	43	108	54	95	65	160	80	0	0	0	0
Undecided	30	02	32	16	13	17	30	15	12	8	20	10
Disagree	25	35	60	30	12	8	20	10	108	72	180	90

Table 4: Attitude towards the institution of Joint family (District Kullu)

	As far as possible one must try to live in a joint family				A joint family helps in the economic development of the family				A joint family hinders the development of a child's personality			
	No.		Total		No.		Total		No.		Total	
	M*	F*		%	M*	F8		%	M*	F*		%
Agree	45	35	80	80.11	47	38	85	82.4	0	0	0	0
Undecided	3	4	7	6.7	5	7	12	11.6	4	3	7	6.7
Disagree	6	10	16	15.5	2	4	6	6.79	50	46	96	92.3

*** M-Male, *F-Female**

the families were nuclear in form. But after migration to India 36% of families are of joint families while 82% of families are of nuclear in form.

In Kullu, it is observed, that 92.7% of families were of joint family and 13.39% of families were of nuclear in form before migration. On the other hand after migrating to India it is observed that 74.16% of families are of nuclear type and 25.7% of families are of joint type. Tibetans after migrating to India have lost their traditional family structure. On the other hand, most of the families are adopting nuclear families. Most of the respondents agree that the change in their traditional family structure i.e. joint family to nuclear family is the result of impact of local population, economic condition and lack of space in the new place. On the other hand, in Tibet they had their own agricultural land, sheep and cows etc. but in Dharamsala and district Kullu these Tibetan refugees do not have such facilities. Respondents were asked on their attitude towards joint family (Tables 3 and 4).

The tables 3 and 4 show that the attitude of the respondents towards joint family system in Dharamsala and district Kullu. 54% of respondents from Dharamsala and 80.11% of respondents from Kullu agree that as far as possible one must live in a joint family. 80% of respondents from Dharamsala and 82.4% of respondents from district Kullu agree that a joint family helps in the economic development of a family. Out of total respondents there was about 83.3% of male and 71.4% of female respondents in district Kullu and 54.1% male and 53.3% female respondents in Dharamsala agree that one must live in a joint family. About 77.5% male and 88.8% female respondents in district Kullu and 79.16% male and 81.2% female respondents in Dharamsala agree that joint family helps in the economic development of a family. In Dharamsala as well as in district Kullu, the respondents said that it was not possible to stay as joint family here in India. It is because they do not have proper accommodation in exile. Most of the families are living in one or two rooms only. In Dharamsala most of the families are living in *pukka* houses. On the other hand, in district Kullu some of the families are living in small *kaccha* houses, built of mud. These refugees have been given land temporarily in the bank of river Beas to build houses. Most of these houses have not more than two rooms. Another factor of adopting nuclear family in these places is lack of any agricultural land. Most of the families are either engaged in small petty business like sweater-selling or services sector of different departments of Tibetan govt. in exile. Thus the traditional system has lost its values for most part and the youngsters have new values of life in the present changed situation. The present economic conditions also led them to adopt nuclear family in exile. The new generation with its new perspective and values are now moving away from their natal family. Thus consciousness to maintain the traditional family, its property, mainly its name and breed through descendents, is now shifted to the maintenance of their own individual families with their own independent occupations.

The younger generation also has disregarded the authoritarian dominance of the head of the joint family. The emergence of modern education and the consciousness of their status and roles by women have contributed to the preference for a nuclear family. This has affected the joint family system since the women coming from different family background with different views prefer to live separately with their husbands. Because of this, number of elementary families has increased in exile. About older people in such families these days do not have any interference in family activities. Their knowledge about the society and culture too has become obsolete in the modern situation. However, they continue to stay with their sons comfortably, concentrating on meditation. Some attend to such jobs as looking after their children and guarding their houses when others go for work.

Residential Pattern: In a household, residential pattern of the members is a basis for the structure of the family. In a family, members are linked by either consanguine or affinal relationship. These members of the household are grouped together to form a single family or have broken out into smaller groups. This may bring some changes in the structure of an ideal family in which the household consists of a man, his wife and children. Nuclear or extended families have their own ideal structure. This ideal structure cannot be realised by some of the families. Such families are affected by the biological or socio-economic factors such as divorce, bachelorhood, widowhood or barrenness. These have resulted in the adequacy of self- support for economic and social means. These families which were living separately have now reunited with the families of their original affinal or consanguinal ones to form either nuclear or joint families which are dependent on each other for economic co-operation. There are, therefore a few deviations from the ideal types of families. There are some causes which bring deviation in the ideal pattern of the families. These are such as due to economic co-operartion between brothers and brother-in law to take up different occupation like readymade sweater selling, running small restaurants

etc. all at one time. In some cases those who are married while their place of residence is not finalised by the respective parents, are staying with their parents to support them economically. Today there is also much social and spatial mobility.

In the present day society in Dharamsala and the district Kullu it is my observation that individual member can go out and established the family of their own independently. Earlier in Tibet, the residence used to be either patrilocal or matrilocal. But here in the studied areas the shift is towards patrilocal and neolocal. Because of these changes, rise in the number of nuclear families increased. It was also observed during field study, in those families where older parents were alive they try to hold the family intact in the traditional form, more for the emotional and social reasons than economic. A number of factors which have put the strain on the family structure of immigrant Tibetans in Dharamsala and Kullu. The strain producing factor are namely loss of family member while fleeing from Tibet, break-up of families due to family tension and widowhood. Due to strain on the family structure the members of the affected families face difficulty in supporting themselves economically and socially. There are few cases in rich families once separated, united and setup a new unit.

The family's authority structure: Today most of the families in the studied areas are patrilineal. However, such a family has lost most of its function and authority derived from those functions. In traditional family the economic basis of the authority was on head of the family, which was derived from the control of the family property, has now changed in new place. It is observed during the field study, the perception of change in authority among older age group is higher than of younger generation. The older age group expressed that a change has come about in the authority of the father or male head of the family.

Notwithstanding the fact that the younger age group might not have been in position to compare the two family settings, the new and the old, thus most of the youngsters said that there may not be change in authority in family. Besides age, education could be considered as another important variable in the perception of change in family behaviour. Hence, effort was made to outline family change by taking the educational status of the respondents. The educational achievement norm of the younger generation was found to be appreciably higher than that of the older generation. Most of the youngsters thus are more educated than that of the older generation. Though the educational status does not appear to be highly significant in the perception of change in father-son relationship, it does indicate that educated ones, in a slightly higher proportion than others, view the traditional relationship structure as having remained unchanged. During the fieldwork it was also observed among young respondents that the Tibetan youth, particularly the educated have evolved some sort of notions that the father should also deserve respect here in the new place, whereas in the native traditional society the father automatically got respect from his children.

The intimate emotional bonds prevalent in traditional Tibetan households are gradually becoming less cohesive. Family, being the primary unit of every social structure when subjected to immense pressure of change, becomes a motivation for bringing about changes in the other spheres of social life. It was observed that in the refugee communities the percentage of nuclear family units was large than the joint-type ones.

It is, of course, understandable that the situation could not have been different. Firstly, it was in very rare cases that whole families were able to make their way out of Tibet. In the early stages of refugeehood many desperate men formed new marital alliances and in most of the cases without any religious or social formalisation. It could be said that in a large majority of cases the family cycle started afresh after migration into India. Secondly, the economic basis of the joint family could not be recreated in India even in the agricultural settlements because the size of land holding allotted to individual families was too small to support a large family. Thirdly, the dwelling space provided to a household was sufficient only for a small family. Fourthly, the increased mobility common to people of non-agricultural occupations, who also came over to India along with the others, acted as a strong deterrent against formation of large joint families. The economic forces of the family rooted in joint inheritance have drastically weakened in the new set-up. The polyandrous family has become very rare and there is greater freedom of choice in contracting matrimonial relationships. Simultaneously the family has lost many of the traditional functions. Another remarkable feature observed was that to offset and fully absorb the decreasing role of the family, the community has created a whole range of welfare institutions.

There is a growing trend among the Tibetan youth to set themselves up in independent professions and occupations, which has led to the loss of parental authority over their children, calling the parents old-fashioned, ignorant and uneducated persons unable to guide, is

another ominous sign of change. This is one of the aspects of change about which the older generation is greatly apprehensive; and for which they frequently express their feelings of emotional insecurity.

The farm holdings having become meager are only suitable to serve as kitchen gardens. Therefore, majority of the Tibetan youth are looking for worthy opportunities in the sphere of trading and marketing.

While analysing the structural conditions for the change which the Tibetan refugee community is undergoing, it is necessary to highlight the role of the new generation that has grown up outside Tibet. The study has revealed some rapidly emerging inter-generational differences among the Tibetans, which is a well known, universal phenomenon faced by a almost all rapidly modernizing industrial societies. Though the traditional Tibetan society barely had any concept of youth as a social category, there is now a distinct youth sub-culture emerging in the new setting. The emerging Tibetan youth, as can be clearly seen, are foremost and most vocal in questioning the relevance of the traditional structure and set-up. One may call them the dissidents both in thought and action. The younger generation Tibetans exhibit attitudes and values different from those manifested by their elders with regard to family and marriage, religion and politics. There is also a perceptible divergence in their life styles.

Among the Tibetan refugees income rather than wealth has become the main criterion for defining one's class or social position. The members of the old aristocracy who thrived on incomes from land and property have suffered a sharp fall in their economic and social status, whereas those from the commoner's rank, who have now acquired professional skills, have substantially raised their income. Admittedly, power in the community, to some extent, is still derived from the exercise of political – administrative authority through the Dalai Lama's Administration, which is, by and large, still monopolised by the members of the old nobility who continue to enjoy a superior status. Yet there is no denying that they have lost much of the traditional force and their authority is no longer uncritically accepted.

The Tibetan youth, as a representative of second generation of immigrant Tibetans, reflects the desire of the developing mind to accept the new values without rejecting the old tradition. The cultural configuration evolved by them bear a semblance of harmonious coexistence between the old and the new.

The conclusion thus is inevitable that on the whole the Tibetan refugees in India have shown a remarkable capacity of synthesis. and adaptability between their pristine cultural traditions and modernity.

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QUERCETIN: A PLANT SECONDARY METABOLITE OF MEDICINAL INTEREST

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Abstract

Quercetin is a plant pigment in the form of secondary plant metabolite and belongs to flavonoids group of substances. It is found in many common foods, blue green algae and other plants. It is available in plants as glycone carbohydrate conjugate. Bio-synthetically, it is derived from a chalcone that is synthesized by chalcone synthase, an enzyme ubiquitous in plants. The molecular mechanism, at least in higher organisms, is still a matter of debate. A number of quercetin's effects are due to its antioxidant and anti-inflammatory activity. It shows many beneficial effects in human beings such as cardiovascular protection, anti-inflammatory, anti-allergic, anti-diabetic and anticancer activity.

Key Words: bio-synthesis, mechanism of action, medicinal properties, quercetin.

Plants are capable of synthesizing an overwhelming variety of low molecular weight organic compounds, called secondary metabolites, usually with very unique and complex structures, for defense and survival in the ecosystem (Kutchan, 2001 and Oomah, 2003). Many of these compounds are differentially distributed among limited taxonomic groups within the plant kingdom and conversely, each plant species has a distinct profile of secondary metabolites. Currently, exceeding 100,000 identified substances have been characterized, and their bioavailability and bioefficacy in humans is studied (Manach *et al.,* 2005). Secondary metabolites belong to three major chemical classes - Terpenes (a group of lipids), phenolics (derived form carbohydrates) and alkaloids (derived from amino acids).

Quercetin (3, 3', 4', 5, 7 pentahydroxyflavone), a polyphenol and a chemical cousin of the glycoside rutin, is a unique flavonoid that has been extensively studied by researchers around the world, starting with the discovery of both vitamin C and flavonoids by Albert Szent- Gyorgyi who received the Nobel Prize in 1937 for research in this area. Both Vitamin C and flavonoids benefit plants by providing them with antioxidant protection and also confer protection against climatic variations (in wind, rainfall, temperature and sunlight). Like vitamins, these compounds are not produced endogenously in the body, and must be supplied either through diet or nutrition supplements.

Quercetin is a plant pigment and belongs to a group of polyphenolic substances known as flavonoids. Quercetin is flavonol that constitutes the major bioflavonoid sources in the human diet (Strack, 1997). It is found in many often consumed foods, including apple, onion, tea and Brassica vegetables, as well as Blue Green Algae, many seeds, nuts, flowers, barks and leaves. It is also found in medicinal botanicals, including *Ginkgo biloba*, *Hypericum perforatum* and many others. Due to its potentially favorable impact on human health, the polyphenol quercetin has come in focus of medicinal interest (Oomah, 2003). Quercetin appears to have many beneficial effects on human health, including cardiovascular protection, anti cancer activity, anti-ulcer effects, anti-allergy activity, cataract prevention, antiviral activity and anti inflammatory effects.

STRUCTURE

Plant phenolics are generally characterized as aromatic metabolites that possess, or formerly possessed, one or more "acidic" hydroxyl groups attached to the aromatic arene (phenyl ring) (Swain *et al.,* 1979). Flavonoids are phenolic glycosides and the structure of their aglycones are based on the flavan structure which consists of two aromatic rings (A & B), joined in a chroman structure by a three carbon unit (ring C) and thus they are, phenylpropane (C_6-C_3-C_6) derivatives with OH groups attached. A multitude of other substitutions can occur, giving rise to many types of flavonoids. Quercetin is known chemically as 2-(3, 4-dihydroxyphenyl)-3, 5, 7-trihydroxy-4H-1-benzopyran-4-one and 3, 3', 4', 5, 7- pentahydroxy flavone. It is also known as meletin and sophretin.

Quercetin is typically found in plants as glycone or carbohydrate conjugates having a sugar molecule (rhamnose, glucose, galactose etc) attached to the centre

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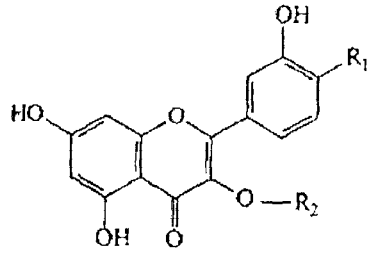
(C) ring. (Manach *et al.*,1998). Quercetin itself is an aglycone or aglucon (meaning minus the sugar molecule) that is, quercetin does not possess carbohydrate moiety in its structure. Recently, the flavonol glycosides have gained renewed interest and it has been suggested that absorption of flavonoids is not limited to the aglycone form. (Watson and Oliveira, 1999; Goh and Barlow, 2004).



Flavonol	R_1	R_2
Rutin	OH	Rutinose
Quercitrin	OH	Rhamnose
Quercetin	OH	H
Kaempferol	H	H
Isorhamnetin	OCH_3	H

Fig. 1: Chemical structure of selected flavonols.

Quercetin glycone conjugates include rutin, thujin and hyperoside. These molecules have the same structure as quercetin except that they have a specific sugar molecule in place of one of quercetin's hydroxyl groups on the C ring, which dramatically changes the activity of the molecule. Rutin is also known as quercetin-3-rutinoside. Thujin is also known as quercetin, quercetin-3-L-rhamnoside, and 3-rhamnosyl-quercetin. Onions contain conjugates of quercetin and the carbohydrate isorhamnetin including quercetin-3, 4'-di-O-beta-glucoside, isorhamnetin–4'-O-beta–glucoside and quercetin-4'-O-beta-glucoside. Quercetin itself is practically insoluble in water. Quercetin carbohydrates have much greater water solubility than quercetin.

BIOSYNTHESIS

The biosynthesis of flavonoids is unique in that the two component aromatic rings arise via different pathways. The phenyl propane residue (ring B and C 2, 3 & 4) derives form p-coumaric acid, itself formed via the shikimate pathway. Ring A, on the other hand, is basically formed from acetate and is a rather special case of polyketide synthesis.

The first view of phenol synthesis was that four molecules of acetate condensed to form polyketide (3, 5, 7-triketo-octanic acid) which could cyclize in various ways. One way appropriate to our discussion is illustrated below.

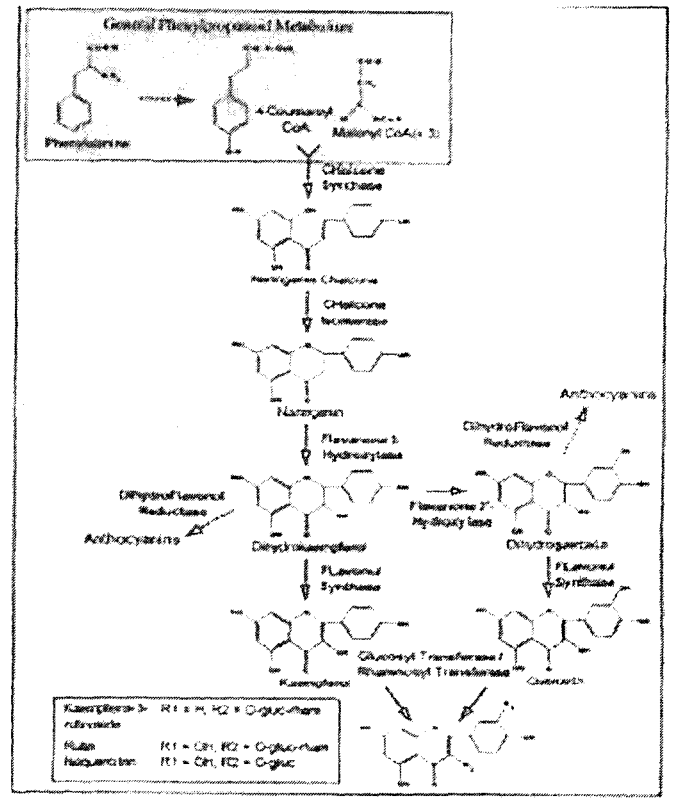


Fig. 2: Flavonoid biosynthetic pathway included are structures of the major flavonol glycosides in tomato, rutin and its precursor isoquercitrin and kaempferol-3-rutinoside.

Basic mechanism for forming aromatic rings from polyketides (other possibilities of cyclization are not included, in the simplest form. R = CH_3).

Further investigations revealed that the starter R.COOH was acetyl - CoA and the other 'acetates' added were in fact malonyl-CoAs. Now, if R. CoSCoA is P-coumaryl – CoA rather than acetyl - CoA, then the cyclization envisaged in Fig 3 can yield a chalcone or a flavanone. (wong, 1976, and Strack, 1997). Quercetin is produced form chalcone involving various steps (Austin et al 2004). An enzyme system from parsley cell cultures will convert p-coumaryl – CoA and malonyl - CoA into 4, 2', 4,'6'-tetrahydroxy chalcone which rapidly and spontaneously isomerizes to naringenin.

II Dihydroflavonols

Feeding experiments indicate that these are formed directly form chalcones, but the mechanism of the hydroxylation involved has not yet been reported.

III Flavones & Flavonols

Flavones can be formed by the oxidation of flavonones by flavanone dioxidase. Dihydroflavonols are probably the

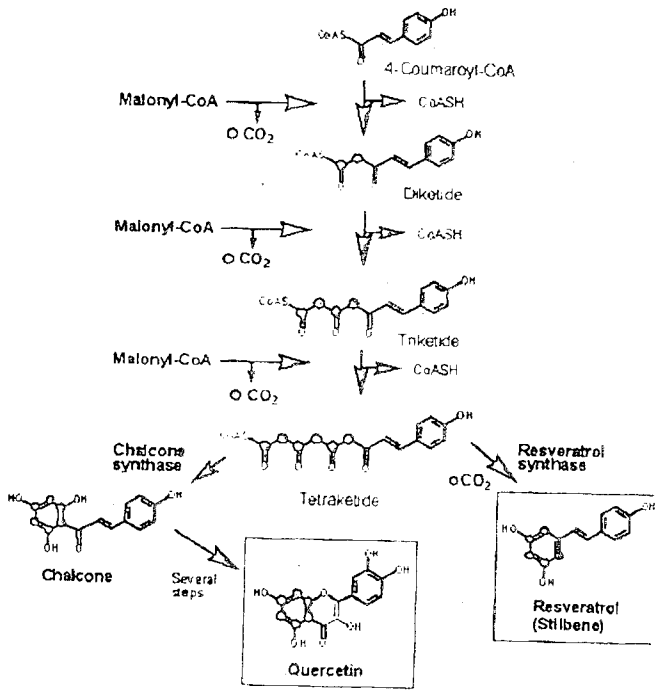


Fig. 3: An aldol switch discovered in stilbene syntheses mediates in cyclization specificity of type III polyketide syntheses.

precursors of flavonols because isotope experiments have demonstrated the conversion of dihydroquercetin (dihydroflavonol) into quercetin (flavonol). The enzymology of the reaction is not clear but peroxidase-like enzymes may be involved.

MECHANISM OF ACTION

Quercetin contributes the major bioflavonoid in the human diet. It shows various effects, which are:

Anti oxidant activity

Normal cell metabolism results in a continuous generation of reactive oxygen species, such as superoxide radical or the nonradical hydrogen peroxide. An imbalance between reactive oxygen species and antioxidant defence mechanism of a cell, leading to an excessive production of oxygen metabolites, creates a condition frequently termed "oxidative stress". Oxidative injury leads to lipid peroxidation, DNA breakage and enzyme inactivation, including free radical scavenger enzymes.

Flavonoids as a rule are antioxidants and a number of quercetin effects appear to be due to its antioxidant activity. Antioxidants stop oxidants (free radicals), which disrupt the integrity of other molecules (their electrons) from attacking nearby molecules, such as mitochondria, and membranes. Its antioxidant activity is due to the reactivity of its phenolic group, which reacts with free radicals to form the more stable phenoxy radicals (Lamson and Brignall, 2001).

Quercetin inhibits xanthine oxidase and inhibits lipid peroxidation *in vitro*. It may delay oxidant injury and cell death by scavenging oxygen radicals, protecting against lipid peroxidation (Dechameux *et al.*, 1992) and thereby terminating the radical reaction (Torel *et al.*, 1986), chelating metal ions (Afanas'ev, 1989) to form inert complexes that cannot take part in the conversion of superoxide radicals and hydrogen peroxide into hydroxyl radicals.

Afanas' ev *et al.*, (1989) studied the inhibitory effects of flavonoids, rutin and quercetin on ferrous ion-dependent lipid peroxidation of lecithin liposomes and NADPH and C^{14} – dependent lipid peroxidation in rat liver microsomes to elucidate the chelating and free radical scavenging activities of these compounds. It was concluded that rutin and quercetin are able to suppress free radical process at three stages: the formation of superoxide ion, the generation of hydroxyl or cryptohydroxyl radicals in the fenton reaction and the formation of lipid peroxy radicals.

As another indicator of its antioxidant effects, quercetin inhibits oxidation of LDL cholesterol *in vitro,* probably by inhibiting LDL oxidation itself (De Whalley *et al.*, 1990a, Chopra 2003), by protecting vitamin E in LDL from being oxidized or by regenerating oxidized vitamin E, by itself. Paired with ascorbic acid, quercetin reduced the incidence of oxidative damage to neurovasculature structure in skin, and inhibited damage to neurons caused by experimental glutathione depletion (Skaper *et al.*, 1997).

Anti-Mutagenic activity

Ray Sahelian (2005) performed study to evaluate the antimutagenic activity of quercetin, ascorbic acid and their combination against an oxidative mutagen. Antimutagenicity testing was done in *Ames salmonella* assay system using *Salmonella typhimurium* TA 102 against butylhydroperoxide as an oxidative mutagen. Quercetin when combined with ascorbic acid showed an increase in the antimutagenic activity. The study indicated that the antimutagenic activity of quercetin was not solely accountable by its antioxidant nature. However, *in vitro* free radical scavenging activity of quercetin correlated well with the antimutagenic activity.

Anti - hypertensive effects

Quercetin exerts antihypertensive effects and reduces left ventricular hypertrophy, endothelial dysfunction, and

the plasma and hepatic oxidative status in spontaneously hypertensive rats.

Anti-inflammatory and anti-allergic properties

Quercetin's anti-inflammatory activity appears to be due to its antioxidant and inhibitory effects on inflammation-producing enzymes (cyclo-oxygenase, lipooxygenase) (De Pascual Teresa *et al.*, 2004). The proposed mechanism of action is inhibition of lipoxygenase and cyclo-oxygenase resulting in reduced production of inflammatory mediators eg. Leukotrienes and Histamine. Quercetin appears to inhibit cyclo-oxygenase to a greater degree than lipoxygenase (Kim *et al.*, 1998).

Quercetin is a strong inhibitor of basophil and mast cell degranulation (Amella *et al.*, 1985). Degranulation of mast cells is an active process that requires the influx of calcium, along with an increase in cAMP (Saponara *et al.*, 2002). This activity leads to phosphorylation of the membrane around mast cell granules, which renders them permeable to water and calcium. The mast cell uses calcium-activated enzymes to assemble contractile microtubules, which pulls the granules towards the cell membrane, where the inflammatory contents are spilled outside the cell, unleashing an allergic reaction (Robins *et al.*, 1984). Quercetin prevents calcium from influxing into the cell.

Antiviral Activity

Quercetin functions like other bioflavonoids in enhancing the collagen network (structure integrity) of blood vessels, and is also known for its antiviral activity. It is further thought to decrease the infectiousness of certain RNA – DNA viruses by inhibiting their replication. Quercetin exerts antiviral activity against reverse transcriptase of HIV and other retroviruses, and was shown to reduce the infectivity and cellular replication of herpes simplex virus type, polio virus type (Saija *et al.*, 1995), parainfluenza virus type (Chang *et al.*, 1993) and respiratory syncytial virus (RSV) (Kaul *et al.*, 1985).

Anti-Diabetic effects

Many of the complications associated with diabetes, such as glaucoma, cataracts and neuropathy are caused by the sorbitol pathway, or process through which high levels of glucose are converted to sorbitol and fructose via the enzyme aldose reductase. Aldose reductase is especially important in the eye and plays a part in the formation of diabetic cataracts. Quercetin is a strong inhibitor of human lens aldose reductase (Varma and Kinoshita, 1976).

Anti-cancer effect

Propsoed anti- cancer mechanisms of action include down regulation of mutant p 53 protein; G1 phase arrest (Lamson and Brignall, 2001), tyrosine kinase inhibition (Ferry, *et al.*, 1996), estrogen receptor binding; inhibition of heat shock proteins; and RAS protein expression inhibition. Quercetin inhibits the growth of several cancer cell lines and that the anti-proliferative activity of this substance is mediated by a so-called type II Estrogen-Binding site (Type II EBS). It may also increase the effectiveness of chemotherapeutic agents.

BIO-ACTIVITY

Few investigations have studied the influence of the bioactivity of flavonoids on metabolism. Reported biological activity of quercetin includes essentially cytotoxic effects, such as induction of apoptosis (Brusselman's *et al.*, 2005), cell cycle arrest and anti-proliferative effects on the one hand, inhibition of apoptosis (Watgen *et al.*, 2005), anti inflammatory effects and protection against oxidative stress on the other. These reflect the contradictory reports.

BENEFITS OF QUERCETIN

Quercetin is a powerful antioxidant and many potential benefits of it are still being discovered. Some of the important functions are summarized below.

Protection from oxidative stress

Quercetin increased cutaneous vascular permeability occurring in conditions of experimentally induced inflammation. (Nakadafe *et al.*, 1985). Quercetin or rutin alone or combined with ascorbic acid may be effective in protecting neurovascular structures in skin and likely also those in other districts (*eg.* mucosa and nerves) from oxidative stress and free radical induced toxicity.

Pain & Inflammation/Allergies

Quercetin is best known as an anti-inflammatory/anti-allergy agent because it stabilizes mast cell membranes and prevents the release of histamine and other inflammatory agents. It is often prescribed for food and inhalant allergies, asthma, eczema, prostatitis and gout. This may be especially helpful in asthma as leukotrin B4 is a potent bronchial constrictor. In particular, quercetin is an inhibitor of allergic (Ig- E- mediated) mediator release from mast cells, Ig-G mediated histamins and basophils (another type of white blood cells involved in immune reactions).

Due to its antioxidant effect, quercetin can inhibit inflammatory processes mediated by "leukotrin" (inflammatory agents, a thousand times more powerful than histamine), hyaluronidase (collagen-destroying

enzymes), and lysosomal enzymes (other promoter of localized inflammation). Some of the important functions of quercetin are:

1. *Gout:* Quercetin inhibition of xanthine oxidase decreases the formation of uric acid (the cause of painful attacks), and thus it may be of value in the treatment of gout.
2. *Eczema:* Natural agents which address excessive histamine release may be beneficial in enhancing the healing response among eczema patients. With asthma and other allergic conditions serum Ig-G levels are greatly elevated in eczema patients and many eczema patients either suffer from or go to develop asthma, and/ or hay fever. Quercetin is one of the more important of such natural compounds in eczema therapeutics.
3. *Hives:* Because Quercetin inhibits manufacture and release of histamines and other allergic inflammatory mediators by mast cells and basophils, it may be useful in treating hives, another condition characterized by increased serum IgE levels.
4. *Prostatitis:* Chronic prostatitis syndrome (non bacterial chronic prostatitis and prostatodynia) is a common disorder with few effective therapies. Bio flavonoids have shown to improve the symptoms of these disorders in significant proportion of men.

Diabetic complications

Quercetin aldose –reductase inhibiting properties makes it a useful addition to diabetic nutritional supplementation, to prevent cataract and neurovascular complications. Quercetin can significantly decrease the accumulation of "sorbitol" in the lens of diabetic animals, effectively delaying the onset of cataract. It is also indicated in diabetes for its ability to enhance insulin secretion, protect the pancreatic beta cells from the damaging effects of free radicals, and inhibit platelet aggregation. It has now been linked to kidney damage in those with diabetes (Chaudhary *et al.,* 1988).

Cardio vascular diseases

Quercetin's cardiovascular effects center on its antioxidant and anti inflammatory activity and its ability to inhibit platelet aggregation *ex vivo* (Pace-Asciak *et al.,* 1995).

High intake of quercetin and other flavonoids predicted lower mortality rates and incidences of heart attack (myocardial infarction) (Hertog *et al.,* 1993). Quercetin and other flavonoids reduce risk of heart disease by lowering the formation of plaque binding substances, specially oxidized low density lipoproteins damage. Cardiologist believes that damage to LDL cholesterol is an underlying cause of heart diseases.

In woman, a strong connection was found between high intake of dietary flavonoids and reduced risk of fatal heart attack. The woman's extra protection may be related to the flavonoids role as phytoestrogen (plant estrogen) which can offer heart protection benefit, similar to those of soy.

Quercetin inhibits the proliferation and migration of artic smooth muscle cells, along with inhibition of mitogen activated protein kinase phosphorylation. These findings provide new insights and rationale for the potential use of quercetin in the prevention of cardiovascular diseases (Ray Sahelian, 2005).

Cancer – Prevention:

Much of the recent research on quercetin has shown it to be an anticarcinogen to numerous cancer cell types, including breast, leukemia, colon, ovary, squamous cell, endometrial, gastric and non-small -cell lung cancer. (Akagi *et al.,* 1995).

Quercetin is considered phytoestrogen (e.g. a plant substance with similar functions as that of estrogen). Some phytoestrogens are believed also to have antiestrogenic effects that might lead to reduced risks of certain cancers. Quercetin was found to have this antiestrogenic activity by inhibiting breast cancer cells in a test tube.

Anti ulcer effect:

Animal studies have shown quercetin to be protective of gastric ulceration caused by ethanol, probably by inhibiting lipid peroxidation of gastric cells and by inhibition of gastric acid secretion. (Alarcon *et al.,* 1994). An interesting aspect of quercetin's anti ulcer effect is that it inhibits growth of *Helicobacter pylori* in a dose dependent manner *in vitro* (Beil *et al.,* 1995).

Brain Damage:

Quercetin also is a powerful antioxidant that may protect brain cells from damage. An apple a day really keeps the doctor away, thanks to strong antioxidants that fight cell damage. Rat brain cells exposed to the antioxidant quercetin resisted damage much better than those not treated. Eating plenty of fresh fruits and vegetables might

reduce risk of developing Alzheimer's and similar brain diseases.

High cholesterol

Quercetin from red wine or orange juice may help lower cholesterol levels.

Prostate health

Some studies suggest that quercetin improves pain and other symptoms in men with chronic prostates (Shoskes *et al.,* 1999). It is of great value in the fight against prostate cancer, possibly because of its ability to inhibit inflammation.

Canker sores

Quercetin may reduce the frequency of mouth sores and produce mild symptomatic relief.

Other functions

Researchers have been evaluating medicinal plants in the democratic republic of Congo that have been used traditionally to treat diarrhea and dysentery. They have found flavonoids and quercetin among the active ingredients in these plants. Studies done in Russia regarding the use of quercetin, along with other supplements and/or conventional medications, to treat dysentery caused by infections such as Shigella have some promise.

PHARMACOKINETICS

Absorption

Like many other plant chemicals, quercetin is sold as a supplement. Oral quercetin is relatively well absorbed and a few human quercetin absorption studies exist. It appears that only a small percentage of quercetin is absorbed after an oral dose, possibly only two percent, according to one study (Hollman *et al,* 1996). A recent study of absorption in "healthy" ileostomy patients revealed absorption of 24 percent of the pure aglycone and 52 percent of quercetin glycosides from onion. These glycosides may then undergo hydrolysis in the enterocyte via B - glucosidases before draining into the portal veins. Absorption rate from dietary source is influenced by the position and chemical nature of the glycoside in combination with the various compounds in the food matrix (Lamson and Brignall, 2001).

Quercetin undergoes bacterial metabolism in the intestinal track, and is converted into phenolic acids. Absorbed quercetin is transported to the liver bound to albumin, where some may be converted via methylation, hydroxylation or conjugation. For best absorption it should be used in conjunction with a protein – digesting enzyme (eg: bromelian) in equal amounts.

Distribution

Quercetin is found predominantly in plasma in the form of its conjugates (e.g., quercetin glucuronides and/or sulfates) and small amounts of unconjugated quercetin aglycone. Maximum plasma concentrations are achieved within the first two hours of administration. This suggests that the absorption site is the upper gut compartment, and may rule out intestinal bacterial degradation. (Erlund, 2000)

Excretion

Previous pharmacokinetic studies using intravenous administration suggest that quercetin is quickly eliminated in humans, with an approximate elimination half-life of less than two hours.

WARNINGS AND PRECAUTIONS

Because of lack of long-term safety data, pregnant women and nourshing mothers should avoid quercetin.

ADVERSE REACTIONS

Adverse effects reported with oral quercetin include gastrointestinal effect such as nausea and rare reports of headache and mild tingling of the extremities. Oral quercetin is generally well tolerated. Intravenous administration of quercetin has been associated with nausea, vomiting, diaphoresis, flushing and dyspnoea.

AVAILABLE FORMS

Quercetin is available in capsule and powder form. When it is being used for its inflammatory properties, it should be combined with pineapple enzyme bromelian (in equal amounts) for its own anti-inflammatory activity and possibly enhanced absorption of quercetin.

DOSAGE

An oral dose of 400 – 500mg three times per day is typically used in clinical practice. Since solubility is an issue in quercetin absorption, a new, water soluble quercetin molecule, quercetin chalcone, might be used in smaller doses, typically 250 mg three times per day. For chronic prostatitis, a quercetin dose of 500 mg was used administered twice a day for one month. For other diseases, the dose recommended is:

Asthma and Hay fever – 250 to 500 mg 3 times a day (20 minutes before each meal).

Crohn's disease – 400 mg 3 times a day.

Gout – 500 mg twice a day.

Heart burn – 500 mg 3 times a day (quercetin or mixed bio flavonoids with or without added bromelian).

Insect bites and stings- 500 mg thrice a day (20 minutes before meal).

Canker' sores- 400 mg (20 minutes before each meal).

Eczema - 400 mg (20 minutes before each meal).

How supplied- Capsules -250 mg, 300 mg, 500 mg.

Tablets - 50 mg, 250 mg and 500 mg.

Use in conjunction with-

Arthritis - glucosamine, boswellia, antioxidants, flax seed oil.

Allergies – ester –C, antioxidants, echinacea, multi-vitamin/ mineral.

Inflammation - grape seed, flax seed oil, antioxidants, multi vitamin/ mineral.

Hyperglycemia - chromium complex, trace minerals, green food blend.

Eczema - hemp seed oil, zinc complex, multi-vitamin/ mineral.

Psoriasis - milk thistle, grape seed, flax seed oil, antioxidants.

DRUG INTERACTION

Papain - and Bromelain: May assist the absorption of quercetin in the intestine (Shoskes *et al.*,1999).

Quinolone- antibiotic: quercetin may compete for DNA gyrase binding sites on bacteria. Therefore, theoretically, it can serve as a competitive inhibitor to the quinolone antibiotics which also bind to this site.

Cisplatin- Because of theoretical risk of genotoxicity in normal tissues in those using cisplatin along with quercetin, those taking cisplatin should avoid quercetin supplements.

CONCLUSIONS

Quercetin is a flavonoid that serves as the backbone for many other flavonoids. It is consistently the most active of the flavanoids in experimental studies, and many medicinal plants owe much of their activity to their high quercetin content. Quercetin appears to fight a host of disorders, from asthma to cancer to heart disease. As an antioxidant, it combats the destructive "free radical" molecules that play a part in many diseases. Among people with high dietary intakes of quercetin and other major flavonoids, studies show lower rates of stomach, lung, pancreatic, and breast cancers. Quercetin inhibits the build up of a type of blood sugar that could otherwise lead to cataract formation in lens of the eye. Quercetin, which is primarily found in apples, onions and black tea, may be the major reason why the old adage about eating "an apple a day" has been associated with good health. And why a daily cup of tea is more than a comforting ritual.

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OBSERVATIONS ON THE HAZARDOUS IMPACT OF SILK WASTE ON MITOTIC OPERATION IN *VICIA FABA* L.

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Abstract

The present paper is an outcome of the observations made to assess the impact of silk dye effluent on the mitotic indices in *Vicia faba* L. The chief chemical ingredients present in the effluent included Azo dyes and Bleaching Powder .The effects were studied in terms of certain established parameters. Hard pressed under the pollution load, the chromosomal behaviour during cell division came under target resulting into great deal of malfunctions. *Vicia faba* L. has been selected as the target plant.

Key words: silk dye wastes, *Vicia faba* L., mitotic indices, chromosomal-malfunctioning.

INTRODUCTION

Plant species do thrive in a chemical environment of in and outside. Inside chemical environment responds to the chemical changes witnessed time to time outside. A delicate and intricate balance has to be maintained for proper metabolic functioning. If the harmony is disturbed, malfunctioning begins, which is reflected practically at every level of the life's deal. This is an established principle and basic learning of ecophysiological pursuits in any organism. The present approach has been to assess the quanta and magnitude of aberrations in genetic deliberations when a plant species is placed under a stressed ecological niche. Some earlier studies carried out to evaluate impact of chemical changes on the environment of the Gene-pool have been successfully carried out by Upadhyaya and Sinha (1986), Srivastava and Mathur (1987), Upadhyaya & Mishra (1998).

Since time immemorial, silk yarn has been under human preference owing to some exceptional quality it posses. This provides the back- bone of textile industry since long in India and also outside. Bhagalpur has the glamour to be called as 'Silk city of India'. Textile industry is one of the greatest industries in India and also one of the major industries, which contributes maximum to environmental pollution. Water pollution is the major environmental issue related to textile industry. Hynes (1980) has focused attention on physico-chemical aspect of water quality on phyto and zooplanktons, microbes, benthos and fishes. Recent field studies shows that different plants differ in their responses to domestic and industrial effluents and many species disappear under heavily polluted conditions (Weigleb, 1981).

Most textiles are dyed and printed. More than 10,000 different dyes are available for this purpose. Approximately, 70 percent of all dyes used in the textile industry come under the group of azo dyes. They constitute the single largest group of dyes having almost complete range of colours. They are characterized by the presence of azo (N=N) group. In addition, they contain sulphonic acid group and hydroxyl and / or amino group. They are prepared by coupling a diazotized amine, known as primary component, with a phenol or amine, known as secondary component. Azo dyes are mostly direct dyes or azoic/naphthol dyes. Azo dyes can also be disperse acid or basic dyes. Benzidine and other aromatic amine cause the greatest concern regarding azo dyes, as they are known carcinogens.

At the silk city of Nathnagar-Champanagar it was observed that the effluent flows through kaccha drain spread and over in the open crop field for several kms. A large number of cultivated plants are subjected to such a bad exposure. The overall productivity of the area under review has witnessed a sharp decline a fact that appeared after personal communication with the local people.

In the background of the above a cyto-ecological study has been made with an intention to find the effect of effluents on the chromosomal behaviour during cell division. The present attempt is to screen the cytological abnormalities induced by the chemicals present in silk dye.

MATERIAL AND METHODS

Waste water from dye industry was collected from the drains of Champanagar, Bhagalpur, which is the most active center of textile industry. This was filtered through filter paper and used.

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The seeds of *Vicia faba* L. were germinated in petridishes having blotting paper soaked in distilled water. After 2-3 days, when the roots attained an average length of 1 (one) cm., the seeds were taken and placed in beakers of suitable size containing water sample in such a manner that the roots are kept immersed. Five different concentrations of effluent were prepared by adding distilled water in collected and filtered sample. For each experimental set one control set was set up. All these experiments were done at room temperature. The roots treated and control-sets were fixed in acetic acid and ethanol (3:1) mixture for cytological preparations.

Cytological slides were prepared from the root meristem of the germinating seeds of *Vicia faba* L. Root tips were pre-treated to soften the material, in Para-dichlorobenzene (PDB) for 2.5 hours at $16^0 \pm 2^0$ C. Fixation of material was done in freshly prepared aceto-alcohol for 24 hours. Slides were prepared by squash technique using 2% aceto-carmine as the stain. Freshly prepared slides were used for observation; slides were micro-photographed and made permanent for future record.

RESULTS AND DISCUSSION

The findings suggest that silk waste in high concentration exhibits retardly effect on seed sprouting and seedling establishment. The result under different concentrations of effluent treatment has been shown in *Table No.1*. A significant observation at higher concentration of effluent treatment has been very abnormal growth of radicle. At high concentration of 80% and 100%, radicle swelled,

Table 1: Effect of effluent on seed germination in *Vicia faba* L.

Sl. No.	Effluent concentration	Time (in hrs.)	No. of Seeds Taken	Radicle appearance	Germination percentage	Seedling appearance	Abnormalities during germination
1.	Control	24	50	46	92%	46	Normal
2.	20%	24	50	47	94%	47	Normal
3.	40%	24	50	46	92%	46	Normal
4.	60%	24	50	44	88%	40	Slow and stunted growth of radicle
5.	80%	24	50	34	68%	08	Deformations and eventual decay of meristematic region
6.	100%	24	50	20	40%	00	Branching of radicle followed by tumour like appearance. Tendency of decay.

deformed and proliferated. The embryo gets rotten and decays ultimately. The deformity observed is shown through *Fig. No.1*. Such exhibitions are the morphological manifestations of physiological and genetic disorders generated by the effluents.

The first cytological parameter studied under different concentrations has been the study of mitotic index, which is an indication of smoothness of cell cycle operation. Fall of mitotic index in relation to increasing effluent concentration has been a significant observation. Results are shown in *Table No.2* and *Figure No.2*. It is apparent that mitotic index value has fallen to 5.6 from 14.13 as shown in control. Smooth operation of cell cycle is a highly delicate genetic operation, which requires a perfectly set, and friendly ecophysiological environ. Any deviation in this equilibrium will add to predicaments and set aside the whole process. Normal cell cycle requires synthesis of many enzyme needed for DNA synthesis and many other proteins, most importantly 'Tubulin'(De Robertis & De Robertis, 1995). So, the regulatory genes under handicapped chemical conditions fail to perform and the mitotic cycle is jeopardized. The chief ingredients of effluent used consists of azo dyes which can be thought to be antimitotic in action and all such ingredients are generally referred to as mitotic poisons (Sharma, 1976), whose action may affect different developmental stages such as nuclear membrane cycle, chromatin condensation leading to chromosomal sparalization, organization of chromosomal centromere and spindle,

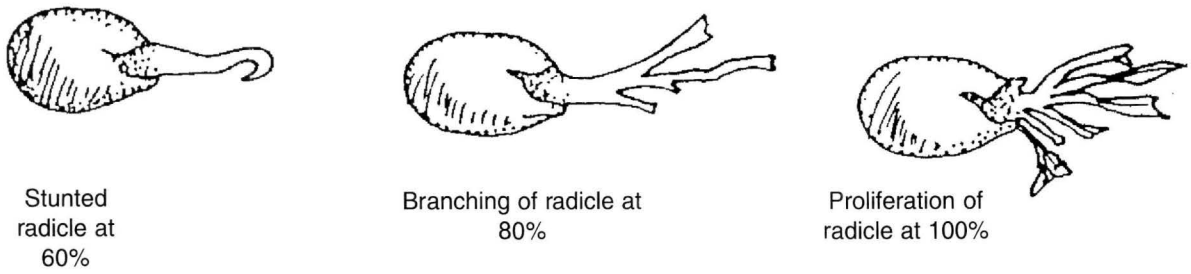

Fig. 1: Deformities in radicle emergence & growth.

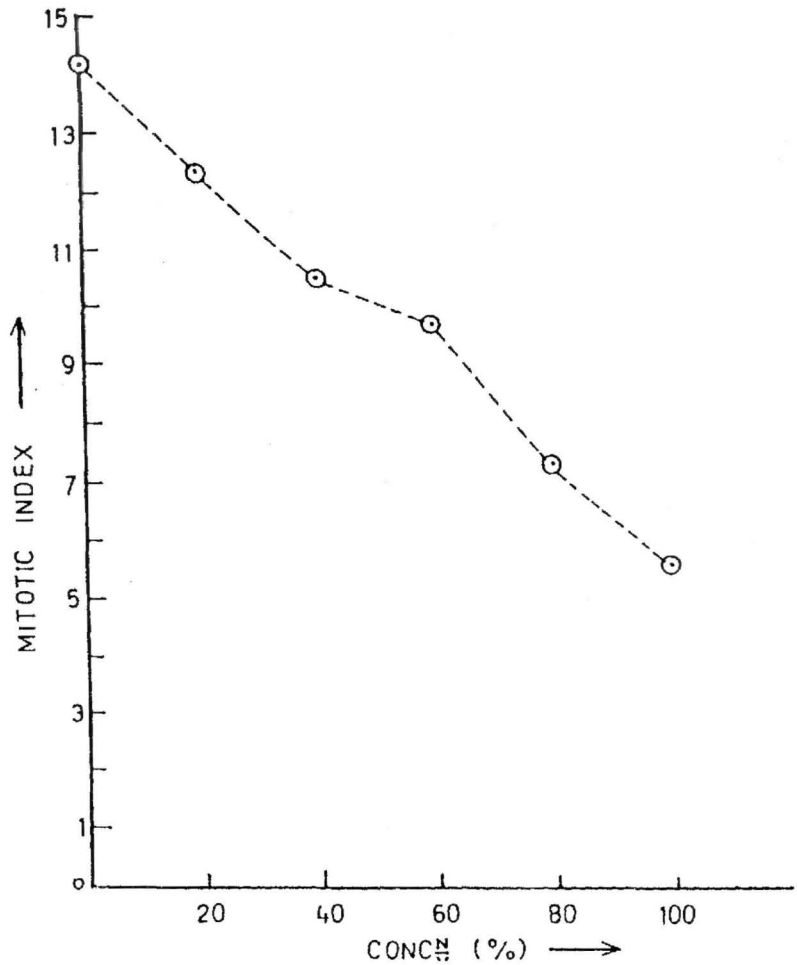

Fig. 2: Graph showing decline in mitotic index with the increase in effluent concn.

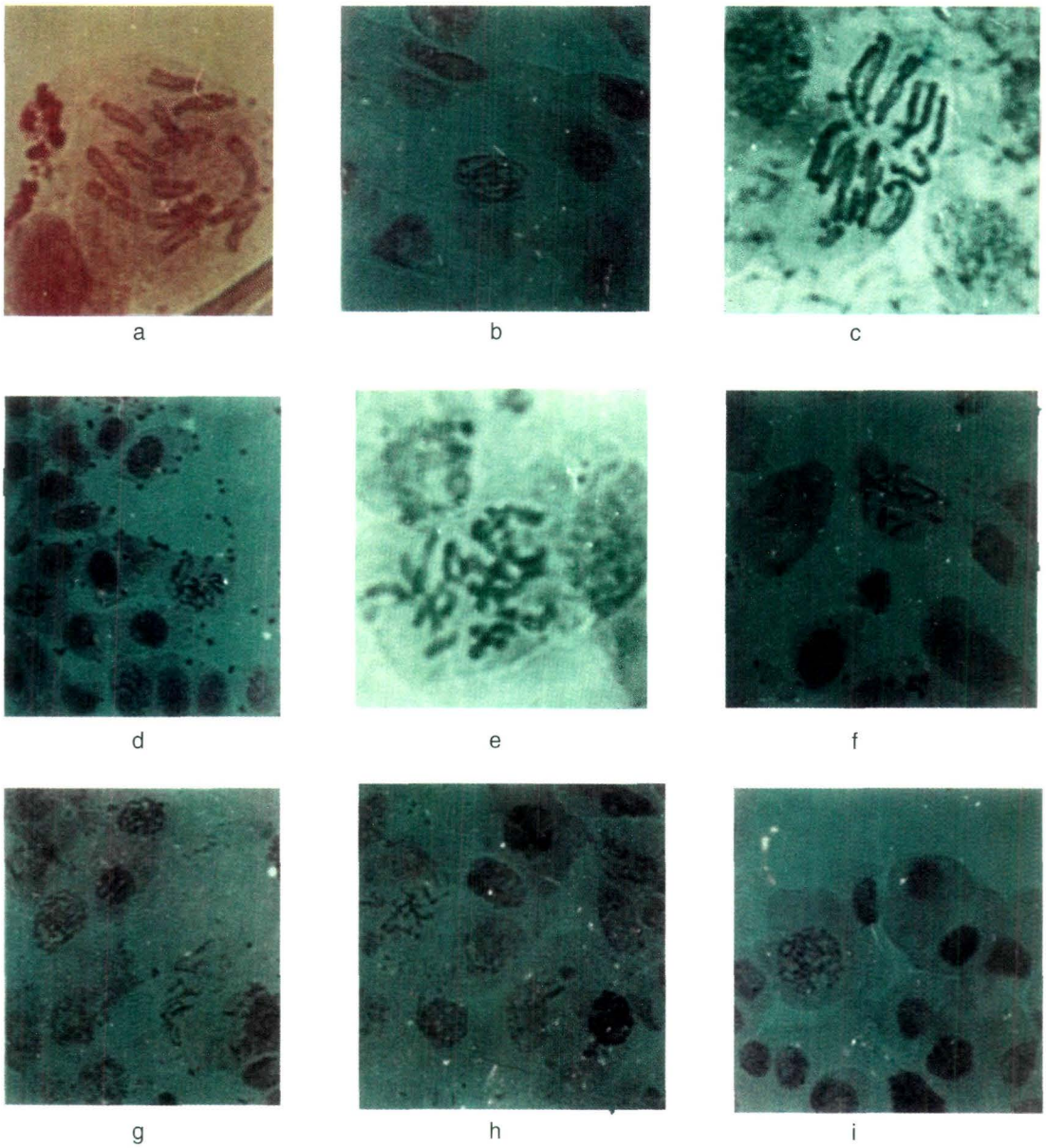

Fig. 3: Mitotic Metaphase in *Vicia faba* under effluent treatment

a. Normal configuration of mitotic chromosomes, **b.** Chromosomes becoming larger and interwoven at 20% concn., **c.** Chromosomal fragmentation begins at 40% concn., **d.** Endoduplication of chromosomes at mitosis at 60% concn., **e.** Polyploidization at 60% concn., **f.** Chromatin dispersion at 60% concn., **g.** Dispersion of nuclei and depletion of chromosome at 100% concn., **h.** Cytomixis at 100% concn., **i.** Endopolyploidization at 100% concn.

Table 2: Mitotic operation under the effluent treatment in *Vicia faba* L.

Observation	No. of cells Obser-ved	Mean of Mitotic Index	Mitotic aberrations **									
			1	2	3	4	5	6	7	8	Total	Total percentage of abnormality
Control	400	14.13	-	-	-	-	-	-	-	-	-	-
Effluent Concentration 20%	400	12.38	16	28	44	40	00	-	-	-	128	32%
40%	400	10.49	20	44	40	36	20	26	-	-	186	46.5%
60%	400	9.56	38	60	46	40	36	30	26	28	304	76%
80%	400	7.32	36	40	48	38	40	36	44	36	318	79.5%
100%	400	5.60	40	12	04	46	24	62	92	84	364	91%

** **1.** Accessory nuclei
2. Precautious separation
3. Chromosomal bridge
4. Fragmentation
5. Laggards
6. Endopolyploidization
7. Nuclear depletion
8. Chromatin depletion

nuclear and cytoplasmic DNA and RNA metabolism.

Apart from the cell cycle, chromosomal behaviour at mitosis has been a matter of prime concern of the cytological screening. The detail results obtained at different concentration treatments causing nuclear and chromosomal casualties have been shown in Table 2 and Fig. 3. One notable observation has been the appearance of accessory nuclei and micronuclei in the increasing frequency with the increase of effluent concentration. The possible reason behind the formation of accessory nucleus is failure of cytokinesis after the nuclear division has taken place. Micronuclei may appear from the lagging chromosome at anaphase resulted after aberrant mitotic or meiotic operation; therefore they are smaller in size. Another reason behind the formation of micronuclei is cytomixis. (Kumar *et al.*, 1997) This involves migration of chromatin materials from one cell to another. Incase, the whole chromatic material of one cell is transferred to another cell, this may eventually leads to binucleate condition, but if the migration of nuclear material is not complete or partial, this would lead to a cell having a micronucleus. Cytomixis again is an abnormal event, which has been scored during the course of present investigation.

The effect of the effluent has been of narcotic nature resulting into endopolyploidization. Spindle apparatus could not develop and work properly so the duplicated chromosomal complement remain asserted within the same nucleus and so cells are under stressed ecological condition due to radical change in chemical medium the active genes come under threat of destruction. Therefore, as a safety measure probably, the organism multiply its genomic complement just to keep their genomic constitution intact. An exposure of Fig. 3 and Table 2 suggested that enhanced chromosomal complement is quite common and this is a positive response to another wise negative chemical change of the surroundings.

Many other chromosomal abnormalities, which were visible, included formation of chromosomal bridge and fragmentation of the chromosomes. Mitotic bridges are produced because of dicentric chromosomes produced after non-reciprocal translocations. This also results in formation of short chromosomal fragments or the laggards. Another visible observation has been high degree of nuclear dispersion and chromosomal disintegration.

The cytological observation discussed above, are the indicatives of a very high degree of malfunctioning included under the influence of hazardous chemicals present in silk waste. So, the area through which the effluent passes influences plants growing nearby remarkably.

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ROLE OF ARBUSCULAR MYCORRHIZAL FUNGI IN NATURAL AND MANAGED ECOSYSTEMS

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Abstract

Arbuscular mycorrhizal fungi (AMF) are widely associated with temperate and tropical plant species. The associations are normally mutualistic, based functionally on reciprocal transfer of sugars from plant to fungus and soil derived nutrients from fungus to plants. Worldwide, interest in AMF has reached a point wherein any task of agricultural biotechnology is incomplete without their role in plant productivity. The intent of this review is to highlight the functions of arbuscular mycorrhizal (AM) fungi.

Key words: AMF, nutrient acquisition, plant productivity, ecosystems.

INTRODUCTION

Mycorrhiza is the natural association between the group of soil fungi and the roots of higher plants. A.B. Frank, a German researcher more than 100 years ago, coined the term "mycorrhiza". Mycorrhiza falls almost exclusively into two broad groups: 1) Ectomycorrhiza of woody angiosperms and gymnosperms, in which Basidiomycetes, Ascomycetes or Zygomycetes develop intercellular hyphae, form a mycelial sheath covering the surface of short lateral roots; and 2) Endomycorrhizas, characterized by intraradical mycelial growth and intercellular fungal proliferation, which are formed by Basidiomycetes in the Orchidaceae (orchidoid mycorrhiza); Ascomycetes in the Ericales (ericoid mycorrhiza) and Zygomycetes in the most other plant taxa (Harley and Smith, 1983). Endomycorrhizas, alternately called vesicular arbuscular mycorrhiza, is the most widely distributed associations in plants. They belong to taxonomic order Glomales, which currently comprises eight genera namely *Glomus, Acaulospora, Gigaspora, Sclerocystis, Entrophospora, Scutellospora, Archaeospora and Paraglomus*. Plant compatibility with mycorrhizal fungi is a generalized and ancient phenomenon. About 80% of all terrestrial plant species from this type of symbiosis (Smith and Read, 1997) and 95% of the world's present species of vascular plants belong to families that are characteristically mycorrhizal (Pirozynski and Dalpe, 1989; Remy *et al.*, 1994; Quilambo, 2000). AM is found in wide range of habitats usually in the roots of the angiosperms, gymnosperms and pteridophytes. They also occur in gametophytes of some mosses, lycopods and psilotales, which are all rootless (Pocock and Duckett, 1985). They were also found in aquatic plants (Beck-Nielsen and Madsen, 2001).

There are plants, however, that have been shown to be mycorrhiza free such as Proteaceae (Brundrett *et al.*, 1996), Cruciferae, Zygophyllaceae (Varma, 1998), Dipterocarpaceae, Betulaceae, Myrtaceae and Fagaceae (Nicholson, 1967). Cactaceae, Chenopodiaceae, Cyperaceae, Amaranthaceae and Juncaceae earlier thought to be mycorrhiza free, had been found to be infected under natural stressed range-land conditions (Neeraj *et al.*, 1991). Since AM is the most prevalent and widespread underground mutualistic association, and has been found under natural stressed rangeland conditions, and has numerous beneficial effects on the plant species, this review summarizes some of its beneficial roles such as improved nutrient acquisition, increased resistance to some diseases, tolerance to abiotic stresses, improved water relations and improved soil structure and soil stability. These can be exploited for enhancing agricultural productivity under stress conditions.

NUTRIENT ACQUISITION AND AMF

The demand for a particular mineral nutrient depends on plant's internal requirements, while the supply of that nutrient depends on its availability and mobility in soils. AM fungi absorb mineral nutrients from the soil and then translocate these nutrients to the plants with whose roots they are associated (Newsham *et al.*, 1994). Mineral nutrients such as P, Cu, and Zn have very limited mobility in soils so that depletion zones where all the available nutrients have been utilized quickly form around the roots. To obtain more nutrients, plants must bypass these depletion zones by further root activity elsewhere in the soil. To rescue itself from this critical situation, plant uses mycorrhiza. AMF modulate plant growth by enhancing

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the availability of nutrients, mainly phosphorus, for plant nutrition (Karandashov and Bucher, 2005). Mycorrhizae extend their external hyphae from root surfaces to areas of soil beyond the nutrient depletion zones, thereby exploring a greater volume of the soil than is accessible to the unaided root (Jakobsen *et al.*, 1994). VAM helps in the uptake of not only the immobile nutrients (such as P, Cu, and Zn) but also the relatively mobile nutrients such as N and Ca (Chandrashekara *et al.*, 1995; Jamal *et al.*, 2002; Liu *et al.*, 2002; Chen *et al.*, 2003). Therefore, AMF association is helpful for the maintenance of balanced supply of nutrients in the plants.

AMF AND DISTURBED ECOSYSTEMS

The functioning and stability of terrestrial ecosystems are determined by plant biodiversity and species composition. Below ground diversity of arbuscular mycorrhizal fungi (AMF) is a major factor contributing to the maintenance of plant biodiversity and to ecosystem functioning (van der Heijden *et al.,* 1998). Mycorrhizae play an important role in the ecosystems, disturbed and degraded, by recent faulty agricultural practices (Cooke and Lefor, 1990; Bethlenfalvay and Linderman, 1992). AMF helps in the development of beneficial microorganisms in the soil and can replace the external inputs in the form of fertilizers (Linderman, 1997). AMF also has an environmental benefit. High levels of P in the soil can result in the pollution of water bodies, when eroded soil rich in P is deposited in them (Sharpley *et al.,* 1992). When plants rely on AMF association than heavy P fertilization, risk to water quality is reduced. AMF are an important component to reduce environmental pollution and are promising tools for the restoration of degraded lands, hence reduce the use of inputs of chemical fertilizers, deleterious to environment. Indigenous arbuscular mycorrhizal fungi and rhizobial nitrogen-fixing bacteria not only enhance the establishment of key plant species but also increase soil fertility and quality. AM can interact synergistically with other microbes in the mycorrhizosphere to enhance both plant growth and survival. Nitrogen fixing bacteria (both free living and nodulating) in combination with AM are more effective at nitrogen fixation than in the absence of AM. The dual symbiosis increases the soil nitrogen (N) content, organic matter, and hydrostable soil aggregates and enhances N transfer from N-fixing to nonfixing species associated within the natural succession to aid the recovery of desterified ecosystem (Requena *et al.*, 2001).

AMF IN RECYCLING OF NUTRIENTS

Mycorrhiza has been shown to play an important role in recycling of nutrients from organic matter to plants. It is now established that there are some categories of mycorrhizas which by means of extracellular, cell wall-bound and intracellular enzymes, can hydrolyse, oxidise, assimilate and transform many of the major nitrogen-and phosphorous-containing organic molecules in plant, microbial and animal detritus (Leake *et al.*, 1997). There is increasing direct and indirect evidence that at least some arbusuclar mycorrhizal associations may have a direct role in mineralizations and uptake of phosphorus from organic compounds. Acid phosphatase activity is greater in the roots and mycorrhizosphere of infected plants compared to non-mycorrhizal plants (Doddet *et al.*, 1987; Mohandas 1992; Tarafdar and Marschner, 1994).

AMF IN AMELIORATION OF ABIOTIC STRESSES

Drought and salinity stress are major agricultural constraints affecting plant productivity and land development in arid and semiarid regions. Drought and salinity share a common osmotic component (in terms of water relation for plants) as the main factor responsible for their negative effects on plant development. AMF symbiosis can protect host plants against detrimental effects caused by drought stress (Ruiz Lozano *et al.*, 1999). The increased drought tolerance in AM plants may be due to increased phosphorous uptake (Nelson and Safir, 1982) or due to increased leaf conductance and photosynthetic activity (Dell- Amico *et al.*, 2002) or because of better osmoregulation (Davies *et al.*, 2002). Improved plant water status and changes in water relation have been attributed to wide variety of mechanisms not directly related to phosphorus nutrition or water uptake (Davies *et al.*, 2002). AMF may influence plant hormones or improve water uptake. Different AMF species differ in their effectiveness to enhance plant water uptake from soil and this ability seems to be related to amount of external mycelium produced by each AM fungus and to frequency of root colonization in term of alive and fungal structures (Marulanda *et al.*, 2003). Other mechanisms may include osmotic adjustment, which assists in the maintenance of leaf turgor and effects on physiological processes such as photosynthesis, transpiration, conductance and water use efficiency.

AMF have been shown to decrease plant yield losses in saline soils (Baker *et al.*, 1995; Azcon and El-Atrach, 1997; Al - Karaki, 2001; Garg and Geetanjali, 2004). Mychorrizhal plants were better able to withstand salt stress as a result of mechanisms based on physiological processes (proline accumulation, photosynthetic activity, transpiration, carbondioxide exchange rate, stomatal

conductance and water use efficiency, rather than on uptake of nitrogen and phosphorus (Ruiz Lozano *et al.*, 1996). Alguacil *et al.* (2003) have shown that increased antioxidant enzyme activities could be involved, at least in part, in the beneficial effects of mycorrhizal colonization on the performance of species grown under semi-arid conditions.

AMF also improves the plant tolerance to heavy metal toxicity (Bethlenfalvay, 1992). Tonin and coworkers (2001) showed the accumulation of the toxic metal in non-toxic forms in the roots. Due to greater absorbing surface, the uptake of Cu, Zn and Cd were increased by the mycorrhiza from polluted soil, but the volume of translocation to the shoot was reduced (Loth and Hofner, 1995; Joner and Leyval, 1997). Plants grown in acidic soils have reduced productivity due to limited mineral nutrients and in such acidic soils, AMF are important for optimal growth and productivity (Bethlenfalvay, 1992; Alloush and Clark, 2001).

AMF IN PATHOGEN RESISTANCE

In addition to improved nutrient uptake, establishment of AM symbiosis induces a number of other physiological changes. AM fungi influence plant survival and growth in various ways, one of which is the enhancement of host tolerance to plant parasitic nematodes and other pathogenic organisms. AMF and plant parasitic nematodes are rhizosphere inhabitants that often interact with each other. Since in most of the interactions observed, the fungi appear to suppress nematodes, there is potential for managing AM fungi as biological control agents against plant parasitic nematodes (Habte *et al.*, 1999). Increased P nutrition uptake leads to suppression of plant diseases by enhancing disease resistance of AM plants which may be due to increased phytoalexin like compounds in AM plants (Newsham *et al.*, 1994; Trotta *et al.*, 1996). AMF stimulates hormone production in plants, improves soil structure (Bethlenfalvay, 1998) that may be the indirect effect of improved P nutrition.

CONCLUSIONS

AM fungal hyphae network are the nutrient switches in the plant roots that enhance water uptake, disease resistance, phytohormone production and osmo regulation. These changes in turn account for the capacity of AMF plants to grow and survive in disturbed/stressed agro-ecosystems. So AM, the three-component system involving plant, fungus and soil, is an important biological tool for sustainable agriculture. It is vital that soil scientists and agriculturalists pay due attention to the management of AMF in any schemes to increase, restore or maintain soil fertility.

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DIVERSITY, DISTRIBUTION AND ECONOMIC IMPORTANCE OF LIVING GYMNOSPERMS IN INDIA

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Abstract

The paper throws light on species diversity, distribution, economic importance and conservation of living gymnosperms including indigenous and introduced taxa in India. The present study has revealed the occurrence of 101 species, 9 varieties and 1 forma belonging to 33 genera under 10 families of gymnosperms (indigenous and exotic) in India. *Cycas sphaerica* Roxb.- a native of Mayurbhanj hills of Orissa state is less known endemic. *Cycas edentata* de Laub.- a native of Andaman & Nicobar Islands is widely cultivated throughout plains of India. One hundred and five taxa are conserved in botanical gardens situated in different parts of the country. The Indian Botanic Garden at Howrah and the Botanical garden associated with ICFRE (Old FRI) at New Forest, Dehradun are practically the field gene banks of gymnosperms. The whole town of Kodaikanal up to Sacred Heart College is full of exotic taxa of gymnosperms. *Araucaria rulei* is still restricted to Govt. Botanical Garden, Ooty.

Key words: gymnosperms, diversity, distribution, economic importance, India.

INTRODUCTION

Flowering plants or more specifically the seed bearing plants which are called as *'Spermatophytes'* (*Sperma*-seeds; *phyta*-plants) are usually classified in two main groups *viz.* Gymnosperms (*Gymnos*-naked; *sperma*-seed) and Angiosperms (*Angios*-covered; *sperma*-seed), on the basis of the protection afforded to the ovules. In Gymnosperms, the ovules are exposed while in the Angiosperms they are enclosed in an ovary.

The word 'Gymnosperm' was first used by Theophrastus-a pupil of Aristotle. Almost all the Gymnosperms are woody perennials (shrubs or trees); erect or climbing. Most of them are evergreen except the 'Larches'. Gymnosperms possess two types of leaves, which may be scale-like, and awl shaped as in the Genera viz. *Cupressus*, *Juniperus* etc. but the genera like *Podocarpus, Agathis* and *Ginkgo* possess broader leaves. The inflorescences are unisexual. The secondary wood is also of two types viz. *manoxylic* (soft, relatively sparse with wide parenchymatous rays) and the *pycnoxylic* (dense, with small woody rays). The latter is of much commercial value.

In addition to their ornamental value, the Gymnosperms are of much use to the human beings as they provide the very valuable timber, wood pulp, soft wood, resins, edibles, medicine etc. Recently the 'Himalayan Yew' has turned out as the savior of the human race against breast/stomach cancer. Seeds of *Pinus gerardiana,* known as *'chilgoza'* are consumed as nutritious dry fruit. Similarly the ripe cooked seeds of *Cycas rumphi,* young leaves and strobili of *Gnetum gnemon* and tender fleshy shoots of *Cycas pectinata* are eaten, *Ephedrine* obtained from *Ephedra* species is an important medicine for treatment of 'Asthma', Hey fever and other bronchial troubles. *Cedar* oil is also valuable in treatment of pulmonary/urinary disorders, piles and rheumatism.

The living Gymnosperms belong to five orders *viz.* Cycadales, Ginkgoales, Taxales, Coniferales and Gnetales. These orders are quite distinct and widely divergent. Several important contributions have been made from India on various aspects of living Gymnosperms by Maheshwari & Biswas (1970), Meheswari & Vasil (1961). Maheshwari & Konar (1971), Jain (1976), Mehra (1988), Pant (1973), Raizada & Sahni (1960), Sahni (1990) and Srivastava (1992) and many others. Hooker (1888) in *Flora of British India*, described 42 taxa (40 spp. and 2 varieties) belonging to 16 genera under three orders *viz.* Gnetaceae, Coniferae (with 6 tribes *viz.* Cupressineae, Taxidieae, Taxeae, Podocarpeae, Araucarieae and Abietineae) and Cycadaceae (Table-I). Out of these 33 species and 2 varieties were reported from present political boundaries of India. He treated only one species *viz. Cupressus torulosa* D.Don distributed in W. Himalaya, but described other 3 spp. *viz. C. lusitanica* Mill., *C. semipervirens* L. and *C. funebris* Endl. as planted ones. His observation regarding distribution of these taxa still stands good. *Agathis loranthifolia* Salisb. was also described by him, but from Penang only. However, as on today, this species is quite common but still restricted to gardens only.

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Similarly *Pinus merkusii* Jungh & De Vriese described by him from Martaban and Upper Tenasserim (in Myanmar) is now recorded from Arunachal and is planted as well.

SPECIES DIVERSITY

Hooker (1888) recorded 40 species and 2 varieties from British Indian region out of which 33species and 2 vars. were from the present political boundaries of India (Table I).

Table I: Genera of Gymnosperms in British India
(cf. Hooker. f., 1888)

	Genera	No. of taxa in Brit. India			Number of taxa in present India		
		Sp.	Var.	Forma	Sp.	Var.	Forma
Order: ***Gnetaceae***							
1.	*Ephedra*	3	—	—	3	—	—
2.	*Gnetum*	6	1	—	4	1	—
Order: ***Coniferae***							
3.	*Abies*	1	1	—	1	1	—
4.	*Agathis*	5	—	—	—	—	—
5.	*Cedrus*	1	—	—	1	—	—
6.	*Cephalotaxus*	2	—	—	2	—	—
7.	*Cupressus*	4 (1+3)	—	—	4 (1+3)	—	—
8.	*Dacrydium*	1	—	—	—	—	—
9.	*Juniperus*	4	—	—	4	—	—
10.	*Larix*	1	—	—	1	—	—
11.	*Picea*	1	—	—	1	—	—
12.	*Pinus*	1	—	—	4	—	—
13.	*Podocarpus*	3	—	—	2	—	—
14.	*Taxus*	1	—	—	1	—	—
15.	*Tsuga*	1	—	—	1	—	—
Order: ***Cycadaceae***							
16.	*Cycas*	1	—	—	4	—	—
Total 16 Genera		**40 spp.**	**2 var.**	**—**	**33 spp.**	**2 var.**	**—**

Sahni (1990) provided a comprehensive and well illustrated account of Gymnosperms of India and Adjacent countries viz. Bangladesh, Bhutan, Maldives, Myanmar, Nepal, Pakistan and Sri Lanka (*cf.* Table-II). *Pinus bhutanica* discovered by him (Sahni) in 1977 (but described by Grierson, Long & Page in 1980) and *Pinus wallichiana* var. *parva* were recorded as additions to the Gymnospermous flora of India.

Table II: Gymnosperms in India and seven adjacent countries (*cf.* Sahni, 1990)

	Genera	India			India Adj. Countries		
		Sp.	Var.	Forma	Sp.	Var.	Forma
1.	*Abies*	4	—	—	4	—	—
2.	*Amentotaxus*	1	—	—	1	—	—
3.	*Calocedrus*	—	—	—	1	—	—
4.	*Cedrus*	1	—	—	1	—	—
5.	*Cephalotaxus*	1	—	—	2	—	—
6.	*Counninghamia*	1	—	—	1	—	—
7.	*Cupressus*	5	—	—	5	—	—
8.	*Cycas*	4	1	—	5	1	—
9.	*Ephedra*	7	3	—	8	4	—
10.	*Gnetum*	5	3	1	8	4	2
11.	*Juniperus*	5	—	—	6	—	—
12.	*Larix*	1	—	—	2	—	—
13.	*Picea*	3	—	—	4	—	—
14.	*Pinus*	7	—	—	7	1	—
15.	*Podocarpus*	2	—	—	4	—	—
16.	*Taiwania*	—	—	—	1	—	—
17.	*Taxus*	1	—	—	1	—	—
18.	*Thuja*	1	—	—	1	—	—
19.	*Tsuga*	1	1	—	2	—	—
19 Genera		**50**	**6**	**1**	**64**	**10**	**2**

PRESENT WORK

The present communication aims to present a state-of-art-report on the status of the living Gymnosperms in India. The study is based on field surveys, scrutiny of Indian Herbaria and published literature. The indigenous taxa and those exotics that are naturalized have been included for their taxonomic/distributional status, could not be ascertained during the short period of compilation of this report. The introduced taxa, which are still restricted to the gardens, have been listed with brief details.

The accepted names are given in bold letters with their citations followed by basionyms and synonyms in italics. Local or common names, brief description, flowering & fruiting period, distribution, habitat, uses (wherever known) and in some cases additional information are presented under 'Notes'. Artificial keys to the families, genera, species and varieties are also provided.

Present study records the occurrence of 10 families, 33 genera, 101 species, 9 varieties and 1 forma in India (Table-III). The endemic taxa are listed in Table-IV.

Table III: List of living Gymnosperms in India (present study)

	Genera	Number of Species	Variety	Forma
1.	*Abies*	4	—	—
2.	*Agathis* (Exotic)	1	—	—
3.	*Amentotaxus*	1	—	—
4.	*Araucaria* (Exotic)	6	—	—
5.	*Callitris* (Exotic)	2	—	—
6.	*Cedrus*	1	—	—
7.	*Cephalotaxus*	2	—	—
8.	*Chamaecyparis* (Exotic)	1	—	—
9.	*Cryptomeria* (Exotic)	1	—	—
10.	*Cunninghamia* (Exotic)	1	—	—
11.	*Cupressus* (1 species exotic)	9	—	—
12.	*Cycas* (one species exotic)	6	—	—
13.	*Dioon* (Exotic)	1	—	—
14.	*Encephalartos* (Exotic)	1	—	—
15.	*Ephedra*	8	3	—
16.	*Ginkgo* (Exotic)	1	—	—
17.	*Gnetum*	5	4	1
18.	*Juniperus*	8	1	—
19.	*Larix*	1	—	—
20.	*Libocedrus* (Exotic)	1	—	—
21.	*Metasequoia* (Exotic)	1	—	—
22.	*Picea*	3	—	—
23.	*Pinus* (8 taxa indigenous)	26	1	—
24.	*Podocarpus*	3	—	—
25.	*Sequoia* (Exotic)	1	—	—
26.	*Taxodium*	1	—	—
27.	*Taxus*	1	—	—
28.	*Tetraclinus* (Exotic)	1	—	—
29.	*Thuja* (Exotic)	4	—	—
30.	*Tsuga*	1	—	—
31.	*Welwitschia* (Exotic; only at NBG, Lucknow)	1	—	—
32.	*Widderingtonia* (Exotic)	1	—	—
33.	*Zamia* (Exotic)	4	—	—
33	**Genera**	**101**	**9**	**1**

Table IV: Endemic Gymnosperms of India

	Botanical Name (s)		Distribution
1.	*Amentotaxus assamica*	o	Arunachal Pradesh (Delia Valley)
2.	*Cycas beddomei*	o	Andhra Pradesh (Cuddapah district)
3.	*Cycas circinalis* var. *orixensis*	o	Orissa (Puri & Angul)
4.	*Gnetum montanum* var. *megalocarpum*	o	Arunachal Pradesh (Dafla Hills)
5.	*Gnetum contractum*	o	Kerala & Tamilnadu (Nilgiri)
6.	*Gnetum latifolium* var. *macrocarpus*	o	Andaman & Nicobar Islands
7.	*Pinus wallichiana* var. *parva*	o	Arunachal Pradesh (Tawang)

DISTRIBUTION

Based on author's own observatios and also the published contributions of other previous botanists, the distribution of the gymnosperms along with the associated angiospermic species are presented below:

EASTERN HIMALAYAN REGION

Darjeeling district (27°3'N & 88°18'E) of West Bengal state of India is a part of the Eastern Himalayan region, with an average altitude of 2044m on the outer Himalayan ranges. Main base camp areas include Badamtam (600m), Kurseong (1350m), Kalimpong (1180m), Darjeeling (2044m) Tonglu (3022m) Sandakphu (3579m) and Phalut (3666m) on Singallila range. The vegetation of the area may be classified in to *Low Hill Forests* (upto 900m); *Middle Hill Forests* (750-1500m); *Upper Hill Forests* (1500-2700m); *Rhododendron-Conifer Zone* (2700-3600m) and *Alpine scrub & grasslands* (3600-4300m and above). *Cycas pectinata,* locally Known as *'Thakal'* in Nepali language, occurs scarcely on hill slopes as one proceeds up towards Darjeeling or Gangtok. Most of the mountain slopes between 600-2100m in Darjeeling district have been cleared off the forests for tea plantations. *Cryptomeria japonica* has been extensively cultivated in and around Darjeeling since 1891. It is now covering large areas forming dense forests between 1200-2400m.The forests in *Cold temperate* to *Sub-alpine Zone* (2700-3600m) are evergreen and mainly composed of Rhododendrons and Conifers. *Taxus wallichiana* grows in the forests from Tonglu to Sandakphu. Interspersed with these are patches of *Tsuga dumosa* and *Abies densa.* At 2700-3000m all along Singallila range below Phalut in Darjeeling district and upto Lachen valley (N.Sikkim Dt.) and also above Bakhim in West Sikkim district in Sikkim State, *Tsuga dumosa* grows in abundance. *Picea spinulosa* is abundant on all the hills around Lachen (Sikkim) intermixed with *Tsuga dumosa* but does not reach above 3000m. On the last ridge between Tonglu and Sandakphu (Darjeeling district), *Abies densa* is very common. It grows profusely on Singallila range above 3000m upto the top of the ridges, but it forms dense forests where it has more tolerable shelter, as on Phalut spur. Dense undergrowth of *Arundinaria aristata* (Poaceae) is seen in silver-fir Forests, especially where the fire has destroyed the tree canopy below Sandakphu (3300m).

Sikkim State (27°10'-28°5'N & 88°30'-89°E) is bounded on the west by Nepal, on the north by Tibet and to the east by Bhutan and Tibet. The upper basin of the West Bengal hills and Sikkim is 80km wide. The topography of this pretty Himalayan state is varied. The altitudes range from 244m to over 8595m. *'Teesta'* is the main river. The upper reaches of the river teesta are known as *Lachen Chu* and *Lachung Chu,* which have their confluence at Chungthang and form the *Teesta,* which is the biggest drainer of the Eastern Himalaya. The remarkable feature of the landscape are its numerous peaks above 5500m, Chho-Lhamo (5250m), Dankia-La (5540m) and undulating hills rising to 5560m. Bordering Tibet are the vast gently undulating cold level deserts. In western, Sikkim, Mt. Sinolichu is 6890m high. Most of the 7300sq. km area of this State, is interlaced with Jungle-clad ridges and deep ravines created by, and through which, the raging torrents of the mountain rivers speed and emerald valleys alternating with their terraced hill-sides and dense forests; a variety of plant beauties along with water-falls, rivulets, Lakes and snow-capped mountains girdling the state in a protective embrace, embellish the land.

Pure formations of *Abies densa* also occur between Karponang and Chhangu (E. Sikkim dt.) and Simdong to Thangu (North Sikkim dt) extending up to 3600m or slightly above. At the heights above 3600m, where the tree line ends on the exposed sunny hillsides *Juniperus pseudosabina* Fisch. & Mey and *Juniperus recurva* Buch-Ham. ex D.Don grow in bushy formations in East and North Sikkim districts expecially above Changu (4300m) and Thangu (4200m). *Ephedra saxatilis* Royle var *sikkimensis* (Stapf) Florin covers vast areas near the top of hills around Thangu (*ca* 4200m).

Srivastava (1992) reported the occurrence of twenty-five species belonging to 18 genera and 10 families of gymnosperms in this pretty Himalayan State. *Cryptomeria japonica*-an exotic species is naturalised in many parts of the state. It is one of the main species taken up for afforestation by the Sikkim Govt. in view of its fast growth. However, it was noted that important elements of the ground flora are getting lost due to its impact on soil probably by increasing soil's acidity. Similarly, *Cupressus corneyana* is also commonly grown almost throughout the state (up to 1800m) because it is considered as a *sacred tree* by the *'Bhutia'* community and is planted near the Monastries. In addition to these taxa, species of *Agathis, Araucaria, Callitris, Cunninghamia, Ginkgo* and *Thuja,* are also grown. *Ginkgo biloba* however, is still restricted to only few gardens. D.G. Long *et al,* R.C. Srivastava, D.C.S. Raju and co-workers have conducted survey & exploration in Sikkim. R.C. Srivastava & co-workers completed a checklist of 5070 species of Flora of Sikkim State. Srivastava (1996) published an account of trees of Sikkim, which included gymnosperms also.

In Arunachal Pradesh, gymnosperms are fairly well distributed from 500-4000m elevations throughout Arunachal Pradesh with their maximum concentration between 1500-3500m. They are mostly seen in sub-tropical, temperate and Alpine zones. In the last named forest type, tree forms are comparatively rare. Conifers generally are gregarious as pure stands. The pine forests are characteristic in that they are abundant where rainfall is comparatively sparse and annual fire is a constant phenomenon.

At some places near the local habitations or camps of Forces, there has been over exploitation of the conifers but as such the gymnospermous plant wealth of Arunachal Pradesh has not been commercially exploited. Most of the conifers are used for fuel wood or construction of houses except for *Cupressus conrneyana* (for timber). Mainly *Pinus roxburghii* and to some extent *Pinus merkusii* are commercially tapped for resin.

In the areas that are left untouched either due to steepness or remoteness, trees over 10m girth and 60m height especially of *Abies spectabilis* and *Picea spinulosa* (in Melinja-Hotspring areas of Lohit District) are seen. Except Raizada and Sahni (1960), Sahni (1990), Kanjilal *et al.* (1934-40), Haridasan (1990), and Beniwal and Haridasan (1992), very little is known about plant wealth of the state in general and gymnosperms in particular. Information on the distribution and status of regeneration of gymnosperms in different areas of Arunachal Pradesh is provided. Exotic gymnosperm species, which are introduced to the State, are also listed in this work.

Twelve genera and 21 species of Gymnosperms are found wild in Arunachal Pradesh while 17 species and 8 genera under are introduced and cultivated. In the distribution pattern of Gymnosperm in Arunachal Pradesh, there is a definite ecological succession seen as the elevation increases. The general trend is: *pine* in lower positions, followed one above the other, by *Tsuga, Larix, Picea, Abies, Cupressus* etc. *Taxus, Cephalotaxus, Amentotaxus* are seen mixed with other species.

Among the pines, *Pinus wallichiana* has the widest distribution covering all districts where as the other species are restricted to a few localities. *Pinus armandii, P. bhutanica* and *P. roxburghii* are seen in Kameng District and *P. merkusii* in Lohit District. Other conifers like *Abies, Picea, Tsuga, Juniperus*, also have a fair distribution almost throughout the State. Introduced ones are mostly as forest plantations or garden plants of avenue trees of ornamental value.

Plants of the family 'Araucariaceae' are cultivated. The trees have needle like stiff leaves (like *Araucaria*) or flat thick ones (like *Agathis*). Both genera *Agathis* and *Araucaria* are cultivated in gardens. *Araucaria* is more commonly seen in tropical areas. *Agathis robusta* F.M. Bailey is a cultivated tree with flat thick leaves. Few trees are under cultivation in Deomali and Kharsingsa areas. The growth of the trees is satisfactory. *Araucaria columnaris* (Forster) Hooker (syn. *A. cooki* R. Br.) is another widely cultivated species of the family. Tall trees with horizontal branches and needle like leaves, this plant is quite popular as an ornamental tree in gardens and parks in Arunachal Pradesh.

'Cupressaceae' family is represented by three genera in Arunachal Pradesh *viz. Cupressus, Juniperus* and *Thuja*. The last named is exclusively cultivated. *Cupressus cashmeriana* Royle ex Carriere (trees with long drooping flat branches) is introduced in Bomdila, Yachuli, Ziro and similar cold places and are doing well. *Cupressus corneyana* Carriere, locally known as *'Dhupi'*. is found scattered throughout the State (both wild and cultivated) between 2500-3000m elevation. These trees grow extensively in West Kameng District. Lofty trees with 8m girth and over 30m height could be seen along the Rupa-Jigaon belt. Timber is durable and is in great demand. This species has been extensively exploited from their natural regions in West Kameng District. Natural regeneration is poor to moderate. Small trees or shrubs of *Juniperus recurva* Buch.-Ham. with recurved drooping pointed branches with prickly spines are distributed in the alpine zones (2500-4500m) throughout the State, but is more common in Tawang and Lohit Districts. Regeneration is poor. *Thuja orientalis,* an ornamental shrub or small tree with vertical flat branches, is doing very well all over the State in tropical and sub-tropical areas. It is introduced and cultivated.

Cephalotaxus griffithii Hook. (Cephalotaxaceae) occurs in Lohit, Lower Subansiri, West Kameng Districts and other areas of the state between 1000-2000m elevation. This small tree has drooping bifurcating branches. Although it is wild, yet, grows well under cultivation also. Regeneration is very poor.

Gnetum montanum Mgf. (Gnetaceae) -a large evergreen dioecious climber, occurs in Tezu area of Lohit District.

'Pinaceae' family is represented by 6 genera in Arunachal Pradesh, of which one (*viz. Cedrus*) is only under cultivation. The five other genera are: *Abies, Picea, Pinus, Larix* and *Tsuga. Abies delavayi* Franchet is found between 2700-3200m elevations only in West Kameng

District in *Peri-la* mountaintop near *Eagles nest, Sela* and above *Tawang*. This *Fir* is restricted to a very small area. Most of the crop is either over mature trees or stunted seedlings. Natural regeneration is almost absent. *Abies densa* W. Griff. is found at 2700-3900m elevations in *Lohit, Dibang Valley, Changlang, West Siang, Upper Subansiri, West Kameng* and *Tawang districts*. Regeneration is good in areas where forests have been worked out or in open places as seen in *Noorangang, Se-la, Hostpring*, etc. It is particularly dense along moist stream banks along slopes; very poor or almost absent in untouched areas. Good regeneration is noticed in open areas in West Kameng (Sa-la) where the area has been almost cleared; trees were utilised as firewood, for timber purpose and during road construction. This species occurs mostly as pure crop and have very few associates like *Larix, Juniperus, Tsuga*, etc. Attempts have been made on a very small scale to raise the species artificially but growth is not encouraging. *Abies spectabilis* (D. Don) Spach. is found between 2500-3500m elevations. Its distribution is same as that of *A. densa*. It grows in association with *Pinus wallichiana, Tsuga dumosa, Picea spinulosa* etc. Though the species is not raised artificially, it has good regeneration and better growth as compared to the former species. Natural regeneration along hill slopes particularly moist areas is very good in *Simbi-Melinja-Hotspring* area of *Lohit District*.

Large evergreen conical trees of *Cedrus deodara* (Roxb.) G. Don are widely seen in the Western Himalayas. Its plantation was tried on a very small scale in Lower Subansiri (Hapoli) and West Kameng (Dirang and Bomdila) District, and its performance particularly in West Kameng District at Dirang (1800m) elevation seems to be promising.

Larix griffithiana Hort. *ex* Carr. is found mixed with *Tsuga dumosa, Abies densa* (and broad leaved Angiosperm trees like *Sorbus* spp.) above 2500 to 3600m altitude. Its distribution is very scattered in Lohit, Tawang and West Kameng Districts. Regeneration is generally poor except in worked out areas where seedlings come up in abundance along with *Tsuga* seedlings.

Lofty trees of *Picea spinulosa* Henry, having branchlets pendulous branches and attaining over 9m girths and 60m heights are distributed in Lohit District. Generally they form pure patches often associated with *Abies spectabilis*. Natural regeneration is good and occurs in patches.

Picea brachystyta Franchet is a handsome *Spruce* with pale brown shoots, horizontal branches and pendulous branchlets. It is found at Mago, W. Kameng District. Regeneration is poor.

Pinus armandii Frachet. is reported to occur in Lohit District (Ward, 1952) and resembles *P. wallichiana* but no specimens could be seen. *Pinus bhutanica* Grierson. is found in Tenga Valley. West Kameng District, Arunachal Pradesh. The species resembles *P. wallichiana*. The growth is very satisfactory and regeneration is good. *Pinus merkusii* Jungh et de. Vr.-the *two needle pine* is found over extensive areas in Lohit District and to some extent in Changland District. It occurs mostly as pure crop between 550-2200m evevation. It also occurs in association with Oaks and Rhododendrons. The species is naturally seen over Lohit Valley and Dichuy Valley from Kharang-Walong-Kibithoo-Dichu and Melinja. Best growth is observed around Kibithoo and Dichu. Regeneration in natural zones is profuse but introduction trials were not positive. *Pinus roxburghii* Sargent.-a "Three needle Pine" grows naturally in West Kameng District only. The crop is growing extensively in Tenga Valley, Dirang and Nafra. One of the best crops is found in Nafra area. Extensive tapping is being carried out, which started recently. Profuse natural regeneration can be seen. Artificial regeneration has been tried on a very limited scale. *Pinus wallichiana* Jackson, is found almost throughout the State between 1600-3000m. Natural regeneration is satisfactory. The species is raised artificially in Apatani Valley of Lower Subansiri District under agroforestry system. *Pinus wallichiana* Jackson var.*parva* K.C. Sahni is similar to *Pinus wallichiana*, but differs in shorter needles, smaller female cones, smaller seeds and wings. It is known only from Tawang area. It may also be available in other areas of Arunachal Pradesh for which intensive fieldwork is required.

Pinus kesiya Royle ex Gordon was introduced from Meghalaya and has been tried in Yachuli and Itanagar of Lower Subansiri District and has proved exceedingly well for afforestation of '*Jhum fallows*'. Large scale insect attack is prevalent making it difficult to manage the plantations. A number of exotic pine species were tried but among them *P. patula* Scheide & Deppe has done extremely well at 700-1000m elevation as seen in Yachuli and Hapoli in Lower Subansiri District. Growth at Yachuli is better than *P. kesiya*. Other pine species like *Pinus elliotii* Engelmann, *P. caribaea* Morelet and *P. oocarpa* Scheide ex Schil. are tried on experimental basis at the Arboretum Van Vigyan Kendra, Chessa. All the species are doing well. These species will be particularly suitable for afforestation of lower belts of Arunachal Pradesh. Tall trees of *Tsuga dumosa* (D. Don) Eichler, with massive trunk are

gregarious and found in Lohit, Changlang, Dibang Valley, West Siang, Lower Subansiri, West Kemeng and Tawang Districts between 1800-3000m elevation, associated with *Pinus wallichiana, Abies densa, Larix giffithiana* etc. Regeneration is very good in open areas and worked out places; otherwise poor.

Family Podocarpaceae is represented by the genus *Podocarpus* and its species in Arunachal Pradesh. *Podocarpus neriifolius* D. Don (locally known as *Titmin*) is a lofty tree found upto 700m elevation but not very common. Its growth and natural regeneration are very good near Ganga Lake, Itanagar. Introduction trials are being carried out in Van Vigyan Kendra, Chessa that are very promising. *Podocurpus wallichianus* C. Presl is said to occur in this State but no specimens could be seen.

Family Taxaceae is represented in Arunachal Pradesh by two genera viz, *Amentotaxus* and *Taxus*. Both are seen only in wild condition. Large trees of *Amentotaxus assamica* Ferguson are found mixed with broad-leaved trees at 1600-1800m elevations in Lohit District. It is seen at present, only in Mithumna ridge in Delai Valley, Lohit District.

Family Taxodiaceae is represented by cultivated trees in Arunachal Pradesh. Two genera viz. *Cryptomeria* and *Taxodium* are cultivated. *Cryptomeria japonica* D. Don an exotic, tall ornamental tree with drooping branches is cultivated in Arunachal Pradesh. It thrives best between 700-1500m elevations. Growth is observed to be good in Yachuli, Hapoli and in Lower Subansiri District. *Taxodium distichum* (L.) Richard an ornamental, deciduous tree with distichous branchlets is under cultivation at the Van Vigyan Kendra, Chessa and Banderdewa. Nursery growth is observed to be good. This species is promising for parks and gardens at lower elevation.

WESTERN HIMALAYAN REGION

North-West Himalayas covers the altitudinal range of 300-4200m in the states of Jammu and Kashmir, Himachal Pradesh and Uttaranchal States. The climate is very variable in the vast ranges of the Western Himalayas. It is much more moist in the eastern part in contrast to the extreme west owing to gradual westward decrease in precipitation.

Some important localities explored so far include: JAMMU AND KASHMIR: Srinagar (34°.06'N, 74°.49'E, altitude 1800m): Anantnag (1590m), Baramula (1800m), Gulmarg (2655m), Jammu (335m), Pahalgam (23250m), Sonmarg (2700m). Tangmarg (2535m), enroute Kolahoi glacier (4200m), enroute Amaranth cave (4200m). HIMACHAL PRADESH: Dalhousie (32°. 31;N, 75°.59'E, altitude, 2000m): Chamba (600m), Dainkund (2700m), Kalatop (2400m), Khajjiar (1600m), Sherpur (600m), Kulu (1200m), Manali (2000m), Manikarn (1800m), Rohtang pas (4200m). Shimla (31°.60'N, 77°.11'E, altitude, 2170m): Chadwick falls (1500m), Glen (1500m), Chhrabra (2430m), Hattoo Peak (3150m), Mashobra (2400m), Mattiana (2230m), Narkanda (2680m), Kalpa valley (900-3000m). UTTARANCHAL: Mussoorie (30°.28'N, 78°.10'E, altitude 2000m): Campty falls (1300m), Dehradun (680m), Jabarkhet (2400m), Mossy falls (1500m), Rajpur (800m), Sarsar Dhara (750m), Badrinath (3100m), Hemkund (4300m), Valley of flowers (3300m). Nainital (29°. 22'N, 79°.29'E, altitude, 1930m): Bhimtal (1300m), Cheena peak (2800m), Haldwani (350m), Kaldhungi (600m), Land's end (2200m), Lariakanta (2800m), Ranibagh (750m), Tiffontop (2300m), Pindari glacier (4200m), Jageshwar (1800m), Tanakpur (350m).

Situated in the eastern corner of the region, Nainital receives an average 2,597.5mm of annual rainfall. The major amount (2,239.4mm) falls during the monsoon season from June to October The monsoon is heaviest in July and August with a monthly precipitation of *ca* 750mm. The winter months (November to March) are quite dry with less than 60mm of rain per month except January when a few good showers and snowfalls are experienced. The period between November and December. is almost dry. The yearly mean maximum temperature is 18.33°C and mean minimum temperature touching the freezing point. Situated at the western end, Srinagar receives the minimum annual rainfall of 664mm. The major amount of precipitation (390.7mm) is in the form of snow and is received in the months of January to May. The rainy months are July and August. January is the coldest month with temperature much below the freezing point but humidity is maximum (88%).

The shallow brown soils in Kalpa and Pangi valleys are suitable for the growth of *Pinus gerardiana*.

The western Himalayas harbours rich flora and differ from their eastern counterpart in the greater representation of conifers. The vegetation shows a considerable resemblance to the European element, especially in Kashmir valley. The wide range of altitude, temperature, rainfall and soil result in diversified forests in the region. The northern slopes, which retain snow and moisture for a longer period, are often thickly clad with dense forests. On the contrary, the southern slopes support poor vegetation.

The plains, the Siwaliks and the outer Himalayan ranges up to 1000m comprise the tropical zone, is associated with *Shorea robusta*-the dominant species of many forests. The only gymnosperm species *viz. Pinus roxburghii* (towards its upper limit). Commonly mixed with it in the lower hills are *Terminalia sp., Haldinia cordifolia, Syzygium cumini, Cordia dichotoma, Toona ciliata, Lannea coromandelica and Anogeissus latifolia.* The lower storey of *Mallotus philippinensis, Emblica officinalis, Ehretia laevis, Wendlandia exserta, Flacourtia indica and Acacia catechu.*

In tropical dry deciduous forests common elements are *Sapium insigne, Acacia catechu, Albizzia lebbek, Pyrus padus, Bauhinia variegata, Zizyphus maurtiana* and *Dendrocalamus strictus.* At certain places the effect of biotic factors (like lopping, overgrazing and fires) is seen in form of scrub vegetation wherein the dominant element is *Euphorbia royleana.*

Pure forests of *Pinus roxburghii* are found in sub-tropical Zone (1000-1800m) at upper limits, with *Quercus leucotrichophora, Rhododendron arboreum, Lyonia ovalifolia, Querous glauca, Acer oblongum, Myrsine semiserrata* and *Ficus palmata* etc. Scattered trees of *Terminatia chebula, Pyrus pashia* and *Syzygium cumini* are found in the lower hills. Pine forests are absent in Kashmir. The pioneer spices in dry evergreen forests of the subtropical zone are *Acacia modesta* and *Dodonaea viscosa* and the climax vegetation consists of *Olea cuspidata* forests.

In the forests of temperate zone (1800-3600m) evergreen oaks and conifers cover extensive areas, very often in pure communities. Each of the three altitudinal oak belts has its specific conifer. *Quercus leucotrichophora* occupies the lowest zone of moist temperate forests between 1800-2400m occasionally descending to 900 m on southern slopes in moist ravines. The typical associates are *Rhododendron arboreum, Lyonia ovalifolia, Ilex dipyrena, Betula alnoides, Carpinus viminea, Cedrela serrata, Euonymus pendula* and *Acer* spp. *Quercus glauca* is locally abundant at lower elevations in shady places. The characteristic conifer of this belt is *Cedrus deodara*, which ascends to 2700m. *Picea smithiana* and *Pinus wallichiana* are thinly mixed with it. Most frequently broad-leaved associates are *Quercus dilatata* and *Prunus cornuta.* The middle oak zone between 2000-2700m is occupied by *Quercus dilatata* with *Picea smithiana* as the commonest conifer associate. *Quercus* leucotrichophora and *Quercus semecarpifolia* penetrate freely into this belt. The other commonly dispersed trees are *Euonymus pendulus, Ilex dipyrena, Rhododendron arboreum, Rhamnus purpureus, Cedrela (Toona) serrata, Machilus* sp., *Fraxinus micrantha, Acer caesium* and *Betula alnoides.*

From Mussoorie (Uttaranchal state) northwards, prominent amongst the forest vegetation are the stately *Cedar*, the rigid *Silver Spruce*, and the graceful *weeping Pine.* The twisted *Cypress* towes aloft and the soleman *yew* stretches out its sombre arms.

The high altitude oak is *Quercus semecarpifolia.* A thin mixture is found with this species of *Abies pindrow, Picea smithiana, Cedrus deodara, Rhododendron arboreum, Salix daltoniana, Taxus wallichiana, Pyrus lanata* and *Betula utilis.*

The temperate mixed coniferous forests consist of varying mixture of conifers such as *Abies pindrow, Picea smithiana, Pinus wallichiana* and *Cedrus deodara.* The broad-leaved trees, intermixed with them or occasionally forming strips and patches are *Quercus semecarpifolia, Q. dilatata, Juglans regia, Prunus cornuta, Acer* spp. and *Aesculus indica.*

In moist depressions and along streams the temperate deciduous forests are constituted of *Aesculus indica, Juglans regia, Fraxinus micrantha, Betula alnoides, Ulmus wallichiana, Carpinus viminea, Quercus semecarpifolia, Acer* spp., *Prunus cornuta, Rhodoeendron arboreum* and *Taxus wallichiana.* In the inner ranges, the monsoon is feeble and the precipitation, which is usually under 1000mm, falls in the form of snow. *Pinus gerardiana* is the main species mixed with *Juniperus macropoda, Artemesia maritima, Quercus ilex, Fraxinus xanthoxyloides* and *Olea cuspidata.*

Alpine scrub vegetation stretches throughout the western HImalayas above 3600m. The elements are bushy and have short and much branched stems. Dwarf *Willows* and *Junipers* are abundant in some localities. *Rhododendron campanulatum* and *R. barbatum* are conspicuous at other places. Under them *Cotoneaster* spp. commonly form mats on rocks. *Betula utilis, Syringa emodi* and *Salix spp.* constitute the deciduous scrub near Aparwat (3700m) in Kashmir.

The Garhwal Himalayan region of Uttaranchal state includes the districts of *Uttarkashi, Chamoli, Tehri-Garhwal, Pauri-Garhwal,* and *Dehradun.* It lies between 29°26'-31°28'N and 77° 49'-80°6'E and the total surveyed area is *ca* 25000 sq. km. In the south, this region starts as the *Sub-Shiwalik bhabar* (325m) and extends up to *Indo-Tibetan* border in the north, with series of snow clad

lofty peaks of *Yamnotri, Gangotri, Kedarnath, Badrinath*, and Valley of Flowers-*Hem Kund Sahib* sectors. The highest peak in the *Garhwal Himalaya is Nandadevi*, 7817m high. The region is *traversed* by deep gorges of *Bhagirathi, Alaknanda*, and the *Dhwal Ganga*. These merge together to form the Holy *Ganges*.

The moist temperate forests are found in the whole of the Garhwal Himalayan region between 1500-3000m. The *subtropical pine-forest* forms its lower limit while at its upper end it merges into subalpine forests. Between 1700-2500m at certain locations at Mussoorie, Kanasar and Patlidevi, *Cedrus deodara* conspicuously replaces Common Oak or grows along with it. The canopy of such forests is fairly close. In certain inner cool localities of Barkot and Uttarkashi, *Deodar* also comes at an appreciably lower altitude to form associations with *Pinus roxburghii,* or at places it reaches as high as 3200m to form associations with the moist mixed coniferous forests. Normally, it forms a pure crop or may be associated with some scattered trees of *Ban Oak, Moru Oak, Rhododendron arboreum* etc. The shrubby growth is almost similar to that of the Oak forests. Epiphytic ferns on *Cedrus deodara* are *Lepisours nudus* and *Polypodium microrhizoma*.

At still higher altitudes of 2400-3000m in this region (Deoban and Hannuman Chatti), very beautiful mixed coniferous forests are met with, wherein dominating trees are *Abies pindrow* and *Picea smithiana*. At lower altitude, *silver fir* is also associated with *Cedrus deodara*. Very few other broad-leaved species are associated with these forests.

The mixed coniferous forests merge into the pure *Kharsu* oak forests, which extend up to 3300m. Such forests are met with at *Yamnotri* and *Deoban* areas. With *Quercus semecarpifolia,* other associated trees are *Abies pindrow, Picea smithiana* and *Cedrus deodara*. Its broad-leaved associates are *Ilex dipyrena, Pyrus lanata, Quercus dilatata, Euonymus lacerus, Syringa emodi* and *Betula utilis*. The undergrowth is composed of *Salix elegans, Strobilanthes atropurpureus* etc. After the snow melts, herbaceous elements alongwith fern species appear on the forest floor.

Above *Yamnotri-Gangotri* (3000-3800m), the *Himalayan birch-fir forests* are met with. These are mainly composed of *Abies spectabilis* and *Betula utilis.* Other very rarely associated broad-leaved species are *Rhododendron companulatum, Quercus semecarpifolia* and *Sorbus foliolosa*. Commonly recorded shrubs are *Cotoneaster acuminata, Ribes* spp., *Lonicera spp., Rubus niveus, Salix* spp. and *Viburnum nervosum.* Herbs appear only for a short duration after the snow melts.

At *Mussooree* many species of gymnosperms have been planted. Of these special mention may be made of *Taxus wallichiana, Pinus wallichiana, Ginkgo biloba, Cupressus torulosa* and *Thuja orientalis.*

Himalayan Subtropical Pine forest may be categerised in two types *viz.*, *'Siwalik Chirpine forest'* and *'Himalayan Chir pine forest'*. In *Siwalik Chir pine forest, Pinus roxburgii* forms the upper story with elements of the tropical mixed deciduous forest. The undergrowth is fairly continuous and composed of xerophytic shrubs. These are found at 600-1000m on dry slopes of Siwalik *conglomerates* and sand stone. At Assaori (600m) the ridges are occupied by *Pinus roxburghii* as a dominating species as a top canopy with associated angiosperm species like *Shorea robusta* and *Terminalia tomentosa*.

'Himalayan Chir Pine forest' is found all along the subtropical zone of the Western Himalaya from 1200-1800m (-2300m). Typically *Pinus roxburghii* occurs here as a pure crop which usually is not so dense and is marked by the absence of the other trees in the top canopy. Shrubs are rare but the forest floor is covered by grasses. Climbers, epiphytes and bamboos are absent but some broad-leaved species are present along watercourse at Kathnaur Barkot, Gangnani (1200-1800m). The top story is purely constituted by *Pinus roxburghii*. At Mussoorie only scattered trees of *Pinus* roxburghii are restricted to the exposed dry situation such as crest of spurs or south facing slopes and well-drained areas.

In moist and fire-protected places, the shrubby undergrowth and grasses are distinct. In certain inner valleys of Garhwal Himalayas, near Barkot and Uttarkashi, *Cedrus deodara* has been found growing under *Pinus roxburghii*. Large tracts of the *subtropical pine forests* in the Garhwal Himalayas near *Chamba* and *Gangnani* have been adversely affected by various biotic factors such as large scale cutting for timber and pressure for agricultural and also due to increasing population. Presently trees of *Pinus roxburghii* are left either at very steep slopes or at the hilltops which are unfit for cultivation.

The Kumaon Region of Uttaranchal state includes Almora, Bagheshwar, Champawat, Nainital, Pithoragarh, and Udham Singh Nagar districts. Haridwar district of Garhwal region and Udham Singh Nagar of Kumaon region fall under Tarai region of agroclimatic zone while

remaining 11 districts of Uttaranchal are hilly (*cf.* Gupta, 1968)

The forest vegetation of the Kumaon region has been subdivided as under:

I.1. Forests lying south of the line of the main Himalayan peaks (namely Kedarnath, Trisul and Nandakot) where the annual precipitation varies between 160-480mm.

I. 1. Forests of the Tarai and Bhabar and throughout the hills up to 1800m on south aspects and 1500m on north aspects, namely where the climate is tropical or subtropical and the mean minimum temperature does not fall below 21°C.

I.1.1. Forests of broad-leaved species up to about 1200m elevation where frosts are absent or light and snow practically unknown.

I.1.1.1. Deciduous rivereine forests of *sissu, khair* and chilbil (*Holoptelia integrifolia*) which follow the banks of the principal rivers up to about 600m elevation.

I.1.1.2. Deciduous forests of miscellaneous species in the 'Bhabar'.

I.1.1.3. Subdeciduous forests of *'sal'* in the sub Himalayan tract and outermost hill ranges up to about 750m elevations.

I.1.1.4. Evergreen forests of *Eugenia jambolana* and other miscellaneous species in the sub-Himalayan tract and along the banks of streams in the outer ranges up to about 750m elevations.

I.1.1.5. Deciduous forests of *Anogeissus* and other miscellaneous species in the central and outer hill ranges up to about 1200m elevations.

I.1.2. Forests of *'Chir'* extending between 900-2100m elevations, where frosts are often severe and snowfall is frequent.

I.2. Oak and low-level *silver fir* forests extending throughout the hills between 1500-3300m elevation where the climate is temperate and the mean maximum temperature does not rise above 21° C.

I.2.1. *Banj oak forests* between 1800-2400m elevations.

I.2.2. *Morus oak forests* between 2100-2400m.

I.2.3. *Karshu oak forests* between 2250-3300m.

I.2.4. Low-level silver fir forests between 2400-2850m.

II. Forests lying north of the line of the main Himalayan peaks, where the annual precipitation varies between 25-100cm.

II.1. *Banj-pine forests* between 1800-3600m elevations.

II.2. Spruce forests between 2100-2400m.

II.3. *Cypress* forests between 1950-2850m.

II.4. Deodar forests between 2400-3300m.

II.5. Forests of mixed hardwoods between 2100-2700m.

II.6. High-level Birch forests between 2850-3600m.

II.7. High-level Silver fir forests between 2850-3750m.

II.8. Alpine bushland between 3000-4050m.

III. Bushland lying within a narrow belt of area bordering Tibet where the annual precipitation is below 25 cm.

III.1. Riverside bushland between 3300-4200m elevations.

III.2. Hillside bushland between 2700-4500m.

Forests of Chir (*Pinus roxburghii*) ordinarily extend on south aspects from 1050-2100m and on north aspects from 900-1950m elevations. *Chir* is found on all geological formations, which occur within its range, though on certain southern slopes with limestone subsoil it is unable to exist on account of the dryness of the soil combined with high temperatures. Nevertheless *chir* forests can establish themselves on dry southern aspects where owing to the xerophytic conditions and the prevalence of fires few other tree or shrub species survive. In all *chir forests* the *pine* is the main dominant tree and it is not at all unusual to find it the only tree species present, though on north aspects where there is more moisture in the soil and fires are less destructive, scattered trees of other species may occur. They are, however, rarely present in sufficient numbers to produce anything approaching a second story. Moreover, the *Chir* itself seldom forms very dense forest. The ground is always covered with a more or less dense crop of grasses whatever the aspect or density of the overhead canopy, and there is also a discontinuous undergrowth of shrubs often so widely scattered that at a short distance their presence is scarcely noticeable.

The open nature of the forests, the absence of other tree species and the poverty of undergrowth are attributable partly to fires which have in the past occured annually throughout their length and breadth, partly to the xerophytic conditions under which they grow and partly

to the fact that the Chir zone is often closely associated with cultivation, heavy lopping and felling The *Chir* itself is especially adapted to withstand fires by means of its thick bark, its coppicing powers whilst young and its rapid growth when once established.

The *Kharshu* occupies the highest zone amongst the oaks, and forests of this species are found between 2250-3450m over very extensive tracts. It is found equally on north and south aspects, and in the extensive areas occupied by this type of forest the *kharshu* has few successful competitors besides *Abies pindrow* and *Quercus dilatata*. The *fir* however only occurs on a small fraction of the area occupied by *kharshu*, and *moru* can only compete with success on north aspects towards the lower limit of the *kharshu*. At its lower limit it most frequently passes into *banj* forest though on north aspects it often gives place to *moru*, *Spruce* or *Silver fir*. At its upper limit it passes into forests of *birch*, *silver fir* and *Rhododendron*, though on south aspects it more usually passes directly into pastureland, the passage being always abrupt without any gradual transition.

Typical forests of *kharshu* on both, north and south aspects are dense. A second story of tree species is sometimes present but is often only represnted by scattered individuals and may be entirely absent. A third story of shrubs is always present under normal conditions and varies in density inversely with that of the overhead over. *Rhododendron arboreum* is still almost as abundant in the *'Kharshu forests'* as it was 900m lower in the *banj*. Among the commoner tree species in the second story may be mentioned *Ilex dipyrena, Acer caesim, Acer pictum, Acer acuminatum* and locally *Taxus wallichiana* (yew). Shrubs occur in great variety but some of the commonest are: *Rosa sericea, Rosa macrophylla, Cotoneaster acuminata, Salix elegans, Ribes galciale, Strobilanthes atropurpureus, Viburnum fatens. V. stellulatium and V. continifolium*. The last named is equally abundant in all types of oak forest from *banj* to *kharshu*. Two species of *ringal* are characteristic, namely- *Yushania jaunsarensis* (Gamble) Yi and *Y. spathiflora* Munro, both of which form dense and continuous undergrowth beneath the oak over extensive areas. The combination of Oak with *Y. jaunsarensis* is estimated to occupy more than 50 square miles in Garhwal alone.

The low-level *silver fir (Abies pindrow)* is found between 2100-3150m elevation and forms continuous forests between 2400-2850m. The only occurrence of this species on the outer hill ranges is a small community at Debthal about 22km east of Naini Tal. There is also an outlier in the *Thali reserve* in East Almora. With these two exceptions, it is restricted to the higher central ranges and the main Himalayan range itself. Wherever the *silver fir* occurs it is a dominant species though when mixed with spruce (as is not infrequently the case) the two are conjointly dominant. It is very exceptional to find a forest in which the *silver firs* are sufficiently dense for their crowns to be actually in contact, and in the majority of *silver fir forests* they are more or less widely scatterd. This however, does not imply that the ground is incompletely covered. On the contrary even in the forests where the trees are widely scattered, the soil is seldom exposed because a second story of trees is always present, except in the very dense forests, and a third story of shrubs is also never entirely absent and may be comparatively dense also. In forests lying south of the main Himalayan range the second story is most frequently composed of either *kharshu* Oak or *Moru* Oak, but north of this as the oaks disappear their place is taken by a mixture of broad-leaved deciduous species the commonest of which are *Ulmus wallichiana, Aesculus indica, Acer caesium* and *Corylus jacquemontii*. Common shrubs composing the third story are *Rosa macrophylla, Deutzia staminea, Viburnum stellulatum, V. nervosum, Berberis affinis* and *Strobilanthes wallichii*, whilst the two *Arundinaria jaunsarensis* and A. *spathiflora* are often found in abundance.

Fairly extensive forests of 'blue pine' (*Pinus wallichiana*) exist beyond the main range within the drainage of the Alakhnanda river, whilst south of the main range only small patches of forest occur and then almost invariably on limestone. This species is not found in the valleys immediately east of the Pindar, but reappears in the Dhauli and Kali valleys of the Almora district. Its range of altitude is very variable and is probably largely dependent on the snowfall. The outlying patches south of main range are found between 1800-2550m, immediately north of the main range between 1950-3300m, whilst in the still drier tract around Malari and Niti it is found between 3000-3600m, scattered trees being occasionally found up to 3750m. Immediately north of the main range the principal tree associates vary according to elevation. Thus between 2450-2550m. *'cypress'* and *'deodar'* are often present; from 2250-2700m *'spruce'*; from 2550-2850m *Abies pindrow*, and from 2850-3300m *Abies webbiana* at their upper limit and into *deodar* or *cypress* at their lower. Dense crops are quite exceptional owing to the disastrous fires, which sweep periodically through the areas. These fires may at times practically exterminate the whole forest and it is due to its wonderful reproductive powers that the species has been enabled to maintain itself. Under most forests there is a more or less dense crop of grass

and in all such cases shrubs are conspicuously absent. Those that occur are usually well adapted to resist damage by fire, for example *Indigofera gerardiana, Hypericum cernuum, Rhus cotinus, Indigofera dosua* and *Excacaria acerifolia*. Other common shrubs are *Aralia cissifolia, Artemisia vestita* and *Salix elegans*.

Spruce (*Picea smithiana*) is not found east of the Garhwal district and, as in the case of the *blue pine*, such forests as occur south of the main range are usually found on limestone or in localities where the rainfall is believed to be below the average for the surrounding area. Here, they are usually restricted to elevations between 2100-2700m though to the north of the main range they reach 3300m. The spruce is restricted entirely to north or northerly aspects. Forests in which it is dominant are exceptional and south of the main range it is usually found associated with *Quercus semecarpifolia or Q. dilatata* whilst north of the main range the principal associates are *Abies pindrow* and *Pinus wallichiana*. Forests of *spruce* mixed with broad-leaved deciduous species also occupy extensive area and are a marked feature of the landscape, since the tall conifers stand out like sentinels towering high above the broad-leaved forest which surrounds them. Spruce forests are often dense and usually contain a distinct second story composed of broad-leaved trees such as *Aesculus indica, Corylus jacquemontii, Juglans regia, Acer calsium* and *Rhus punjabensis*. A distinct and often dense third story of shrubs (such as *Rhamnus virgata, Berberis chitria, Desmodium tiliaefolium, Viburnum stellulatum and Sarcococca saligna*) is also found.

'Cypress' (*Cupressus torulosa*) forest occurs south of the main Himalayan range as well as to the north, but in the former case only in small and scattered patches and then invariably on a limestone soil on account of its comparative dryness. That lime is not in any way necessary to the *cypress* is shown by the fact that in the drier-climate to the north of the main range it grows freely on quartzite as well as gneiss. Forests of this species are found between 1850-2850m. It is capable of existing on rocky precipitous slopes with little soil beyond what lodges in the crevices of the rocks though in such situations the growth is always stunted and the trees are scattered. *Cypress* is extremely susceptible to fire at all stages of its life and perhaps this susceptibility more than any other cause which has driven it to cling to the steep rocky precipices and to shun all grassy areas. Exceptions occur in the case of a few isolated patches south of the main range in which a deep soil favours a dense growth of associated species, which, by reacting on the naturally moist conditions, create a barrier to the advance of fire. North of the main range it invariably forms open forest composed of scattered individuals, usually on steep rocky ground. In these localities the trees have to contend with heavy sheep grazing in addition to periodic fires of a destructive nature. Such forests as exist south of the main range are often associated with angiosperms like *Quercus dilatata* with or without undergrowth of *Arundinaria falconeri, A. jaunsarensis* and other shrubs. North of the main range, the principal tree associates are *'blue pine'* and *'deodar'*, whilst common angiosperm shrubs include *Abelia triflora, Berchemia lineata, Coriaria nepalensis* and *Spiraea raccinifolium*.

South of the main Himalayan range, deodar (*Cedrus deodara*) only occurs sporadically in a few *'blue pine'* and *'cypress'* forests. North of the main range it is found within the drainage of the Alakhnanda River where it forms more or less pure forests between 2400-3000m. The forests are open and the trees do not attain a great height, but heavy sheep grazing and the steep rocky nature of the slopes where it is mainly found, are factors, which have undoubtedly had a material effect on its condition. Few tree species are found associated with *deodar* in these forests and the undergrowth is usually scanty consisting largely of *Abelia triflora, Lonicera hypoleuca, Juniperus macropoda, J. communis, Prunus jacquemonttii. Ribes grossularia* and *Rosa sericea*.

The high-level *'silver fir'* (*Abies webbiana*) never forms continuous forest of large extent; on the contrary it tends to occur in small patches here and there with angiosperms like *Quercus semecarpifolia* or *Betula utilis*, mainly the latter. It is practically never found on a southern aspect and even on north aspects; pure forest of this species is a rare occurrence. There are a few small forests south of the main range at elevations between 2850-3300m, where it is found along the uppermost limit of the Oak forest, and if birch forest is absent it may pass directly into pastureland. North of the main range, forest is found up to 3750m. In mixtures of *birch* and *silver* fir the latter is dominant and forms an upper open story with crowns widely separated. The lower story of birch is often fairly dense and below this again there may be dense undergrowth of shrubs of which the most common ones include: *Cotoneaster acuminata, Rosa sericea, Ribes glaciale, Ribes rubrum* and more locally *Rhododendron barbatum*.

Lonicera rupicola and *L. spinosa* develop stiff spinescent branches; whilst *Juniperus communis* and *J. pseudosabina* have resinous distasteful foliage. The broad-leaved species are deciduous and possess various

well-marked adaptations to retard transpiration. In addition to the species already mentioned *Eurotia ceratoides* and *Lonicera glauca* are common.

Vegetation of the 'Himachal Pradesh' can be broadly classified into *Thorny scrub forests* (of *Acacia* and *Ziziphus*); *subtropical pine forests;* sub-tropical dry evergreen forests; Himalayan Moist temperate forests, Himalayan dry temperatre forests; sub-alpine forests; Moist Alpine scrub and Dry alpine scrub.

Thorny scrub of *Acacia* and *Ziziphus* type of association is generally found at the foothills near Punjab border. *Sub-tropical Pine forests* occupy the lower area of all ranges from an altitude of 600m-1700m and are dominated by *Pinus roxburghii* (Chir pine). The undergrowth consists of scattered bushes of *Berber* is, *Rubus, Carissa* etc. On exposed slopes in *Chir pine belt, Euphorbia royleana* association with *Carissa* sp. is common. In *Sub-tropical dry evergreen forests; Olea cuspidata, Acacia modesta, Pistacia* sp. and different species of grasses like *Themeda, Eulaliopsis, Heteropogon, Cymbopogon, Saccharum* etc are common. The other associates are *Pyrus pashia, Coriaria nepalensis, Rhus cotinua, Rhus semialata* etc. The shrubby layer is composed of angiosperms like *Indigofera gerardiana, Desmodium tiliaefolium, Rubus ellipticus, Prinsepia utilis, Zanthoxylum alatum* etc.

In *Himalayan moist temperate forests, Cedrus deodara, Pinus wallichiana, Quercus, Ilex, Rhododendron arboreum, Juglans regia* etc are the common tree species and species of *Symplocos, Berberis, Spiraea, Artemisia, Indigofera, Daphne, Polygonum, Cynoglossum* and *Iris kumaonensis* etc are some of the common shrubby and herbaceous plants.

In *Himalayan dry temperate forests, Pinus gerardiana, Cedrus deodara,* species of *Prunus, Fraxinus, Lonicera, Artemisia, Daphne, Caragana and Picea smithiana, Acer pictum, Aesculus* sp., *Rhododendron arboreum, Celtis australis, Salix* sp., *Morus serrata, Cornus macrophylla* etc., are generally found.

Sub-alpine forests, characterised by *Rhododendron campanulatum, Betula utilis, Abies spectabilis, Abies pindrow etc.,* occur between 2400-3600m i.e. near tree limit. *Ephedra gerardiana, Morina coulteriana, Eremurus himalaicus, Saxifraga saginoides* are some of the herbaceous plants, growing on the dry rocky edges.

Moist Alpine scrub occurs at about 3600m wherein species of *Rhododendron, Berberis, Salix, Lonicera. Cotoneaster, Aconitum, Astragalus, Cassiopie, Rheum, Potentilla, Iris, Allium, Pedicularis* etc. are common.

In *Dry Alpine scrubs,* shrubby *Juniperus* species are dominant with many herbaceous plants like species of *Primula, Sedium, Draba, Saussurea, Galtheria, Corydalis, Leontopodium, Anaphalis, Anemone, Gentiana, Saxifraga, Thalictrum, Taraxacum, Carex, Kobresia* and grasses like *Festuca, Poa* spp. etc.

In Lahul & Spiti district, the flora is of the steppe type and is rich at the lower elevations but becomes poorer upwards. There are very few scanty natural forests of *'Kail'* (*Pinus wallichiana*), *'devidiar'* (*Juniperus recurva*) and *'birch'* in Lahul. The density of the forest areas in general is very poor and in most of the areas, designated as forests. There are only scattered or stray trees with forest canopies only in few situations. However, in the lower Pattan Valley some good forests of *Pinus wallichiana, Picea smithiana* (Spruce), *Juniperus recurva* and *Cedrus deodara* are found, although they are frequently interrupted by huge cliffs and rock precipices and are often cut up into long narrow strips by snow drifts and at places by the avalanches.

The vegetation of the temperate zone is characterized by *Pinus wallichiana* (blue pine), *Juniperus recurva* (Junipers), *Picea smithiana* (Spruce), *Cedrus deodara* which form the main natural forests alongwith other angiosperm tree associates like *Betula utilis. Juglans regia, Prunus armenica,* species of *Malus, Sorbus, Populus, Salix* etc. The shrubby vegetation consists of *Ephedra gerardiana, Juniperus communis* var. *saxatilis, J. indica* alongwith angiosperms like *Artemisia, Hippophae* spp., *Rosa* (*R. macraphylla* and *R. webbiana*), species of *Berberis, Crataegus, Fraxinus, Viburnum, Cotoneaster, Lonicera, Ribes, Sorbus* etc. The main herbs constituting the forests undergrowth include angiosperms like *Anemone, Aquilegia, Codonopsis, Gaultheria, Geranium, Podophyllum, Primula* etc. *Polygonaceous* members like *Rheum webbianum. Polygonum affine* form thick mats over rocks. A rich growth of herbs is seen along the streams fed with melting snow. These include species of *Polygomum.* At marshy places species of *Polygonum viz. P. amphibium, P. hydropiper* etc. grow in plenty. In meadows and slopes species like *Oxyria digyna, Rumex nepalensis,* species of *Polygonum* alongwith other associates, grasses and sedges are found.

In Alpine zone *Pinus wallichiana* alongwith *Betula utilis, Rhododendron companulatum* etc constitutes the scattered alpine forest (up to 3600m). Above the *Alpine forest zone,* up to 4200m, the main components include shrubby species like *Juniperus communis* var. saxatilis,

J. indica, J. recurva alongwith other associates including *Polygonum* species. Species like *Rheum spiciforme,* and species of *Polygonum* occur in alpine meadows. Alongwith other associates and sedges (*Kobresia* spp., *Carex* spp.) and grasses.

In Lahul & Spiti district, *Juniperus communis* var *saxatilis,* is used in incenses and perfumes and *Pinus wallichiana* is used for implements and furnitures. *Cedrus deodara, Juniperus recurva, Picea smithiana, Pinus wallichiana* are used as timber plants.

Ephedra gerardiana, Juniperus communis var. saxatilis, J. recurva, Pinus wallichiana, Rheum spiciforme and *R. webbiana* are reported as threatened plants in Lahul & Spiti district by Aswal & Mehrotra (1984).

Juniperus recurva is parasitised by *Arceuthobium oxycedri,* in this area. *Ephedra gerardiana, Rheum spiciforme and Rheum webbianum* are reputed medicinal plants of the region.

Aswal *et al.* (1984) have reported interesting biological activity in some plants of the Lahul & Spiti region *viz. Ephedra gerardiana* (oxytocic), *Juniperus communis* var *saxatilis* (antiimplantation), *Polygonum amphibium* (diuretic), *Polygonum somdevae* (oxytocic) etc.

N.C. Nair (1977) published detailed account of 'Flora of Bashahr Himalaya' (30°46' - 32°5' N & 76°28' - 79°4'E), which covers Kinnaur and Mahasu (including Shimla) districts of Himachal Pradesh, covering *ca* 10000 sq. km area with altitude range of 650-6930m.

Collet (1902) described the vegetation of Shimla Hills. Nair (1977) found that the distribution pattern indicated by Collett (1902) remained the same although several changes were evident due to biotic pressure resulting in clearing of forests and other vegetation consequently soil erosion and disappearance of rare species. The herbs though abundent and often showy (mainly at higher elevations) do not take a dominant role anywhere near Shimla town. *Pinus roxburghii* (Chilpine) is found from 600-1700m, above which the community of *Cedrus deodara* and *Pinus wallichiana* (*Pinus griffithii*) dominate. Blue pine is particularly dominant near Mashobra, Charabra, Kufri and near Narkanda up to 2800m. At Hutto (2000-2300m) *Cedrus deodara* forms a thick belt but higher up, up to 2700m, *deodar* is replaced by *Picea smithiana*- the 'Himalayan Spruce. *Abies spectabilis*- the 'silver-fir' and *Abies pindrow* are also common between (2400-3600m) near Huttoo. Theog to Mattiana (2400-2500m) is the zone of 'Silver-fir, wherein *Taxus wallichiana* is also found. On the southern slope of 'Shali', the communities of *Ephedra gerardiana* are found and on its northern slope, the communities of *Cupresus torulosa* occur.

Almost similar pattern of distribution is found up to Panda. *Taxus wallichiana* is common near Sarahan. The *Spruce* completely disappears near Nichar and populations of *Chil-pine* (at low altitude) and *deodar* (at high altitude) become prominent. In the altitudinal zones of 2400-2800m, at certain slopes where there is much biotic interference, *Quercus semecarpifolia* is replaced by *Abies spectabilis* and *Taxus* wallichiana etc. Glacial moraines are usually on the northern slope and support the dominant population of *Abies spectabilis.* Near glacial region of the moraines, pure population of *Pinus wallichiana (Pinus griffithii)* and open grassy patches are found.

At 1600-3500m, the temperate inner dry valleys of Sutlej, *Paber, Baspa* and their tributories, *communities* of *Quercus ilex* with *Polygonum verticillatum* etc; communities of *Cedrus deodara-Quercus ilex* are seen at several places above Sholtu and on the Baspa valley. Another community is of *Quercus ilex* scrub with a composition of *Pinus gerardiana* alongwith species of *Desmodium, Plectranthus, Artemisia* etc. *Quercus - Pinus gerardiana* community is found from Shontony to Purbani with barren ground or with scattered *Fragaria* and *Potentilla* spp.

Pinus gerardiana- Quercus ilex community is found in main valley above Tapri on flood plains of Sutlej. *Cedrus deodara-Pinus gerardiana- Quercus ilex* community is found near Purbani and in Baspa valley. The ground flora of this community is not so rich except for species of *Rumex, Cannabis* and *Chenopodium.*

Deodar grows well at 1200-2700m in zone of high rainfall. Beautiful forests of *deodar* are found at Paber Valley and Kinnaur district, with almost negligible ground flora.

Shifting cultivation has been in practice in Himalayas for the past several centuries due to which many coniferous forests were cleaned. In such places, a pasture community develops wherein seedlings of *Pinus wallichiana* are seen with herbaceous associates. Secondary community of *Pinus wallichiana* (*Pinus griffithii*) develops on abandoned dry cultivated fields, with undergrowth of angiosperms like *Anemone, Brunella, Galium* etc. No gymnosperms are seen in secondary dry or moist scrub communities.

Interesting relationship between geology and vegetation types along Indo-Tibet road in Bashahr have been

reported. For example in Rampur Quartzite, *Pinus roxburghii* and *Euphorbia* are charactristic while in Gaora flood plains and Sarhan granite, *deodar* is common. *Cedrus deodara* and *Pinus wallichiana (Pinus griffithii)* are characteristic of the granite dip slope of Taranda, Ponda and Nichar. *Deodar* is very common in flood plains of Wangtu & Urni also. *Pinus* forests occur in Lipi granite and in granite schist of Roghi, Pangi, Kalpa and Ranung, *Pinus gerardiana* and *Quercus ilex* are found. Good population of *Abies pindrow, Picea smithiana* and *Pinus wallichiana* are found in the glacial valley of Paber near Rohru.

The alpine vegetation is found from 3500m to snow line. The tree line varies between 3500m (at Kinnaur) and 3800m (at Mahasu). Above this is, the zone of alpine meadows with prostrate *Juniperus communis, J. squamata,* with the angiosperm associates like *Rhododendron, Primula, Saussurea* (*S. gossypiphora, S. obvallata* etc.) *Cotoneaster, Draba lasiophylla* etc.

In *stony desert alpine vegetation* (between 3600-4000m), species of *Bergenia, Sèdum, Primula* etc are noticed whereas, above 4000m Lichens *viz. Cladonia, Stereocaulon* etc. are found. In *alpine scrub* at 3500-4200m. *Rhododendron anthopogon* with its other associates grows in moist zone whereas *dry type of alpine scrub vegetation* is dominated by *Juniperus*, which prefer the soils, rich in lime. The common associates of *Juniperus* are *Draba, Meconopsis, Potentilla, Salix, Carex, Caragana* etc. *Alpine meadows, which are found above alpine scrub,* have a luxurient growth of variety of herbs.

Alpine *Abies-Betula* forest occupy the topmost tree zone, where *Abies pindrow* and *Betula alnoides* are dominant elements with occasional occurence of *Pinus wallichiana.* Herbaceous elements of this zone include species of *Persicaria* and *Polygonum* with other herbaceous associates and shrubs.

A little higher up, the *Abies-Betula alpine forests*, is the zone of *Betula-Rhododendron* alpine forests wherein species of *Polygonum* grow with other associates.

N.C. Nair (1977) recorded the occurrence of 14 species belonging to 8 genera and 4 families of Gymnosperms in Bashahr Himalaya region of Himachal Pradesh.

As per the scrutiny of the literature and the specimens available during this study, 23 species and one variety under 10 genera beloging to 5 families are known so far from Himachal Pradesh. No record of the cultivated taxa of Himachal Pradesh could be available during present study.

'Jammu & Kashmir' (J & K) State has *ca* 2082 million ha forest area of which *subtropical* pine forest occupy 0.281 million ha. In Jammu district foothills subtropical pine consisting of a pure crop of *Pinus roxburghii*, occupies all the higher ridges and also the rocky places at *ca* 900m altitudes. The canopy is 25-35m high and fairly dense in certain places

The mountainous Doda area (74°30' and 76°30'N & 32°30' and 34°15'E) with an area of *ca* 11212.5sq. km is blessed by nature with beautiful valleys, alpine meadows, high plateaus and with several lakes. *Chenab* is the main river traversing the district. The district is characterised by a perdominantly temperate flora. At 'Patni top' the mountain ridges are covered with deodar and pines. The gentle slopes in 'Chenab valley' have mixed vegetation of *Pinus roxburghii.* On higher elevation *deodar* and chilgoza are dominant.

In Poonch district (33°45'N and 74° 5'- 74° 55'E) the vegetation of *Bufliaz* village is dominated by *Pinus wallichians* with other angiosperm associates. From Dograan to Pushiana conifers are found in plenty with angiosperm associates. The base of the gali from Lallana is dominated by angiosperm like *Betula utilis, Salix denticulate, Viburnums* followed by scrub vegetation mainly composed of *Juniperus recurva.*

Singh & Kachroo (1994) reported the occurrence of 7 species under 6 genera and 2 families of Gymnosperms from Pir Panjal which extends between the rivers Chenab and Jhelum, having an average altitude of 4000m. and stated that Gymnosperms form the bulk of the forest vegetation while the angiosperms form the bulk of herbaceous flora. The slopes are rugged and fully dissected and mostly bare except in the valley. The foothills on the valley side are the famous *Karewa* these together the lower slopes bear a thick growth of *Cedrus, Pinus, Picea* and *Abies.* All conifers are in bloom during late summer. Above 3600m there is no tree; the forests mostly occur between 1950-3200m. The eastern and western aspects are less forested. The forests are quite rich on the southern slopes forming an almost unbroken succession except for the *Yarwan block, Nilang* blok and Chrare-i-Sharif which are poorly stocked. The main forest trees are deodar, kail, fir and spruce, the fir forests, occupying an extensive and uninterrupted thick belt from east to west up to 70km. On the basis of vegetation three distinct zones include '*Deodar. Kail Zone, Fir Zone*, and

alpine zone. Singh & Kachroo (1994) following Tikku (1978) classified the forests of the Pir Panjal area under the group '*Kashmir valley dry temperate type*' where in the broad leaved elements are lacking and the forests are wholly coniferous whose conspicuous branching and pyramidal form keeps the floor sufficiently shaded throughout the year except the forest patches which are under heavy biotic pressure (human exploitation).

A well-marked altitudinal stratification is noted from *Chrar-i-Sharief* (1750m) to *Bargahmaidan* and beyond (3600m). *Pinus wallichiana* is restricted to the lower elevation at Chrar-i-Sharief and Kachkora (1750-2350m). At higher altitudes it is associated with *Abies* and *Picea*, even at 3000m but its quality is poor, growth stunted and distribution scattered. *Picea smithiana* only occurs as an associate of both *Pinus* and *Abies*. It is more concentrated in *Abies* zone (2400-2700m). *Abies pindrow* reaches at 2000m in *Pinus* zone but dominates with increase in elevation (2450m onwards at Darawan, Nilnag, Negbal, Yus, Romshi and Dudhganga).

Abies pindrow attains the height growth aspect than on the slopes facing south. *Pinus wallichiana* thrives better on southeastern aspects at lower elevations; the opposite slopes being increasingly mixed with *Picea smithiana* and *Abies pindrow* as the co-dominant elements. Towards its higher limits, it is replaced by *Abies pindrow* and with northwestern aspect. It continues showing its dominance. *Picea smithiana* and *Taxus wallichiana* both seem to show more association with the *Abies* than with *Pinus*, found mostly at two different aspects.

Pure *Pinus wallichiana* forests occur on south-easternly slopes. The main communities responding to the aspects are *Pinus wallichiana-Parrotiopsis jacquemontiana; Pinus wallichiana-Cotoneaster-Indigofera* which are restricted to southeastern slopes (1750m to 2300m) while the *Abies pindrow, Viburnum nervosum; Abies pindrow-Sambucus wightianus* and *Abies pindrow - Skimmia laureala* communities of the *Abies* zone are restricted to northern to north-western slopes (2400m-3200m). *Pinus wallichiana* has better growth on the gently inclined slopes. *Abies pindrow* is restricted to the steeper inclinations as also *Picea smithiana* and *Taxus wallichiana*.

In Pir Panjal range, there is a forest patch (area of *ca* 313.7ha of pure *Pinus wallichiana ca* 1.5km ahead of Chrar-i-Sharief town to *Kanidajan village* (1750-2500m) whose canopy is more than 20m high. The over story in the forests on south facing gentle to medium slopes along Chrar-i-Sharief (*ca* 192.3ha) with pure kail crop consists of *Picea smithiana* as well.

On the gentle slopes with north easternly aspect of Nib, surrounded by cultivation on 3 sides, has main crop of *Pinus wallichiana* with few scattered trees of *Picea smithiana*, both of which form the over storey. In Kachkora forest of Nilnag block (2200-2600m) *ca* 400.6ha, also *Kail* (*Pinus wallichiana*) is the dominant and *Abies pindrow* and *Picea smithiana* are the associates in the upper strata. At Romshi (2300-2450m) on area of *ca* 234.7ha along the hilly slopes with south to south easternly aspect with clayey loam soil is less dense and mostly dominated by *Pinus* with *Picea smithiana* and *Abies pindrow* like associate. *Abies* is more frequent towards Nagbal forest. In Darwan forest (2050-2500m) largely on northern aspects, *ca* 627.3ha area is dominated by *Pinus wallichiana*, but above 2450m fir appears and dominates at 2700-3000m. *Abies pindrow* and *Pinus wallichiana* form a close canopy of more than 25m. *Taxus wallichiana* occurs scattered on upper ridges. In Nagbal forest (2300-3000m), an area of *ca* 670ha is occupied by a mixed crop of *Abies pindrow, Picea smithiana* and *Pinus wallichiana*.

About 1301.1ha area at 2300-2750 (-3000m), the *Abies pindrow* abounds closely associated with *Picea smithiana* on gentle northern slopes from 2500-2700m, but above it occurs a pure community: *Pinus wallichiana* occurs scattered mostly at the lower elevations and *Taxus wallichiana* also occurs scattered in depressious.

Fir - spruce assoication occupies Romshi forest (2300-3000m) covering *ca* 1301.1ha area on the northern aspects gentle above the (up to 2500m) slopes, *Abies pindrow* abounds closely associated with *Picea smithiana* up to 2700m; and above as a pure community *Pinus wallichiana* occues scattered mostly of lower elevations *Taxus wallichiana* also occurs scattered in depressions.

On the other side of the Munshikul, *ca* 270.5ha. area at 2300-2750m is occupied by *Abies pindow Picea smithian-Viburnum nerwosum-Sambucus wightianus* community. At higher elevations occur *Abies intermixed* with *Picea smithiana* (30-40m high) *Abies pindrow* and *Picea smithiana* are dominant at Romshi forest covering *ca* 481.6ha at 2900m on south eastern aspects. *Pinus wallichiana* is insignificant here. Pure fir-mixed broad-leaf shrub associations are seen at Romshi forest at 2600-2900m covering ca 437ha area. Here the *Abies pindrow* stand is more than 35m high. *Picea smithiana* is insignificant and *Pinus wallichiana* is scarce in isolated and exposed localities, but seedlings of *Pinus wallichiana*

and *Abies pindrow* are found in large numbers.

Fir-evergreen broadleaf shrub association covers *ca* 3004ha area at 2700-3000m. of Pir Panjal forest Division, where almost pure communities of *Abies pindrow* occur with admixture of *Picea* and *Pinus. Pinus* occurs isolated in crevices and *Picea* is inconspicuous. Pure fir-deciduous-evergreen broad-leaf shrub association occupies *ca* 248.9ha area at 2900-3400m in Dudhaganga forest lying on the southern slope at the Kali-ali Kori whose characteristic feature is the high level fir forest with thin canopy *Abies pindrow* depicts average height of 10m.

In the subalpine-temperate meadow lands, seedlings of *Abies pindow, Pinus wallichiana* and other trees are seen in protected area; *the alpine bushwood association* lies at the end of Draghtolan Nala. The transition from the upper line of the fir forest and this alpine scrub is quite abrupt at certain localities between 3200-3400m while at some places there is an ecotone consisting of scattered short sized old to middle aged trees of *Abies pindrow*, along the nall as between 3200-3400m. The most common shrubs restricted to drier situations and forming closely growing thickets seperated from each other by small patches of meadows are species of *Juniperus*, and angiosperms like *Cotoneaster, Viburnum* etc.

Broadly the vegetational features of Pir Panjal are different from those observed in the moist temperate and tropical dry temperate zones. Presence of Oaks, Yew, Spruce, and other traditional components of Oak-Conifer of temperate moist forests are rare and Oaks are totally absent from the area under study. However, *Picea smithiana* and *Taxus wallichiana* are found mixed, yet never forming a dominant component. The traditional elements of typical dry temperate Himalaya viz. *Quercus, Ilex, Pinus gerardiana* and *Juniperus macropoda* are also almost absent. But *Abies pindrow* and *Pinus wallichiana* and to some extent *Cedrus deodara* representing the traditional species of high and low rain fall zones, respectively, are quite conspicuous. Presence of deodar in Kashmir Himalay and its dominance in certain pockets is due to less rainfall during monsoon and heavy snowfall providing sufficient moisture during growth period; similar situation is found in Bhaderwah forests of Himalaya.

Forest vegatation of Pir Panjal is mostly dominated by conifer communities, besides few scrubs at various tiers of altitude. *Pinus wallichiana* abound in lower and *Abies pindrow* and *Picea smithiana* in upper reaches. *Taxus wallichiana* occurs in isolated pockets as an admixture mostly.

Kashmir Himalaya lies between 33°-36°N and from 72°-80°E and occupy an area of *ca* 222800sq. Km. the highest peak is *ca.* 5500m high and considerable area occur as meadowlands or glaciers. A few high altitude lakes also occur. The Pir Panjal range seperates the plains of India from Kashimr. Towards north of this range, the mountains are very high with most of the peaks covered with snow all along the years. Banihal (3000m) at the S.E. cover of the range above Verinag, Vishnupad (4400m). The snow covered peaks on the east of Banihal, Tasamaidan (3500m) are some important peaks and Apharwat, Damamsar and Alapathar are the important high altitudes lakes of this area.

Between Kashmir Valley and Ladakh, the lofty mountain ranges include Zojila (3400m) between Sonamarg and Dras; Mahadev (4500m), above Dachhigam, Nun-Kum (3600m), in Suru, Lidderwart (3500m), North of Pahalgam; Kolahoi (4300m), west of Lidderwart, Amarnath (4500m), east of Pahalgam. There are two important high altitude lakes viz. Tarsar and Marsar near Lidderwart.

Royle (1839), Duthie (1893, 1894), illustrated works of Coventry (1923-1930) and Blatter (1927-1929), enumeration of plants by Rao (1960), Singh (1970-1973a), Stewart (1967-1972), are the ealier work from this region. In additions to these, Singh (1972-1973b, 1973c), Nagshi (1973, 1975, 1976), and Dhar (1976), Dhar and Kachroo (1983) reported addition to the flora of the region Sharma and Jamwal reported flora of Lidder Valley, but they did not include gymnosperms. Rau (1975) listed the high altitude flowering plants of western Himalaya without delving into distributional patterns. etc. Singh and Kachroo (1976) studied the forest flora of Srinagar mountains: this treatise includes some alpines also. Kachroo et. al. (1977) made an ecological assessment of the flora of Ladakh. Little attempt has been made to study the geographic patterns of plants from this interesting region, except that of Troll (1967) who worked on vegetational phytogeography of the Himalaya in general. Meusal and Shubert (1970-1971) who mainly studied the Phytogeography of forest vegetation of western Himalaya and later that of Mani (1978) on the biogeography of high altitude plants of northwest Himalaya. Polunin and Stainton (1984) and Stainton (1988) described and illustrated the Himalayan flowering plants including gymnosperms.

Between Sonamarg and Baltal, the road passes through a forest composed of *Pinus wallichiana, Populus ciliata* and Willow. Many pines are much injured by the attack of

the minute parasite *Arceuthobium minutissimum*. Most of the pines of the valley after Aishmakam (on way to Palgam) are also infected with *Arceuthobium*. The existence of this parasite can be detected from a considerable distance, as it causes a congested growth of the branches and thus completely alters the habit of the tree or that portion of it which is attacked. It is most abundant on the smaller branches surrounding them with a moss like covering, but some times found on the trunk also. It flowers during September. It is a dioecious plant; male and female plants form separate colonies. Shrubby *Junipers* are plentiful just below the pass of the head of the Masjid valley and is the only kind of fuel available there.

Near *Rimpchana*, *Ephedra gerardiana* is very abundant and conspicuous with its scarlet berries, and the associated species *Polygonum tortuosum* having the crimson autmnal tint gives a red tinge to the hillsides.

Near *Kargeh pass* (in Dras region) the hillsides exposed to north are thickly clothed with forest, while the opposite sides are almost bare of trees, with the exception of occasional stunted specimens of Pencil Cedar (*Juniperus polycarpos* Boiss).

Abies pindrow (Silver fir) locally known as Katul is one of the principal constituents of the high elevation forests in the Naltar valley, north of Gilgit.

Juniperus polycarpos, a characterstic tree of Baltistan is found in Kashmir on hillsides exposed to the south. It is called *Chili*, in the Astor valley and *'Shupa'* is the Shingo valley. *Pinus wallichiana* (chil or blue-pine) is abundant all over Kashmir and usually forming the outer or lower belt of forests of *spruce* and *silver fir*. The native name for *Pinus wallichiana* in Astor valley is 'Chui' and in Shigar valley (in Baltistan) it is called as 'Showi', *Pinus gerardiana*-locally called as 'Garol' is abundant in Astor valley between 2400-2700m.

On way to Nomal in the Hunza valley from Gilgit, after entering the Hunza valley, the road traverses for few Kilometers an undulating sandy tract covered in parts by a large bushy species of *Ephedra intermedia*. Towards the north east of the Naltar valley, is the forest of Silver fir at the elevations of *ca* 2700m. Beyond this, the valley opens out, and is clothed with *Pine forests* containing Silver fir, Juniper and the Blue pine (*Pinus wallichiana*). On the slopes facing the north-east these forests, extend to considerable elevation mixed with birch and willow.

In Kashmir, in geneal, the composition of the herbaceous groud cover in temperate and subalpine vegetation appears to be same throughout the state. The temperate vegetation is marked by the presence of the evergreen conifers like *Pinus wallichiana*, *Cedrus deodara*, *Picea smithiana*, and higher up these are replaced by *Abies pindrow*. *Taxus wallichiana* is also found but is nowhere so common. The trees start disappearing at 3000-3200m and the shrubs are also few like *Juniperus communis* with some angiosperms. Higher up is the zone of Primroses. Pissughati leads to a high altitude lake 'Sheshnag' (4000m) where the slopes surrounding the lake are mostly covered by a mat of *Juniperus recurva* and the associated herbaceous angiosperms. On way to Wavjan (3900m) *Betula* starts disappearing and the only shrubs are *Juniperus communis* and *Cotoneaster microphylla*. Dhar and Kachroo (1983) have provided the digrammatic presentation of the vegetation profile of Gulmarg- Khillanmarg- Apharwat according to which the *Pinus* population is restricted to Tangmarg (2100-2400m). Some population of *Picea* is seen below Gulmarg above, which is the zone of *Cedrus deodara*. *Picea* is dominant above Gulmarg and *Abies* is dominant near Khillanmarge. Above Khillanmarge to Aphratvat, is the zone of Junipers above which the primroses adorn the ground.

Pure fir deciduous - evergreen broad leaf shrub association occupies *ca* 248.9ha area at 2900-3400m in Dudhaganga forests on the southern slope at the Kali-ali-Kori whose characteristic feature is the high level fir forest with thin conopy. *Abies pindrow* depicts average height of 2300-3200m.

In the subalpine-temperate meadowlands, seedlings of *Abies pinbrow, Pinus wallichiana* and other trees are seen in protected areas. The alpine bushwood association lies at the end of *Draghtolan nala*. The transition from the upper line of the fir forest and this apline scrub is quite abrupt at certain localities between 3200-3400m while at some places there is an ecotone consisting of scatterad short sized old to middle aged trees of *Abies pindrow* along the *nallas* between 3200-3400m. The most common shrubs restricted to drier situations and forming closely growing thickets seperated from each other by small patches of meadows are species of *Juniperus*, and angiosperms like *Cotoneaster, Viburnum* etc.

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dry temperate Himalaya viz. *Quercus, Ilex, Pinus gerardiana* and *Juniperus polycarpos* are also almost absent. But *Abies pindrow* and *Pinus wallichiana* and to some extent *Cedrus deodara* representing the traditional species of high and low rainfall zones, respectively, are quite conspicuous. Presence of *deodar* in Kashmir Himalaya and its dominance in certain pockets js due to less rainfall during monsoon and heavy snowfall providing sufficient moisture during growth periods. Similar situation is found in Bhaderwah forests of Himalaya.

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In forest flora of Srinagar and plants of neighbourhood Singh, &. Kachroo (1976) reported that in Sonamarg to Trunkhal area (2500-4500m), *Juniperus recurva* forms large patches at higher altitudes on slopes. In Lidder valley, they found that the dominant arboreal vegetation includes *Picea smithiana, Pinus wallichiana* (2000-3334m), *Abies pindrow* and seveal broad-leaved angisperms. At Kaolahoi valley (4164m), the Mountains are covered by *Juniperus recurva* and *Betula utilis.*

A major portion of Gulmarg (74°28'-74°31'E & 34°03'-33°58'N) range is dominated by dense cover of *Abies pindrow.* At lower limits *Pinus wallichiana* and *Cedrus deodara* aggregate but higher up are replaced by *Picea smithiana. Taxus wallichiana* is sparsely distributed at the *lower silver fir zone.* The slopes of the southern aspects are often bordered by grooves of *Pinus wallichiana* and *Cedrus deodara. Betula utilis* forest forming the upper limit of tree zone occurs usually in form of pure colonies though *Abies pindrow* and a few angiosperms are also found in them. Above birch zone and within it extending above towards upper limits of scrub zone are associations of *Juniperus recurva* and *Rhododendron hypenanthum.* Singh and Kachroo (1976) collected 6 gymnosperms from Gulmarg area.

Evergreen forests in Srinagar are exclusively coniferous. *Pinus wallichiana* is dominant conifer at lower altitudes. The conifer forests at higher altitudes are dominated, in the inner ranges by *silver fir* (*Abies pindrow*) with usual association of *Picea smithiana and Taxus wallichiana.* In the lower belt of *silver fir* zone *Pinus wallichiana* occurs commonly associated but disappeas higher up. In open strands of conifer forests, deciduous tree elements are also seen.

In *Evergreen scrub* at upper limits dwarf scrub communities domniated by *Juniperus recurva* and *Rhododendron anthopogon* are found.

In Dacchigam sanctuary area, coniferous forests of blue pine occur as isolated groove of variable size on the slopes overlying. The ravine between Harwan reservior and Draphom, which are quite deeper in the sanctuary. Though blue pine in the inner ranges passes slowly in the *silver fir (Abies pindrow)* above 2300, the former rarely forming pure stands above that altitude. The outer range of the sanctuary has pure stands of blue pine reaching often up to 3000m.

Blue pine forests of lower levels closer to ravines, though essentially lacking in deciduous tree elements of the ravines, include shrubby elements from adjacent slopes. Grooves of blue pine shaded for whole of the year, have poorly developed stratification, the scruby layer being quite sparse.

Large areas of the mountain slopes are primarily composed of deciduous scrubs; these also form major components of the *blue pine* forests. *Blue pine* forests at higher levels (2300m) though having essentially similar structure, have distinctitive species of angiosperms. Seral *blue pine* communities are generally located in the areas subjected to fire, moraine deposits at higher altitudes, and along ecotone between *silver fir* and scrubs. Isolated *groves of blue pine* also occur in scrubby and savana communities intercepted by local topographic change towards northern exposure. Such seral *blue pine* communities that are more common on outer ranages of the sanctuary and adjacent slope sometimes reach the altitude of 3000m. The composition of these *blue pine* communities is largely determined by the type of surrounding vegetation. The communities of blue pine developed on moraine deposits and slided down materials are more distinctive in having better humus content and rich soil material. In their general structure the seral *Blue Pine* communities are usually open allowing associated woody and herbaceous species to grow favourably.

Silver fir (*Abies pindrow*) *forests* communities dominate the landscape on inner ranges and extend from near 2300-3200m. In the lower level where these often descend down to near 2100m. The *silver fir forests* pass *into blue pine* the latter slowly is disappearing upwards. In the upper limits the *silver fir* passes into birch. The lower limit of silver fir usually has the angiosperm associates like *Rhus succedanea, Ulmus wallichiana* ssp. x*nthoderma, Aesculus indica, Acer cappadocicum, Celtis caucasica, Crataegus monogyna* that disappear upwards.

In the middle of *silver fir zone* at about 2700m altitude and northeasterly exposure, the *silver fir* is associated with all the three coniferous tree species (*Picea smithiana, Taxus wallichiana* and *Pinus wallichiana*) present in the woodland though these three species combined contribute a small part of total tree cover as compared to silver fir and occur as isolated individuals in *silver fir* forests. The herbaceous layer in the zone is well developed, the area being well protected.

In its upper limit the silver fir zone passes into birch. In its upper belt (alt. 3100m) where *fir* and birch are the principal components with associated species like *Rhododendran companulatum, Syringa emodi, Betula utilis, Viburnum grandiflorum, Salix wallichiana, Lonicera purpurescens, Rosa macrophylla var minor, Berberis pachycantha, Lonicera discolor,* and *Juniperus recurva*. At lower elevations *Viburnum grandiflorum, Berberis pachyacantha, Salix wallichiana* and *Lonicera discolor* form the woody components.

The usual tree species in *Corylus-Podus* association, which extends between 2300m. and 2900m in the outer ranges of 'Dachhigam reserve' in the form of small grooves, include *Ulmus wallichiana, ssp., Xanthoderma, Acer cappadocicumm, Pinus wallichiana, Taxus wallichiana* and *Aesculus indica.*

In the blue pine community, the maximum cover is contributed by the *blue pine* itself, the under strata being quite sparce, except for ground cover formed by herbs; mostly the low growing species.

Dwarf evergreen scrub communities formed exclusively of *Juniperus recurva* and *Rhododendron anthopogon* often occur as large patches on the alpine slopes. Though smaller patches of either species occur distinctly, such patches are generally contagious forming continuous mixed communities. These species scarcely exceed 1m in height, and in upper limits are often reduced to 20cm. in height. The scrub cover in this association generally exceeds 70 percent, in old communities and these two species contribute upto 90 per cent coverage.

Isolated communities of *blue pine* are distributed on steeper slopes towards the top of the southern range near Harwan to Shalimar. The lower level *blue pine* community, closer to villages has poorly developed stratification due to biotic pressure and associated factors. In Dachigam Sanctuary where from six species of Gymnosperms under 5 genera and two families have been recorded by Singh and Kachroo (1976). The armed scrubs like *Juniperus communis* are protected from browising the trampling. The scrubs show very stunted growth due to mixed biotic influences and close tree canopy of the blue pine. But this influence decreases successively at higher levels, being distant from the habitation below and on more steeper slopes and this effect is reflected in better stratification at higher levels as compared to lower level blue pine community. Pherplogical studies of Singh & Kachroo (1976) revealed the major concentration of the flowering during July-Sept but considerable reduction of flowering was observed in excessively grazed communities at lower levels. Flowering percentage increases at upper levels with concentration towards summer months. The higher levels exhibited lag in flowering. Isolated young individuals of *Pinus wllichiana* are met especially where the mature individuals of this species are at hand.

However, in view of the inaccecebility to Kashmir during the recent past, the account on distribution of *Gymnosperms* in present Kashmir Himalaya is yet to be confirmed by ground survey. But it is hoped that the present situation might have not been able to affect the diversity and population of these precious elements of the flora.

DISTRIBUTION OF DIFFERENT TAXA

Cycas is the most widely distributed member of the Cycadales. Its species are distributed from the Southern part of Japan, in the North, to Australia and beyond, into the Islands of the India and the Pacific Oceans, in the South. A few species grow on the mainland of Asia -in India, Indo-China and China and one or two even as far as Madagascar, Mauritius and the Eastern Coast of Africa. Five species, viz. *Cycas beddomei, Cycas circinalis, Cycas edentata de Laub., Cycas pectinata* and *Cycas sphaerica* are found wild in India. Besides these, *C. revoluta,* a native of Japan, is the most commonly cultivated species in Indian gardens while *Cycas siamensis* which is found in Myanmar, Thailand and other adjoining regions is also sometimes cultivated.

The genus *Gnetum* is confined to the tropical and humid regions of the world. It comprises more than 30 species. Of these *Gnetum latifolium* Bl., *Gnetum gnemon* L. grows wild in the Andaman and Nicobar islands. *Gnetum ula* Brong. occurs all along the Western ghats of India; *Gnetum contractum* is confined to the extreme southwestern tip of India. *Gnetum gnemon* occurs in N.E. India (Old Assam) also (Markgraf, 1930, 1951; Bharadwaja, 1957). Most of the species are endemic within the areas of their distribution. No species has been found which is common to both the hemispheres and none of the Asiatic species reache either Africa or

America. The centre of the present diversification appears to be Eastern Malaysia. All species inhabit the tropical rain forest below an altitude of 1500 m except *G.microcarpum* (not found in India), *which* has been reported from Mt. Tahan (Pahang, Malaya) only at *ca* 2000 metres. Species with large leaves and fruits are found in the moist air of mountain gorges while those with small leaves occur both in the rain forests and in drier localities (Markgraf, 1951). Markgraf (1930, 1951) lists the following species from India, South-East Asia and China.

Five species, 4 varieties and 1 forma of *Gnetum* are found in India. Of these *Gnetum ula* is the most common. This is an extensive woody climber having branches with swollen nodes. It occurs in the Western Ghats near Khanadala, Kanara and the evergreen forests of Coorg, Kerala, Nilgiris; Chenath Nair and Pulni Hills, the Godavari district of Andhra and some parts of Orissa (Kerala), Nilgiri Hills and Coonoor in Madras. *Gnetum gnemon* has six varieties of which two occur in India. *Gnetum gnemon* L. var *brunonianum* (Griff.) Mgf is a slender shrub with sessile seeds borne on a thick axis; it occurs in the South Lushai Hills, (Mizoram) Naga Hills (Nagaland) and Golaghat areas of Assam, *Gnetum gnemon* L. var *griffithii* (Parl.) Mgf is a shrub up to 2m. high having oblong leaves, with parallel margins; it is found in Sibsagar and Kungaba areas of Assam and Naga Hills (Nagaland) *Gnetum montanum* Mgf. is a robust climber with smooth, slender branches swollen at the nodes. It occurs in Sylhet, (Bangladesh) the Nahrabbi localities of N.E. India(Old Assam), Sikkim, and Mayurbhanj in Orissa. *Gnetum latifolium* is a large climber with dark green variable leaves. *Gnetum latifolium* Bl. var. *macropodum* (Kurz) Mgf occurs in Andaman and Nicobar Islands and *Gnetum latifolium* var *funiculare (Bl.)* Mgf has been recorded from the Andamans only (Bharadwaja, 1957).

Only one species of *Taxus* (Taxaceae) is found all along the Himalaya at 1800-3000m elevations. One species of *Amerntotaxus assamica* (Taxaceae) is found in Arunachal Pradesh (Lohit district). Two species of *Cephalotaxus* (Cephalatoxaceae) are found in India. *Cephalataxus mannii* is found in Khasi, Jaintia and Naga Hills from 1370-2590m. *Cephalotaxus griffithi* occurs in Arunachal Pradesh (Kameng district), Naga Hills, Manipur and Mishmi Hills at 1520-1820m. Three species of *Podocarpus* (Podocarpaceae) are found in India. *Podocarpus neriifolius* occurs is Sikkim, Arunachal Pradesh (Kameng district) and Andamans. *Podocarpus wallichianus* is found in Western Ghats from Nilgiris southwards. Great Nicobar, Assam, Martaban and Tenasserim. *Podocarpus gracillior* is an exotic species planted in gardens. It is nicely fruiting at New Forest Campus, Dehradun. Eight taxa of *Pinus* (Pinaceae) are indegenous in India while, eighteen exotic species are in cultivation. *Pinus wallichiana* occurs all along the Himalaya from Pakistan to Arunachal Pradesh at 1800-3700m, rare in Sikkim and a considerable protion of Kumaon. *Pinus wallichiana* var. *parva* is found in Arunachal Pradesh (Tawang dt.) at *ca* 3300m. *Pinus armandii* occurs in Arunachal Pradesh, (NE Frontier, Lohit District *ca* 2100m. *Pinus bhutanica* is found in Tenga valley in Kameng District, Arunachal Pradesh. *Pinus gerardiana* occurs in Bashahr Himalayas, westwards to Kashmir. *Pinus kesiya* is found in Khasi Hills, Naga Hills and Manipur and is planted at large scale in Arunachal Pradesh also. *Pinus roxburghii* is found all along the Himalaya from Pakistan to Arunachal Pradesh at 450-2300m, except the Kashmir valley proper. *Pinus merkusii* is found in Arunachal Pradesh (Lohit District). *Tsuga dumosa* is found from East Kumaon to Arunachal Pradesh. Two species of *Picea* are found in India. *Picea smithiana* is found in the western Himalaya, from Afganistan to Kumaon at 2150-3300m. *Picea spinulosa* occurs in Arunachal Pradesh (Kameng District), 1500-2300m. Four species of *Abies* are found in India all along the Himalaya (Myanmar up to Arunachal Pradesh). *Abies pindrow* is found in Afganistan, Pakistan and India. It is one of the principal constituent of forests in Neltar valley, north of Gilgit. *Abies spectabilis* occurs all along the Himalaya from (Pakistan to Arunachal Pradesh and Tibet; 2800-4300m), but is common between 3300-4000m *Abies densa* is found from Darjeeling to Arunachal Pradesh. *Abies delavayi* occurs in Perila, above Tawang, Se La (Kameng Distirct, Arunachal Pradesh), at 3000-3300m. *Cedrus deodara* is found in Kashmir, Himachal, Uttaranchal Garhwal, 1200-3300m. One species of *Larix* is found in India. *Larix griffithiana* occurs in Arunachal Pradesh, common from 2900-3300m. Eight species and one variety of *Juniperus* (Cupressaceae) are recorded from India. *Juniperus polycarpos* is found in Kagan valley, Kashmir, Lahul to Kumaon, *Juniperus recurva* is found all along the Himalaya in inner valleys to Arunachal Pradesh. *Juniperus recurva* var. coxii occurs in Eastern Himalaya. Eight species of *Ephedra* have been recorded from India. *Ephedra gerardiana* occurs at Karakorum, Chitral, Gilgit, Hazara, Kashmir, Ladakh, Chamba, Lahul, Baltistan, Zanskar, Kistwar, Garhwal (Badrinath) Kumaon, arcas. *Ephedra foliata* is found in Punjab, Rajasthan, Sind and Baluchistan. *Ephedra intermedia* var. *tibetica* is found at Lahul spiti, Pangi, Rowli chamba and Ladakh. *Ephedra nebrodensis* var. *procera* occurs in Kashmir, Himachal Pradesh (Chamba, Lahul) and Baluchistan. *Ephedra*

pachyclada occurs in Chitral, Kuman Valley and Baluchistan. *Ephedra prezwalskii* is found at Naga Parbat in Kashmir and along bed of Shyoko River in Ladakh. *Ephedra regeliana* is known to occur at Ladakh and Karakorum. *Ephedra saxatilis* is found in Himachal Pradesh (Shimla), Uttaranchal (Gaunsar, Badrinath in Garhwal, Kunawar, Kumaon) and south Tibet. A variety of it viz. *E. saxatilis* var. *sikkimensis* has been reported from Cho-Chamo (*ca* 5200m) in North district of Sikkim state of India and S. Tibet.

ECONOMIC IMPORTANCE

Abies pindrow the Himalayan silver fir yields light wood which is used for packing cases; for ceiling and floor boards, planking and camp furniture. Its wood is suitable for paper pulp also. The wood of *Abies spectabilis* "the east Himalayan silver fir", is also used for the same purposes as that of *Abies pindrow*. It is also used for making cheap grade pencils. Its leaves (known as Talispatra) are considered carminative and used in treatment of cough and phthisis. A purple or violet dye is extracted from its cones. A white resin is also obtained from the trees.

Agathis robusta – an exotic, is the source of an excellent timber for general joinery. Its wood is suitable for manufacture of writing, printing and wrapping papers; also for viscose rayon and oleoresin is obtained from plant which is used in adhesives, paints, polishes, inks and lacquers.

The species of *Araucaria* are common man's favourite for plantation as ornamental plant. *A. bidwillii* Hook. yields wood, suitable for indoor finishes of houses, furniture, carving and boxes. Pulp is used for paper making. Its roasted seeds are eaten. Wood of *A. columnaris* (Forst.) Hook. is also used for above purposes and is a better choice for general carpentry works. Wood of *A. cunninghamii* D. Don is excellent for joinery, cabinet work, flooring, railway cars, furniture boxes, small boats, veneers, match splints. The latex obtained from the tree yields a volatile oil called "Turpentine of Araucaria". Bark is used for hard boards. *A. heterophylla* (Salisb.) Franco is grown on a large scale for decoration, as window for table plants. Its wood is used like that of other Araucarias.

The wood of *Callitris cupressiformis* Vent. (syn.: *C. rhomboidea*) is used as fuel and the plants are used for hedges.

The wood of *Cedrus deodara* (Roxb.) Loud. – Deodar, Himalayan cedar, is strongest of Indian coniferous woods and is used for doors, window frames, furniture, packing-cases, beams, masts, spars, shingles and for bridges/ railway sleepers. An oleoresin and a dark colored oil obtained from wood is used in ulcers and skin diseases. Wood is considered diuretic, diaphoretic and carminative. Its bark is used in dioarrhoea and dysentery.

Picea smithiana Boiss- "The west Himalayan spruce", is one of the most useful timber trees of the western Himalayas. Its wood is used for planking, joinery, furniture, tea boxes, packing cases, match boxes, battery separators, fence posts, transmission poles, news print manufacture etc. An essential oil obtained from leaves is used in bath salts, deodourants and room sprays; leaves are also used as manure and litter for the cattle. Bark contains 4.22% tannin. *Picea spinulosa* (Griff.) Henry is the source of wood which is apperantly similar to that of *P. smithiana* but is harder and haeavier; yields pulp for writing and printing papers. Its other uses are similar to *P. smithiana*.

Different species of *Pinus* including the indigenous ones and the exotics are used for several purposes. Seeds of *Pinus armandii* Franch. are edible. Timber of *P. canariensis* C. Sm. is used for building purposes, furniture and joinery. *P. caribaea* More is a good source of commercial resin. *P. sembroides* Zucc. var. *edulis* Voss is the source of edible seeds. *P. gerardiana* Wall. commonly known as chilgoza pine, is the source of chilgoza seeds which are sold as dry fruits. Its seeds are valued as a dessert, carminative, stimulant and expectorant; contain a fatty oil; yields an oleoresin of good quality. *P. halepensis* Mill. Is planted as wind break, for soil conservation and sand fixation. *P. kesiya* Royle is the source of an oleoresin which yields "Turpentine oil" (which is superior to that of *P. roxburghii*) and also a golden yellow resin. Bark yields tannin and the wood is used for indoor construction; as board wood, suitable for planking; packing cases. Its wood is also used for pulp; suitable for bag and wrapping papers. *P. merkusii* Jungh. & De Vriese is the source of wood used for general construction and indoor work. It is also used to cover the eroded hill slopes. The wood of *P. nigra* Arnold var. *calavarica* Schneid. is used as source of pulp and for mine-props. *P. pinaster* Ait. "the cluster pine", is one of the main commercial sources of pine resin. Its timber is used for telegraph poles and railway sleepers. *P. roxburghii* Sarg. - Chir pine is the most useful *Pinus* species in India. It is the source af an oleoresin which yields turpentine oil having low *pinene* and high *carene* contents; mainly used as a solvent for paints and varnishes; also used in pharmaceutical preparations, for perfumery industry, manufacture of synthetic pine oil, disinfectants, insecticides and denaturants. It is one of the most

important basic raw material for synthesis of terpene chemicals. Its is expectorant, useful in chronic bronchitis and very effective in gangrene of lungs; given as a carminative in flatulent colic; to control minor haemorrahages in tooth sockets and nose; externally used as a rubefacient in rheumatic affections and for deep-seated inflammations particularly of abdomen. It is the source of rosin which is used in paper, soap, cosmetics, paint, varnish, rubber and polish industries; also in linoleum and roofing cements, fire works, match compositions, explosives, insecticides and disinfectants. Rosin oil or rosin spirit is used in printing ink and as an adulterant for boiled linseed, olive, rape and sperm oils. Young twigs, fresh needles and cones yield "pine needle oil" which is used in soaps, bath preparations, room sprays, deodourants etc. Exhausted needles are converted into *pine wool* and used for stuffing pillows, cushions and mattresses; also for packing fruits. Wood is used for construction purposes, joinery, furniture, truck and bus bodies, packing cases, electric transmission poles, railway sleepers, railway wagons and for paper pulp. Its seeds are eaten and the bark yields tannin. The seeds of *P. sabiniana* Doug. "the digger pine", are edible. *P. taeda* L. - "the loblolly pine" is the source of American turpentine oil. Its wood is used for ship building and for wood pulp. Wood of *P. thunbergii* Parl.-"Japanese black pine" is used for heavy construction, interior finish, box board and sleepers. It is essentially a maritime species. *P. wallichiana* A. B. Jackson- "the blue pine " or "kail" is the source of best wood next to deodar. Yield of oleoresin from this species is low but the turpentine oil is of superior quality. "Pine needle oil" is obtained from green needles and cones. It is the source of an excellent quality of charcoal.

Thuja oxidentalis L. is cultivated as hedge and wind breaks. An essential oil viz. "Thuja oil" or "White cedar leaf oil" obtained from leaves and twigs are used in room sprays, disinfectants, insecticides, hair lotions, soaps etc. The fruits of *T. orientalis* L. yield an essential oil and the seeds are the source of a fatty oil. An essential oil obtained from leaves is used as tonic, diuretic and anti-pyretic. Tannin is obntained from leaves and twigs. Wood is used for making furniture, fence post, barrels etc. Leaves and twigs of *T. plicata* D. Don yield an essential oil. Its wood is used for shingles, sleepers, boats, cabinet work etc.; also used for paper pulp; source of a fibre, used for making baskets, mats, hats etc.; yield an essential oil. Wood of *T. standishii* Carr. is used for making thin boards for lining walls and ceilings, sliding doors, boxes and bent wood work. Leaves yield an essential oil.

Kernels of *Ginkgo biloba* L. are eaten (roasted/cooked) but, these are toxic if eaten raw. Seeds are used in preparation of a detergent; also used as an expectorant and sedative. Fruits contain a volatile oil. Wood is used for chess boards and toys. A medicine viz. "Bilovas" prepared from this plant is used as general tonic and also to control the loss of memory in diabetic patients.

Leaves of *Zamia angustifolia* Jacq. Contain *Bilobetin, Ginkgetin, Sciadopitysin* and other compounds found in *Ginkgo biloba*. Acqueous extract of *Zamia furfuracia* Ait. Is slightly toxic to American cockroaches but not to german cockroaches and milk weed bugs. Stems of *Z. pumila* L. are rich in starch suitable for extracting arrowroot.

Cycas plants have found a variety of uses; e.g. for food, medicine, horticulture, decoration, rituals etc. In the Tonga Islands, *C. circinalis* is even cultivated as a food plant. The nutritive value of *Cycas* plants has been known to man for a very long time. From very ancient times, in Eastern and Southern parts of India, Japan, South East Asia, Australia and other native regions of *Cycas*, people have used its plants as a source of food. The Japanese have been using the plants of their indigenous species, *C. revoluta*, not only for preparing starch (sago) and alcoholic drinks but in times of famine its stems have served as invaluable living stores of food. The importance of *C. revoluta* plants in 18th century Japan was so great that their export was banned by prescribing capital punishment for it. The starch contained in the stems (chiefly the pith and the cortex) and the endosperm of the seeds of *C. circinalis, C. revolute, C. rumphii* and other species is variously extracted and forms a useful item of food. In some species e.g., *C. circinalis* and *C. rumphii* the annual yield of starch from the seeds and the felled stems is about the same and in the long run it is always more profitable to use the seeds of these slow growing plants.

The stem starch is widely extracted and prepared into 'sago' (Sabudana in Hindi) in the various parts of South and South East, e.g. India. Fiji, Indo-China, Japan, Indonesia, Malaya, New Caledonia, Myanmar, Sri Lanka and other islands of the Indian and Pacific Oceans including Madagascar. The starch is usually prepared by felling the plants flush with the ground when they are about seven years old. The yield of starch is greater if the trunks are cut off just before the production of a crown of leaves and before they produce any cones. The male plants of *Cycas revoluta* are said to yield more starch than the females. A common method of extracting starch consists of peeling off the outer layers of the trunk and thereafter slicing it into small bits or rounded discs. These

are then spread out over mats, dried in the sun and when they have become crisp they are pounded and powdered. The powder is then sifted and stirred with excess of water and then allowed to stand. A white precipitate (starch) now settles down at the bottom of the vessel. The supernatant liquid is drained off and the white deposit is generally washed repeatedly in water and finally the fresh washed precipitate is rolled about, between boards, until it turns into spherical pellets called 'sago'.

The substantial endosperm of the seeds (often called 'fruits') in various species contains 20 to 30 percent starch. In South Malabar, the sun dried and subsequently ground kernels of the seeds of *Cycas circinalis* are repeatedly washed in water and turned into a flour called *'indum podi'* which is stored and used in times of need. The 'Sinhalese' and the 'Weddas' of Sri Lanka, use the washed flour prepared from the seeds of their native species for making cakes. Starch is extracted from the seeds of *Cycas revoluta* in Japan. The original inhabitants of Australia and other Australasian Islands prepare a kind of porridge from the seed kernels of *Cycas media*. The seeds of this species form an important source of their food and often gathered and stored in large quantities.

In the Andamans, Aru and Key Islands, and in New Caledonia the roasted or cooked seeds are eaten as such. The inhabitants of the Fiji Islands eat the boiled kernels of the seeds. The hill tribes of Assam eat the seeds of *Cycas pectinata*. The sweet mucilagenous testa of the seeds of *Cycas revoluta* is edible. The kernels of the seeds too are eaten in Japan after being roasted. The people of Tumbocca, according to Rumphius, have to eat the seeds during mourning. Some tribes of the Northern Territory in Australia use the seeds of *Cycas media* as sacramental foods.

Young succulent leaves of various species, e.g. *Cycas circinalis, Cycas pectinata, Cycas rumphii,* and *Cycas siamensis,* are often cooked as vegetables in N.E. India (Old Assam), Malaya, Philippines, Aru and Key Islands, parts of Indonesia and the Moluccas. In Sri Lanka people add them to their curries. Japanese also eat the tender leaves of *Cycas revoluta.*

The various parts of the plants and the starch extracted from *Cycas* seeds and stems often contain a toxic principle, which is removed by heating and cooking the leaves and the kernels or by liquidating the starch with water. However, *Cycas* starch forms only the poor man's food or is used only in times of scarcity since its continuous use is said to cause intestinal trouble (*cf.* Burkill, 1933).

The inhabitants of the Marianna Islands section and seep the seeds of *Cycas seemannii* in water to prepare an intoxicant from them. Seeds and stems of *Cycas revoluta* are used for preparing wine in Japan.

The plants of various species of Gymnosperms are used widely in indigenous systems of medicine for ailments of different kinds. The juice of young leaves of *Cycas circinalis* is used as a remedy for disorders of stomach, flattulence, vomitting of blood and skin diseases. In South India, a poultice prepared from the crushed bark, seeds or megasporophylls of *Cycas* and coconut oil is applied to wounds and sores and a paste of the megasporophylls is applied to *loins* to relieve nephritic pains. The pounded and crushed stems of *Cycas pectinata* are used in Assam as a hair wash for the treatment of diseased hair roots. In Cambodia leafless terminal bud of *Cycas circinalis* is crushed in rice water or in a watery suspension of fine particles of clay, and used in dressing ulcerated wounds, boils etc. A decoction prepared from the young red seeds is used as a purgative and emetic. The plant of *Cycas revoluta* is said to be tonic and promotes expectoration. The pollen grains of some species are reported to be strongly narcotic and the microsporophylls of *Cycas rumphii* and *Cycas circinalis* are often used as anogynes in India (*cf.* Burkill, 1933). The gum of *Cycas rumphii* is said to be amazingly effective in healing malignant abscesses. (Kurz, 1877).

The various species of *Cycas*, but most commonly *Cycas revoluta* are cultivated as ornamental plants in gardens all over the world. In India and other tropical regions they are cultivated in the open but in the colder countries they are usually grown in hot houses. In Java, the trees of *Cycas* are often planted in graveyards.

In Malabar region of India, the fresh leaves of *Cycas* are used for the decoration of temples and homes on festive occasions. They are used in the rituals of the natives of New Hebrides and the Solomons. In Goa (India) Philippines and Australia they are used in the Palm Sunday processions. In Europe and America the leaves of *Cycas revoluta* are employed in preparing wreaths and other floral decorations. They are often used in making artificial palm trees for indoor display. For such purposes large quantities of specially treated leaves (plunging the freshly cut leaves in boiling sea water as a result of which they become leathery and do not break on drying-cf. Schuster 1932) are annually imported from Japan, chiefly from the Oschima and the Okinawa Islands.

Beautifully spotted wood of *Cycas revoluta* is used in Hakone Mt., west of Yokohama, Japan, for making small

boxes, dishes, and flask bases etc., which are known by the special name of '*Hakone work*'. This industry is now probably extinct. The trunks of *Cycas rumphii* are sometimes used in Indonesia to build small houses. The fine surface hairs (*ramentum*) found at the base of leaves of some species are employed sometimes in stuffing pillows and mattresses. The surface fibres of *Cycas revoluta* have been used for making cloth. Ropes and twine can be made from the structural fibres of the leaves and bark of *Cycas*. An oil extracted from seeds of *C. revoluta* was used in Okinawa during the food scarcity caused by the Second World War. The gum of *C. circinalis, C. rumphii* and other species is said to be useful as an adhesive. The starch of *Cycas* seeds and stems is occasionally used in laundrying. The children sometimes use the dried shells of the seeds of C. circinalis as whistles and playthings. In India, the empty shells are often used as small snuffboxes. The leaves are used for weaving baskets, mats etc. or for making brooms or thatching houses. The crushed seeds of *C. circinalis* are used in Cambodia as fish poison. Nomadic people of Celebes used to give the juice of the seeds of *Cycas* to kill their children when they wanted to remain unburdened by a family.

The seeds of *C. circinalis* contain: *starch* (*ca* 31%);¾a tragacanth-like gum; *pakoein*¾a pale yellow amorphous powder, soluble in water but insoluble in ethanol, methanol, benzene, acetone, petroleum ether and ether; phytosterin¾a cholesterin-like substance associated with *pakoein*; a dextrorotatory reducing sugar; a fatty oil 0.154% and an amorphuous water soluble toxic glucoside whose exact composition is not known. *Pakoein* and *phytoserin* are said to be respectively the poisonous and toxic substances of the seed.

The seeds of *C. revoluta* contain: crude protein 14%, soluble non-nitrogenous substances including some sugar alleged to be fructose 68% and combined formaldehyde 0.164 to 0.22%. The seeds may yield up to 23.37% fat, depending upon the method of extraction, which contains palmitic, stearic, oleic, and a small amount of behenic acid. The fat has acid value 182.25, sap. val. 199.76, iod, val. 59.82 and unsapon matter 4.80%. About 90% of the formaldehyde can be removed by washing the seed flour repeatedly with water or by boiling the seed slices. A toxic glucoside called *cycasin* has been isolated from the seed kernels of *C. revolute. Cycasin* is identical with *glucosyloxyazoxymethane* isolated by him from seeds of *C. circinalis.*

The air dry matter of stem of *C. revoluta contains* 44.5% starch and 9.15% crude protein but the composition varies with age, in different seasons and in male and female plants. Stems contain 0.074mg. of formaldehyde per 100g. of stem tissue. Leaves of *C. revoluta* contain 0.021mg. of formaldehyde per 100 g. of leaf tissue.

Gnetum gnemon is cultivated in Malaya, Indonesia and some Southeast Asian islands for its edible fruits. The yellowish-red outer part of the fruit is discarded and the seeds are eaten after roasting or cooking; the seed kernel is mashed, moulded into cakes or biscuits, dried in the sun and fried in boiling oil. The young leaves and inflorescences are eaten as vegetable. The bark yields a fibre, which is obtained by peeling the branches and beating and splitting the bark into fine filaments. The fibre is strong and durable in seawater and has a good tensile strength both in dry and wet conditions. It is valued for fishing nets and lines. Ropes made from it are strong, pliable and light. It is also suitable for paper pulp. The wood of old trees is dark, brittle and non-durable. It is used for making posts for rafts and junks. Split branches may be used for cooperage (*cf.* Markgraf, 1951). The seeds of *G. ula* are utilized in Penissular India in the same way as those of *G. gnemon.* The kernels yield 14.2% of a fixed oil, which is used in Kerala for massage in rheumatism, for illumination, and to a small extent for edible purposes. In Annam the roots and stems are used as antiperiodic. The bark of *G. latifolium* is used for making ropes and nets. The kernels are eaten after roasting or boiling. *G. monlanum* is reported to possess piscicidal properties.

CONSERVATION STRATEGIES

Although all gymnosperm habitats are protected by law, yet due to greed of some influential persons mass destructions of these climate forests have been observed during the present study. A "Gymnosperm Sanctuary" has been established at Khirsoo in Uttaranchal under Botanical Survey of India. A good number of species (Indigenous and Exotic) are well protected in the Botanical gardens established in different parts of the country (Table V). *In- vitro* multiplication of *Taxus wallichiana* has been successfully done by SFRI, Itanagar.Such attempts are yet to be made for other taxa also.

Table V: Gymnosperms Conserved in Botanic Gardens in India

Species	Family	Garden
Abies delavayi	Pinaceae	Llyod Botanic Garden, Darjeeling.
Abies densa	Pinaceae	Llyod Botanic Garden, Darjeeling.
		Jawaharlal Nehru Botanical Garden, Rumtek, Sikkim.
		Sikkim Himalayan Circle, Botanical Survey of India.
Abies pindrow	Pinaceae	Botanical Garden and Arboretum, Dr. Y. S. Parmar University of Horticulture & Forestry, Solan (Himachal Pradesh)
		Northern Circle, Botanical Survey of India, Experimental Garden.
Abies spectabilis (*Abies webbiana*)	Pinaceae	Llyod Botanic Garden, Darjeeling.
Abies nordmanniana	Pinaceae	Llyod Botanic Garden, Darjeeling.
Agathis browrii	Araucariaceae	(The) Assembly House Garden, Kolkata.
Agathis damara (*Agathis loranthifolia)*		University Botanic Garden, Tilka Manji Bhgalpur University, Bhagalpur.
Agathis lanceolata		Eastern Circle, Botanical Survey of India, Woodlands Laithumkhrah, Shillong.
		Northern Circle, Botanical Survey of India, Experimental Garden, Dist. Pauri, Garhwal, Uttaranchal.
Agathis robusta		Experimental Garden and National Orchidarium, Botanical Survey of India, Yercaud, Salem, Tamil Nadu.
		Central Circle, Experimental Garden, Botanical Survey of India, Allahabad.
		Experimental Garden, Western Circle, Botanical Survey of India, Pune
		Llyod Botanic Garden, Darjeeling.
		Botanical Garden, Forest Research Institute and Colleges, New Forest, Dehradun.
		Government Botanic Garden, Ootacamund (Udhagamandalam), Tamil Nadu,
		Botanical Garden, Guru Nanak Dev University, Amritsar.
		Indian Botanic Garden, Howrah.
		Llyod Botanic Garden, Darjeeling.
Araucaria cookii *(Araucaria angustifolia Araucaria columnaris)*	Araucariaceae	University Botanic Garden, Tilka Manjhibhagalpur University, Bhagalpur.
		Botanical Garden, Forest Research Institute

		and Colleges, New Forest, Dehradun.
		Government Botanic Garden, Ootacamund (Udhagamandalam), Tamilnadu.
		Horticultural Experiment & Training Centre, Saharanpur.
		Indian Botanic Garden, Howrah. Llyod Botanic, Garden, Darjeeling.
		Northern Circle, Botanical Survey of India, Dehradun.
		State Botanical, Garden, Barang, Orissa.
		Experimental Garden and National Orchidarium, Botanical Survey of India, Yercaud, Salem, Tamil Nadu.
		Sim's Park, Coonoor, Nilgiri Distt. Tamil Nadu.
Araucaria bidwillii	Araucariaceae	The Assembly House Garden, Kolkata.
		Llyod Botanic Garden, Darjeeling.
		Botanical Garden, Forest Research Institute and Colleges, New Forest, Dehradun.
		Government Botanic Garden, Ooty.
		Horticultural Experiment & Training Centre, Saharanpur.
		Indian Botanic Garden, Howrah.
		Llyod Botanic Garden, Darjeeling.
		Experimental Garden and National Orchidarium, Botanical Survey of India, Yercaud, Salem, (Tamil Nadu).
		Sim's Park, Coonoor, Nilgiri Distt. (Tamil Nadu).
Araucaria cunninghammi	Araucariaceae	Botanic Garden, Tilka Manjhi Bhagalpur University, Bhagalpur.
		Eastern Circle, Botanical Survey of India, Woodlands Laithumkhrah, Shilong.
		Botanical Garden, Forest Research Institute and Colleges, New Forest, Dehradun.
		Government Botanic Garden, Ooty.
		Horticultural Experiment & Training Centre, Saharanpur.
		Indian Botanic Garden, Howrah.
		Llyod Botanic Garden, Darjeeling.
		National Botanical Research Institute, Lucknow.
		State Botanic Garden, Barang, Orissa.
		Sim's Park, Coonoor, Nilgiri Distt. (Tamil Nadu).
		Western Circle, B.S.I., Pune.
Araucaria heterophylla (*Araucaria excelsa*)	Araucariaceae	Indian Botanic Garden, Howrah.
Araucaria rulei		Government Botanic Garden, Ooty.

Bowenia spectabilis var. serrulata	Cycadaceae	Regional Plant Resource Centre, Nagapalli, Bhubaneswar.
Callitris cupressiformis (Callitris rhomboidea; Cupressus australis)		Llyod Botanic Garden, Darjeeling. Experimental Garden and National Orchidarium, Botanical Survey of India, Yercaud, Salem, Tamil Nadu.
Callitris robusta *(Callitris columellaris; Callitris glauca)*		Llyod Botanic Garden, Darjeeling. Forest Research Institute, Dehradun.
Callitris whytei		Government Botanic Garden, Howrah. Forest Research Institute, Dehradun.
Cephalotaxus griffithii	Cephalotaxaceae	Eastern Circle, B.S.I. 'Woodlands' Laithumkhrah, Shillong (Meghalaya)
Cephalotaxus harringtonia *(Cephalotaxus pedunculata)*	Cephalotaxaceae	Forest Research Institute, Dehradun.
Cephalotaxus mannii	Cephalotaxaceae	B.S.I. Campus Gangtok, (Sikkim)
Chamaecyparis lawsoniana *(Cupressus lawsoniana)*	Cupressaceae	Government Botanic Garden, Ooty. Kodaikanal, Tamilnadu. Llyod Botanic Garden, Darjeeling.
Cryptomeria japonica	Taxodiaceae	Llyod Botanic Garden, Darjeeling.
Cunninghamia lanceolata *(Cunninghamia sinensis)*	Taxodiaceae	Llyod Botanic Garden, Darjeeling. Forest Research Institute, Dehradun. Kodaikanal (T.N.) Northern Circle, B.S.I., Dehradun.
Cupressus arizonica	Cupressaceae	Forest Research Institute, Dehradun. Indian Botanic Garden, Howrah. The State Horticultural Garden (Lalbagh) Botanic Garden), Lalbagh, Bangalore. Northern Circle, B.S.I., Pauri, Garden, Uttaranchal. Experimental Garden and National Orchidarium, Botanical Survey of India, Yercaud, Salem, Tamil Nadu.
Cupressus benthami *(Cupressus lindlehi)*	Cupressaceae	Government Botanic Garden, Ootacamund (Udhagamandalam), Tamilnadu. Sim's Park, Coonoor, Nilgiri Distt. Tamil Nadu.
Cupressus benthami var. lindleyi	Cupressaceae	Government Botanic Garden, Ootacamund (Udhagamandalam), Tamilnadu. Sim's Park, Coonoor, Nilgiri Distt. Tamil Nadu.
Cupressus cashmeriana	Cupressaceae	Llyod Botanic Garden, Darjeeling. Forest Research Institute, Dehradun. National Botanical Research Institute, Lucknow. Northern Circle, B.S.I., Dehradun.
Cupressus funebris	Cupressaceae	Forest Research Institute, Dehradun. Government Botanic Garden, Ooty (T.N.) (Udhagamandalam), Tamilnadu.

		Indian Botanic Garden, Howrah. Lalbagh Botanic Garden, Bangalore.
Cupressus glabra		Lalbagh Botanic Garden, Bangalore.
Cupressus goveniana		Llyod Botanic Garden, Darjeeling. Government Botanic Garden, Ootacamund (Udhagamandalam), Tamilnadu.
Cupressus lawsoniana	Cupressaceae	Government Botanic Garden, Ootacamund (Udhagamandalam), Tamilnadu. Lalbagh Botanic Garden, Bangalore.
Cupressus lusitanica (***Cupressus glauca***)	Cupressaceae	Forest Research Institute, Dehradun. Government Botanic Garden, Ootacamund (Udhagamandalam), Tamilnadu. Western Cicrle, B.S.I., Pune.
Cupressus macrocarpa (*Cupressus horizontalis*)	Cupressaceae	Llyod Botanic Garden, Darjeeling. Government Botanic Garden, Ootacamund (Udhagamandalam), Tamilnadu. Lalbagh Botanic Garden, Bangalore. National Botanical Research Institute, Lucknow. State Botanical Garden, Barang, Orissa. Experimental Garden and National Orchidarium, Botanical Survey of India, Yercaud, Salem, Tamil Nadu.
Cupressus sempervirens		Forest Research Institute, Dehradun. Government Botanic Garden, Ootacamund (Udhagamandalam), Tamilnadu. Botanic Garden, Guru Nanak Dev University, Amritsar. Indian Botanic Garden, Howrah. Lalbagh Botanic Garden, Bangalore. National Botanical Research Institute, Lucknow. Northern Circle, B.S.I., Dehradun.
Cupressus torulosa	Cupressaceae	Llyod Botanic Garden, Darjeeling. Experimental Garden of B.S.I. at Barapani (Khasi Hills,) Meghalaya.
Cycas beddomei	Cycadaceae	Indian Botanic Garden, Howrah. National Botanical Research Institute, Lucknow. Experimental Garden and National Orchidarium, Botanical Survey of India, Yercaud, Salem, Tamil Nadu.
Cycas circinalis	Cycadaceae	Botanic Garden, Bhagalpur University, Bhgalpur, (Bihar) Central Circle, B.S.I., Campus, Allahabad. Experimental Garden of B.S.I. at Barapani (Khasi Hills,) Meghalaya. Forest Research Institute, Dehradun. Indian Botanic Garden, Howrah.

		Kastrurba, Gram, Indore, Madhya Pradesh. Lalbagh Botanic Garden, Bangalore. National Botanical Research Institute, Lucknow. Northern Circle, B.S.I., Dehradun. State Botanical Garden, Barang, Orissa. Experimental Garden and National Orchidarium, Botanical Survey of India, Yercaud, (Tamil Nadu). Teen Murti Bhavan Garden, New Delhi. Western, Circle, B.S.I., Pune.
Cycas pectinata	Cycadaceae	Eastern Circle, Botanical Survey of India. Experimental Garden and National Orchidarium, Botanical Survey of India, Yercaud, (T.N.) Indian Botanical Garden, Howrah. Regional Plant Resource Centre, Nayapalli, Bhubaneswar.
Cycas revoluta	Cycadaceae	Experimental Garden of BSI at Dhanikhari in Andman & Nicobar Isl.; Botanic Garden, Bhgalpur University, Bhagalpur (Bihar). Forest Research Institute, Dehradun. Indian Botanical Garden, Howrah. Lalbagh Botanic Garden, Bangalore. Indira Gandhi Memorial Garden, Bareilly. Northern Circle, B.S.I., Dehradun. State Botanical Garden, Barang, Orissa. Experimental Garden and National Orchidarium, Botanical Survey of India, Yercaud, T.N.) Experimental Garden, Western Circle, B.S.I., Pune.
Cycas rumphii (?)	Cycadaceae	Experimental Garden of BSI at Dhanikhari in Andman & Nicobar Isl.; Botanic Garden, Bhgalpur University Bhagalpur (Bihar); Central Circle, BSI, Campus, Allahabad. Indian Botanical Garden, Howrah. Indra Gandhi Memorial Garden, Bareilly. Lalbagh Botanic Garden, Bangalore. National Botanical Research Institute, Lucknow. Regional Plant Resource Centre, Nayapalli, Bhubaneswar. Experimental Garden and National Orchidarium, Botanical Survey of India, Yercaud, (T.N.)
Encephalartos ferox		Regional Plant Resource Centre, Nayapalli, Bhubaneswar.
Encephalartos hildebrandtii		The State Horticultural Garden (Lalbagh) Botanic Garden), Lalbagh, Bangalore.

Encephalartos villosus		Indian Botanical Garden, Howrah. The State Horticultural Garden (Lalbagh) Botanic Garden), Lalbagh, Bangalore. National Botanical Research Institute, Lucknow.
Ephedra foliata Boiss		Indian Botanical Garden, Howrah. National Botanical Research Institute, Lucknow.
Fitzroya cupressoides (syn. Fitzroya *patagonica)*		Government Botanic Garden, Ootacamund, (T.N.) Sim's Park, Coonoor, Nilgiri Distt. (T.N.)
Gnetum gnemon var brunonianum		Eastern Circle, BSI 'Woodlands' Laithumkhrah, Shillong (Meghalaya).
Gnetum latifolium		Experimental Garden of BSI at Dhanikhari in Andman & Nicobar Isl.
Gnetum latifolium var. funiculare		Experimental Garden of BSI at Dhanikhari in Andman & Nicobar Isl.
Gnetum ula		Indian Botanical Garden, Howrah. Eastern Circle, BSI 'Woodlands' Laithumkhrah, Shillong (Meghalaya). Experimental Garden and National Orchidarium, Botanical Survey of India, Yercaud, Tamil Nadu.
Juniperus bermudiana		Forest Research Institute, Dehradun.
Juniperus chinensis		Forest Research Institute, Dehradun. Indian Botanical Garden, Howrah. Lalbagh Botanic Garden, Bangalore. National Botanical Research Institute, Lucknow. State Botanical Garden, Barang, Orissa. Experimental Garden and National Orchidarium, Botanical Survey of India, Yercaud, Tamilnadu.
Juniperus communis		Experimental Garden of BSI at Barapani, Khasi Hills, Meghalaya. Forest Research Institute, Dehradun. Regional Plant Resource Centre, Nayapalli, Bhubaneswar.
Juniperus mexicana (*J. deppeana*)		Forest Research Institute, Dehradun.
Juniperus oxycedrus		Forest Research Institute, Dehradun.
Juniperus phoenicea		Forest Research Institute, Dehradun.
Juniperus procera		Forest Research Institute, Dehradun. The State Horticultural Garden (Lalbagh) Botanic Garden), Lalbagh, Bangalore.
Juniperus prostrata	Cupressaceae	Horticultural Experiment & Training Centre, Saharanpur.
Juniperus pseudosabina	Cupressaceae	Llyod Botanic Garden, Darjeeling. Eastern Circle, BSI 'Woodlands' Laithumkhrah,

		Shillong (Meghalaya). Jawaharlal Nehru Botanical Garden, Rumtek, Sikkim.
Juniperus recurva	Cupressaceae	Llyod Botanic Garden, Darjeeling. Government Botanic Garden, Ootacamund (T.N.). Botanic Garden, Guru Nanak Dev University, Amritsar. Indian Botanic Garden, Howrah. Experimental Garden and National Orchidarium, Botanical Survey of India, Yercaud, (T.N.).
Juniperus scopulorum	Cupressaceae	Forest Research Institute, Dehradun.
Juniperus virginiana (*Juniperus bedfordiana*)	Cupressaceae	Government Botanic Garden, Ootacamund (Udhagamandalam), Tamilnadu. Sim's Park, Coonoor, Nilgiri Distt. Tamil Nadu.
Picea smithiana		Tropical Botanic Garden and Research Institute, Trivendrum. Llyod Botanic Garden, Darjeeling. (West Bengal) Northern Circle, B.S.I., Dehradun.
Picea spinulosa		Jawaharlal Nehru Botanical Garden, Rumtek, Sikkim. Northern Circle, B.S.I., Dehradun.
Pinus montana var. mughus		Lalbagh Botanic Garden, Bangalore.
Pinus montezumae		Sim's Park, Coonoor, Nilgiri Distt. Tamil Nadu.
Pinus nigra		Northern Circle B.S.I. Experimental Garden, Distt. Pauri, Garhwal, Uttaranchal.
Pinus oocarpa		Forest Research Institute, Dehradun.
Pinus parvifolia (*Pinus pentaphylla*)		Lalbagh Botanic Garden, Bangalore.
Pinus patula		Forest Research Institute, Dehradun. Government Botanic Garden, Ootacamund (Udhagamandalam), Tamilnadu.
Pinus canariensis	Pinaceae	Forest Research Institute, Dehradun. Government Botanic Garden, Ootacamund. Lalbagh Botanic Garden, Bangalore.
Pinus caribaea (*Pinus elliottli*)	Pinaceae	Forest Research Institute, Dehradun. Northern Circle, B.S.I., Dehradun. Northern Circle B.S.I. Experimental Garden, Pauri, Garhwal, Uttaranchal.
Pinus densiflora	Pinaceae	Northern Circle B.S.I. Experimental Garden, Pauri, Garhwal, Uttaranchal.
Pinus echinata	Pinaceae	Northern Circle B.S.I. Experimental Garden, Pauri, Garhwal, Uttaranchal.

Pinus gerardiana	Pinaceae	Forest Research Institute, Dehradun. Northern Circle B.S.I. Pauri, Garhwal, Uttaranchal.
Pinus greggii	Pinaceae	Forest Research Institute, Dehradun. Northern Circle B.S.I. Pauri, Garhwal, Uttaranchal.
Pinus halepensis	Pinaceae	Forest Research Institute, Dehradun. Northern Circle B.S.I., Dehradun. Northern Circle B.S.I. Experimental Garden, Distt. Pauri, Garhwal, Uttaranchal. Experimental Garden and National Orchidarium, Botanical Survey of India, Yercaud, (T.N.).
Pinus pseudostrobus	Pinaceae	Forest Research Institute, Dehradun.
Pinus radiata	Pinaceae	Forest Research Institute, Dehradun.
Pinus roxburghii *(Pinus longifolia)*	Pinaceae	Central Circle, B.S.I. Campus, Allahabad. Llyod Botanic Garden, Darjeeling. Forest Research Institute, Dehradun. Botanic Garden, Guru Nanak Dev University, Amritsar. Indian Botanic Garden, Howrah. Indra Gandhi Memorial Garden, Bareilly. Lalbagh Botanic Garden, Bangalore. National Botanical Research Institute, Lucknow. BSI Campus Gangtok, Sikkim. Teen Murti Bhawan Garden, New Delhi.
Pinus sabiniana	Pinaceae	Government Botanic Garden, Ootacamund (Udhagamandalam), Tamilnadu.
Pinus taeda	Pinaceae	Northern Circle B.S.I. Experimental Garden, Distt. Pauri, Garhwal, Uttaranchal.
Pinus thunbergii	Pinaceae	Lalbagh Botanic Garden, Bangalore. Northern Circle B.S.I. Experimental Garden, Distt. Pauri, Garhwal, Uttaranchal.
Pinus wallichiana	Pinaceae	Eastern Circle, BSI "Woodlands" Laithumkhrah, Shillong (Meghalaya). Eastern Circle, B.S.I., Experimental Garden, Distt. Pauri, Garhwal, Uttaranchal.
Taxodium distichum	Taxodiaceae	Llyod Botanic Garden, Darjeeling. Government Botanic Garden, Ootacamund (Udhagamandalam), Tamilnadu. Indian Botanic Garden, Howrah. National Botanical Research Institute, Lucknow. Experimental Garden Western Circle, B.S.I., Pune.
Taxodium mucronatum	Taxodiaceae	Central Circle, B.S.I., Allahabad. Forest Research Institute, Dehradun.

		Indian Botanic Garden, Howrah.
Taxus wallichiana	Taxaceae	Experimental Garden, of B.S.I. at Barapani, (Khasi Hills.) Meghalaya. Northern Circle B.S.I. Dehradun. Eastern Circle, B.S.I. 'Woodlands' Laithumkhrah, Shillong (Meghalaya). Northern Circle B.S.I. Dehradun. Northern Circle B.S.I. Experimental Garden, Distt. Pauri, Garhwal, Uttaranchal. B.S.I. Campus, Gangtok, Sikkim. SFRI, Chimpu, Itanagar
Tetraclinus articulata	Cupressaceae	Forest Research Institute, Dehradun.
Thuja compacta		Government Botanic Garden, Ootacamund (Udhagamandalam), Tamilnadu. Botanic Garden, Guru Nanak, Dev University, Amritsar.
Thuja japonica		Government Botanic Garden, Ootacamund (Udhagamandalam), Tamilnadu.
Thuja obtusa (*Cupressus obtusa*)		Government Botanic Garden, Ootacamund (Udhagamandalam), Tamilnadu.
Thuja occidentalis		Llyod Botanic Garden, Darjeeling. Experimental Garden of B.S.I. at Barapani, (Khasi Hills.) Meghalaya. Forest Research Institute, Dehradun. Botanic Garden, Guru Nanak, Dev University, Amritsar. Government Botanic Garden, Ootacamund (Udhagamandalam), Tamilnadu. Indian Botanic Garden, Howrah. Experimental Garden and National Orchidarium, Botanical Survey of India, Yercaud, Tamilnadu. Experimental Garden Western Circle, B.S.I., Pune.
Thuja occidentalis var. compacta	Cupressaceae	Forest Research Institute, Dehradun.
Thuja orientalis (*Thuja filiformis; Biota orientalis*)	Cupressaceae	BSI, Campus, Allahabad. Llyod Botanic Garden, Darjeeling. Experimental Garden of B.S.I., Barapani, (Khasi Hills.) Meghalaya. Forest Research Institute, Dehradun. Government Botanic Garden, Ootacamund (T.N.) Botanical Garden, Guru Nanak, Dev University, Amritsar. Indian Botanic Garden, Howrah. Indira Gandhi Memorial Botanic Garden, Bareilly.

		Lalbagh Botanic Garden, Bangalore. Northern Circle, BSI, Dehradun. Regional Plant Resource Centre, Nayapalli, Bhubaneswar. Experimental Garden and National Orchidarium, Botanical Survey of India, Yercaud, (T.N.) Experimental Garden Western Circle, B.S.I., Pune.
Thuja orientalis var. aurea (*Biota orientalis* var. *aurea*)	Cuprassaceae	Government Botanic Garden, Ootacamund (T.N.)
Thuja orientalis var. campacta	Cuprassaceae	National Botanical Research Institute, Lucknow.
Thuja orientalis var. pyramidalis	Cuprassaceae	Lalbagh Botanic Garden, Bangalore.
Thuja plicata (*Thuja gigantea*)	Cuprassaceae	Llyod Botanic Garden, Darjeeling.
Zamia angustifolia	Zamiaceae	Indian Botanic Garden, Howrah.
Zamia floridana	Zamiaceae	BSI, Compus, Allahabad. Indian Botanic Garden, Howrah. National Botanical Research Institute, Lucknow. Northern Circle B.S.I. Dehradun.
Zamia furfuracea	Zamiaceae	Indian Botanic Garden, Howrah. National Botanical Research Institute, Lucknow. Regional Plant Resource Centre, Nayapalli, Bhubaneswar.
Zamia latifolia	Zamiaceae	Horticultural Experiment & Training Centre, Saharanpur. Indian Botanic Garden, Howrah.
Zamia pallidas	Zamiaceae	Indian Botanic Garden, Howrah.
Zamia pumila	Zamiaceae	Indian Botanic Garden, Howrah.

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ENHANCEMENT OF SALT TOLERANCE BY PRE-SOWING SEED SOAKING SALT TREATMENT IN SOYBEAN AND LENTIL

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Abstract

The present investigation was made to study the effect of pre-sowing seed soaking saline treatments on seed germination and early seedling growth in two legume species namely *Glycine max* L. (soybean) and *Lens esculenta* L. (lentil). It was apparent that germination and early seedling stage suffered very heavily under saline condition in lentil while it was less injurious in soybean. The quantum of suppression was mainly guided by increasing salt concentration. However, pre-sowing soaking treatment of 4 dSm^{-1} salt level could alleviate the effects of salt stress and resulted in a significant increase in germination and early seedling growth as compared to control. The seedlings seemed to acclimate themselves to salt stress by attaining hardiness against the changed environment by pre-sowing soaking salt treatment.

Key words: germination, lentil, pre-sowing soaking, salt, soybean.

INTRODUCTION

Legumes, in general, have been reported to be salt sensitive. The early stage of plant growth is the most critical one as later development and crop production depends on germination and seedling emergence. The imbibitional pressure of the seed is conditioned by the moisture content within the range of available soil moisture. Seed germination, therefore, would take place in a shorter period of time at high soil moisture than at low moisture level. With an increase in salinity (NaCl) concentrations, there is a corresponding decrease in imbibition and germination (Moreno-Limon *et al.* 2000). Presence of salt in the germination medium shows negative effects on germination (Khalid *et al.*, 2001). Experiments conducted by Esechie *et al.* (2002) in two chickpea cultivars in response to varied salinity levels showed that salinity had an adverse effect on seedling emergence at the high salinity treatment. Promila and Kumar (2000) reported a reduction in radicle and root elongation, fresh and dry biomass of embryo axis, delayed germination and inhibited hypocotyl elongation and mobilization of reserves from the cotyledons to the embryo axis under NaCl salinity.

However, only a few studies on plant acclimation to salt stress have been conducted. Strogonov (1964) conducted pioneering experiment study in which he showed that salt tolerance of plant increased by pre-sowing salt-treatment. Pre-sowing soaking followed by complete dryness enhanced germination in *Cajanus cajan* (Hansra & Kumar, 1989). Umezawa *et al.* (2000) observed enhancement of salt tolerance in soybean with NaCl pretreatment. Barring a few reports, detailed investigations on the effects of pre-sowing salt treatment have not been carried out. In this context, present study was conducted to compare relative response of soybean (*Glycine max* L.) and lentil (*Lens esculenta* L.) to increasing concentration of salt stress and to study the effect of presoaking salt treatment on germination and growth of seedlings subjected to different salinity dosage.

MATERIAL AND METHODS

Saline solutions of various concentrations i.e. 2, 4, 6 and 8 dSm^{-1} electrical conductivity(EC) were prepared from a mixture of NaCl, $CaCl_2$ and Na_2SO_4 according to recommended specification (Richards, 1954).

Pre-sowing treatment

Experiments were conducted to study the effect of different concentrations of salinity on the germination and early seedling growth of the two experimental species namely *Glycine max* L. cv. *Pusa Hara Soya* and *Lens esculenta* L. cv. *Pusa Vaibhav* in petri dishes. Each Petri dish was washed, dried and lined with filter paper. A preliminary selection of seeds was done for their uniformity of size and colour. All seeds were surface sterilized with 2% $HgCl_2$. Seeds of both plant species i.e. lentil and soybean were soaked in salt solutions of 4 dSm^{-1} EC for 4 h and oven dried for 72 h. Similar soaking and drying treatments were repeated three times to achieve salt acclimation. A distilled water control was maintained. After pre-treatment, 10 seeds were placed in each petridishes moistened with salt solutions of 2, 4, 6 and 8

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dSm^{-1}, maintained at room temperature. Observation on germination and seedling growth under salt stress were recorded after the emergence of both radicle and plumule. The estimations of sodium and potassium content were done by method of Chapman and Pratt (1961) and are represented as K^+/Na^+ ratio.

Sampling procedure

Four seedlings were selected from each treatment for sampling at 12, 20 and 28 DAS in case of each experiment. All the seedlings from one treatment were sampled together for recording the observations and the data were calculated by taking an average of four plants. The data were analyzed statistically and is shown as CD (<5%) in each table.

All data were subjected to analysis of variance using one-way ANOVA, and Critical Difference (CD) was calculated at 5% level.

RESULTS AND DISCUSSION

The study revealed that salt susceptibility and resistance in a species was directly related with the type of plant as well as the salt treatments. Soybean plants seem to adapt themselves to the environmental stress caused due to salinity by showing higher germination (Table 1) and by accumulating higher plumule and radicle lengths as well as dry weights (Table 2, 3, 4, 5) which might have helped in sustaining the plants even under relatively higher concentration of salts. The number of secondary roots (Table 6) declined with increasing saline dosage in both the legume species. The negative effects of salt stress on seedling growth were directly correlated with disturbed K^+/Na^+ ratio. The Na ion concentration increased, while the K ion concentration declined with increasing salt stress and resulted in decreased K^+/Na^+ ratio (Table 7).

The Legume species vary widely in response to salt stress ranging from entirely sensitive to tolerant species (Lauchli 1984; Cordovilla *et al.*, 1995). Present results confirmed the earlier observations on soybean and alfalfa (Serraj and Drevon, 1998), chickpea (Lauter *et al.*, 1981) and fababean (Yousef and Sprent, 1983) showing reduction in the shoot and root growth under salt stress resulting in decreased root/shoot ratio. Saxena *et al.*, (1989) pointed out that growth retardation in seedling might be due to osmotic pressure and toxic effect of specific ions in the medium. Excess sodium chloride in the medium leads to the loss of K^+ due to membrane depolarization by Na^+ ions (Serraj and Drevon, 1998). The reduction in the K^+ concentrations could inhibit growth by reducing the capacity for osmotic adjustments and turgor maintenance or by adversely affecting the metabolic functions (Greenway and Munns, 1980). Cordovilla *et al.* (1995) suggested that a direct relationship between the salt tolerant range in legumes and the macronutrient accumulation in vegetative organs resulted in decreased shoot and root biomass. A decrease in the observed shoot

Table 1: Effect of salt stress on % germination of seeds in lentil and soybean at different stages of growth (figures in parenthesis represent percent increase (+) or decrease (-) over control).

Days after sowing (DAS)	Legume species	Salinity (dSm^{-1})								
		Pre-soaked (Distilled water)					Pre-soaked (4dSm^{-1})			
		0	2	4	6	8	2	4	6	8
3 days	Lentil	90.0	90.0 (0.0)	90.0 (0.0)	70.0 (-22.2)	60.0 (-33.3)	100.0 (+11.1)	100.0 (+11.1)	100.0 (+11.1)	100.0 (+11.1)
	Soybean	80.0	80.0 (0.0)	80.0 (0.0)	70.0 (-12.5)	60.0 (-25.0)	100.0 (+25.0)	100.0 (+25.0)	90.0 (+12.5)	90.0 (+12.5)
5 days	Lentil	100.0	100.0 (0.0)	90.0 (-11.1)	70.0 (-30.0)	60.0 (-40.0)	100.0 (0.0)	100.0 (0.0)	100.0 (0.0)	100.0 (0.0)
	Soybean	10.0	90.0 (-10.0)	90.0 (-10.0)	80.0 (-20.0)	80.0 (-20.0)	100.0 (0.0)	100.0 (0.0)	100.0 (0.0)	100.0 (0.0)
7 days	Lentil	10.0	100.0 (0.0)	90.0 (-11.1)	70.0 (-30.0)	60.0 (-40.0)	100.0 (0.0)	100.0 (0.0)	100.0 (0.0)	100.0 (0.0)
	Soybean	10.0	90.0 (-10.0)	90.0 (-10.0)	80.0 (-20.0)	80.0 (-20.0)	100.0 (0.0)	100.0 (0.0)	100.0 (0.0)	100.0 (0.0)

Critical Difference (<5%) due to Age: 1.24, Treatment: 2.15

Table 2: Effect of salt stress on length of radicle (cm) in lentil and soybean at different stages of seedling growth (figures in parenthesis represent percent increases (+) or decrease(-) over control).

Days after sowing (DAS)	Legume species	Salinity (dSm^{-1})								
		Pre-soaked (Distilled water)					Pre-soaked ($4dSm^{-1}$)			
		0	2	4	6	8	2	4	6	8
12 days	Lentil	1.45	1.36	1.35	1.30	1.20	2.0	1.75	1.2	1.65
			(-6.2)	(-8.5)	(-10.3)	(-17.2)	(+37.9)	(+20.6)	(+17.2)	(+13.7)
	Soybean	6.5	6.25	6.15	5.9	5.6	10.5	9.9	8.45	8.0
			(-3.8)	(-5.3)	(-9.2)	(-13.8)	(+61.5)	(+53.3)	(+30.0)	(+23.0)
20 days	Lentil	1.45	1.25	1.20	1.10	.95	2.05	1.95	1.00	1.75
			(-13.7)	(-17.2)	(-24.1)	(-34.4)	(+41.3)	(+34.4)	(+31.0)	(+20.6)
	Soybean	2.65	2.45	2.35	2.20	1.95	4.65	4.4	4.2	3.9
			(-7.5)	(-11.3)	(-16.9)	(-26.4)	(+75.4)	(+66.0)	(+58.4)	(+47.1)
28 days	Lentil	1.95	1.55	1.25	1.0	.75	2.95	2.65	2.60	2.45
			(-20.5)	(-35.8)	(-48.7)	(-61.5)	(+51.2)	(+35.8)	(+33.3)	(+25.6)
	Soybean	4.05	3.65	3.15	2.55	1.95	7.25	7.0	6.55	6.0
			(-9.8)	(-22.2)	(-37.0)	(-51.8)	(+79.0)	(+72.8)	(+61.7)	(+48.1)

Critical Difference (<5%) due to Age: 1.56, Treatment: 3.62

Table 3: Effect of salt stress on length of plumule (cm) in lentil and soybean at different stages of seedling growth (figures in parenthesis represent percent increase (+) or decrease (-) over control).

Days after sowing (DAS)	Legume species	Salinity (dSm^{-1})								
		Pre-soaked (Distilled water)					Pre-soaked ($4dSm^{-1}$)			
		0	2	4	6	8	2	4	6	8
12 days	Lentil	5.1	4.5	4.2	4.1	3.0	6.2	6.0	5.6	5.5
			(-11.7)	(-17.6)	(-24.3)	(-41.1)	(+21.5)	(+17.6)	(+13.7)	(+7.8)
	Soybean	.7	.65	.60	.55	.50	1.1	1.0	.90	.85
			(-7.1)	(-14.2)	(-21.4)	(-28.5)	(+57.1)	(+50.0)	(+28.5)	(+21.4)
20 days	Lentil	7.8	6.5	5.0	4.5	3.0	10.5	9.90	9.65	8.85
			(-16.6)	(-35.8)	(-42.3)	(-61.5)	(+34.6)	(+26.9)	(+23.7)	(+13.4)
	Soybean	1.0	.85	.75	.65	.60	1.65	1.55	1.4	1.25
			(-15.0)	(-25.0)	(-35.0)	(-40.0)	(+61.5)	(+55.0)	(+40.0)	(+25.0)
28 days	Lentil	6.3	4.45	2.25	1.3	1.0	8.8	8.5	8.15	7.55
			(-29.3)	(-64.2)	(-79.3)	(-84.1)	(+39.6)	(+34.9)	(+29.3)	(+19.8)
	Soybean	.95	.75	.65	.50	.45	1.65	1.5	1.35	1.20
			(-21.0)	(-31.5)	(-47.3)	(-52.6)	(+73.6)	(+57.8)	(+42.0)	(+26.3)

Critical Difference (<5%) due to Age: 2.15, Treatment: 2.62

Table 4: Effect of salt stress on dry weight of radicle (g) in lentil and soybean at different stages of seedling growth (figures in parenthesis represent percent increase (+) or decreaseover control).

Days after sowing (DAS)	Legume species	Salinity (dSm^{-1})								
		Pre-soaked (Distilled water)					Pre-soaked ($4dSm^{-1}$)			
		0	*2*	*4*	*6*	*8*	*2*	*4*	*6*	*8*
12 days	Lentil	.006	.0055	.0045	.0038	.0025	.0091	.0085	.0070	.0065
			(-16.6)	(-25.0)	(-36.6)	(-58.3)	(+51.6)	(+41.6)	(+16.6)	(+8.3)
	Soybean	.045	.042	.039	.032	.026	.075	.60	.055	.050
			(-6.6)	(-13.3)	(-28.8)	(-42.4)	(+66.6)	(+55.5)	(+22.2)	(+11.1)
20 days	Lentil	.0045	.0036	.0031	.0025	.0015	.0070	.0065	.0055	.0051
			(-20.0)	(-31.1)	(-44.4)	(-66.6)	(+55.5)	(+44.4)	(+22.2)	(+13.3)
	Soybean	.0195	.0166	.0148	.0122	.0015	.0335	.0311	.0255	.0235
			(-14.8)	(-24.1)	(-37.4)	(-51.2)	(+71.79)	(+59.4)	(+30.7)	(+20.5)
28 days	Lentil	.0056	.0045	.0035	.0029	.0012	.0098	.0091	.0080	.0078
			(-24.4)	(-35.71)	(-48.2)	(-78.5)	(+75.0)	(+62.5)	(+42.8)	(+39.2)
	Soybean	.0198	.0165	.0139	.011	.090	.0365	.0354	.0305	.0265
			(-16.6)	(-29.7)	(-44.4)	(-54.5)	(+84.34)	(+78.7)	(+54.0)	(+43.8)

Critical difference (<5%) due to Age :0.39, Treatment :1.54

Table 5: Effect of salt stress on the dry weights (g) of plumule in lentil and soybean at different stages of seedling growth (figures in parenthesis represent percent increase (+) or decrease(-) over control).

Days after sowing (DAS)	Legume species	Salinity (dSm^{-1})								
		Pre-soaked (Distilled water)					Pre-soaked ($4dSm^{-1}$)			
		0	2	4	6	8	2	4	6	8
12 days	Lentil	.007	.0065	.006	.005	.0035	.0105	.0095	.0085	.0078
			(-7.1)	(-14.2)	(-28.5)	(-50.0)	(+50.0)	(+37.1)	(+21.4)	(+11.4)
	Soybean	.004	.0038	.0035	.003	.0025	.0063	.0056	.0052	.005
			(-5.0)	(-12.5)	(-25.0)	(-37.5)	(+57.5)	(+40.0)	(+30.0)	(+25.0)
20 days	Lentil	.0065	.0058	.0055	.004	.003	.0105	.0100	.0096	.0086
			(-10.7)	(-15.3)	(-38.4)	(-53.8)	(+61.5)	(+53.8)	(+47.8)	(+32.3)
	Soybean	.0055	.005	.0047	.0039	.0031	.0098	.0090	.0085	.0081
			(-9.0)	(-14.5)	(-29.0)	(-43.6)	(+78.1)	(+63.6)	(+54.5)	(+47.2)
28 days	Lentil	.005	.004	.0038	.003	.002	.0085	.0082	.0075	.0070
			(-20.0)	(-24.0)	(-40.0)	(-60.0)	(+70.0)	(+64.0)	(+50.0)	(+40.0)
	Soybean	.008	.0067	.065	.005	.004	.015	.014	.0135	.0125
			(-16.2)	(-18.7)	(-37.5)	(-50.0)	(+87.5)	(+75.0)	(+68.7)	(+56.2)

Critical Difference (<5%) due to Ag e: 0.12, Treatment :0. 21

Table 6: Effect of salt stress on number of secondary root in lentil and soybean at different stages of seedling growth (figures in parenthesis represent percent increase (+) or decrease (-) over control).

Days after sowing (DAS)	Legume species	Salinity (dSm^{-1})								
		Pre-soaked (Distilled water)					Pre-soaked ($4dSm^{-1}$)			
		0	2	4	6	8	2	4	6	8
12 days	Lentil	5.0	4.5	4.0	4.0	3.0	7.5	7.0	6.5	6.0
			(-10.0)	(-20.0)	(-20.0)	(-40.0)	(+50.0)	(+40.0)	(+30.0)	(+20.0)
	Soybean	13.5	12.5	11.5	11.0	9.5	22.0	20.0	18.0	17.0
			(-7.4)	(-14.8)	(-18.5)	(-29.6)	(+62.9)	(+48.1)	(+33.3)	(+25.9)
20 days	Lentil	6.0	5.0	4.5	4.0	3.0	9.5	8.5	8.0	7.5
			(-16.6)	(-25.0)	(-33.3)	(-50.0)	(+58.3)	(+41.6)	(+33.3)	(+25.0)
	Soybean	10.0	9.0	8.5	8.0	6.0	16.5	15.0	13.5	13.0
			(-10.0)	(-15.0)	(-20.0)	(-40.0)	(+65.0)	(+50.0)	(+35.0)	(+30.0)
28 days	Lentil	8.0	6.5	5.5	5	3.5	13	12	11	10.5
			(-18.7)	(-31.2)	(-37.5)	(-56.2)	(+62.5)	(+50.0)	(+37.5)	(+31.2)
	Soybean	12.0	10.0	9.0	8.5	6.5	20.0	18.5	17.0	16.0
			(-16.6)	(-25.0)	(-29.1)	(-45.8)	(+66.6)	(+54.1)	(+41.6)	(+33.3)

Critical Difference (<5%) due to Age :0. 61, Treatment :0. 92

Table 7: Effect of salt stress on K^+/Na^+ ratio in lentil and soybean at different stages of seedling growth.

Days after sowing (DAS)	Legume species	Salinity (dSm^{-1})								
		Pre-soaked (Distilled water)					Pre-soaked ($4dSm^{-1}$)			
		0	2	4	6	8	2	4	6	8
12 days	Lentil	2.36	2.33	2.09	1.84	1.57	2.50	2.35	2.05	1.90
	Soybean	2.21	2.16	1.97	1.77	1.69	2.30	2.21	1.96	1.87
20 days	Lentil	2.07	2.00	1.92	1.79	1.41	2.10	2.01	1.87	1.74
	Soybean	2.01	1.98	1.91	1.67	1.57	2.05	2.00	1.84	1.64
28 days	Lentil	2.02	2.00	1.84	1.63	1.51	2.04	2.02	1.71	1.55
	Soybean	1.96	1.94	1.82	1.61	1.45	1.98	1.94	1.67	1.47

Critical Difference (<5%) due to Age: 1.04, Treatment: 3.25

growth in the salinized plants could partly be due to a nutrient deficiency mediated by the roots. With an increase in salinity concentrations, a corresponding decrease in imbibition and germination was reported in *Phaseolus vulgaris* by Moreno-Limon *et al.* (2000).

Pre-sowing soaking salt treatment for four hours showed beneficial effects on all parameters under study showing thereby a direct influence on the metabolic processes attributing to an internal metabolic balance which is disturbed by the external saline medium. The presoaking

treatment almost alleviated the species difference when sown in salt medium exhibiting significantly higher radicle and plumule growth with time. Similar observations have also been made by Hansra and Kumar (1989) in *Cajanus cajan*. Strogonov (1964) also has done pioneering work on pre-soaking treatment in soybean and reported complete acclimation by plants under salt stress. In the present study, acclimation to salinity by presoaking salt treatment was revealed in both i.e. lentil and soybean although the quantum of adaptation seem to be higher in soybean as compared to lentil. The germination and rate of seedling growth under severe salt stress was significantly alleviated by moderate salt pretreatment (4 dSm^{-1}) in both the species as well as under all the saline concentrations.

Umezawa *et al.* (2000) observed that while salt stress is generally considered to be made up of both osmotic and ionic effect, osmotic effects of pretreatment have not been detected. They also reported lowering of Na^+ concentration with moderate pretreatment in soybean leaves and suggested that Na^+ translocation was improved. Positive effect of pretreatment on regulation of ion accumulation could involve a Na^+/K^+ selection system at xylem transport (Lacan and Durand, 1996). The present results find support from these reports.

In the present study, the possibility that soybean and lentil acclimated to salt stress due to pretreatment has been demonstrated.

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ASTERELLA PATHANKOTENSIS KASH. : A NEW RECORD FROM RAJASTHAN, INDIA

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Abstract

Asterella pathankotensis Kash. is collected from Kumbhalgarh Wild Life Sanctuary, Rajasthan. This is the first record of the species from Rajasthan.

Key words: liverworts, *Asterella*, Kumbhalgarh Wild Life Sanctuary, Rajasthan.

INTRODUCTION

Rajasthan is the largest state in India with an area of 3,24,274 Sq. Km and lies between latitude 23°3'N and 23° 12'N and longitudes 69°31' E and 78° 17'E at a height of 578 meters above mean sea level. The climate of Rajasthan is one of the great extremes with sudden changes in temperature, precipitation and wind, which restricts the bryophyte flora. As such only a limited number of species, which have a wide range of tolerance, occur in Rajasthan. Kumbhalgarh Wild Life Sanctuary has an area of 608.56 Sq Km. Geographically it is situated between 73°2'-73°30' East longitude and 25°-40' North latitudes. It is a detached hill of the Aravalli ranges. The Sanctuary, given protection to 85 Km long Western face of Aravalli. Sanctuary is just 80 Km in the North of lake city Udaipur. An average height of 1067 m AMSL. The highest peak is Ranakaker Rest House located at a height of 1225 m AMSL. Due to high rainfall and humidity, Sanctuary is one of the richest floristic spot in the Aravalli range.

The genus *Asterella* is widely distributed in tropical region of the world. It is represented by 12-14 species in India. However, there is no record of this genus from Rajasthan. However Mahable and Kharadi (1946), Bapana and Vyas (1962), Chaudhary and Deora (1993) made a good contribution towards the study of bryophytes in the state of Rajasthan.

We collected *Asterella pathankotensis* Kash. from Viro Ka Math, areas of the Sanctuary. The collection is preserved in Bryology laboratory, Department of Botany, College of Science, M.L.Sukhadia University, Udaipur. Specimen No. BR.L. 109.

MATERIAL AND METHODS

Most of the place were visited several times during different seasons of the year, especially following rains. Field notes were taken at the time of collection to observe habit, habitat, abundance and ecological condition. Collection of liverwort from various diverse habitats and localities along with appropriate samples of substrate were made during the period under blotting paper bags. Liverworts were air dried, pressed and stored in well-labeled standard, size pockets. Identification of the liverworts were done with the kind help of for the description of genera, standard books by Kashyap (1916,1929), Pande and Udar (1957) and Bapana (1969), Dry plant material was soaked in water for few hours, than study the plant materials. Before studying the liverworts were collected from Kumbhalgarh Wild Life Sanctuary, Rajasthan in the month of January and February 2004-2005 near always moving water valley. Specimen was placed in FAA. The habit, habitat were noted. Specimen is deposited in the Bryology Herbarium, Department of Botany, M.L. Sukhadia University Udaipur Rajasthan.

TAXONOMY

Division	-	Bryophyta
Class	-	Hepaticae
Order	-	Marchantiales
Family	-	Reboliaceae
Genus	-	*Asterlla*
Species	-	*pathankotensis*

RESULTS AND DISCUSSION

Monoecious Thallus (Fig.A), closely creeping in dense green patches. Thallus once or twice forked, linear or linear-oblong, up to 6-7 mm long and 2.0 mm broad. Dorsal surface green, flat or slightly convex margin wavy purple. Epidermal cell 5-6 angled, walls thin, angles not thickened. Dorsal layer not very deep. Stomata pores small (Fig. C). Air-chamber small (Fig. G), many layered,

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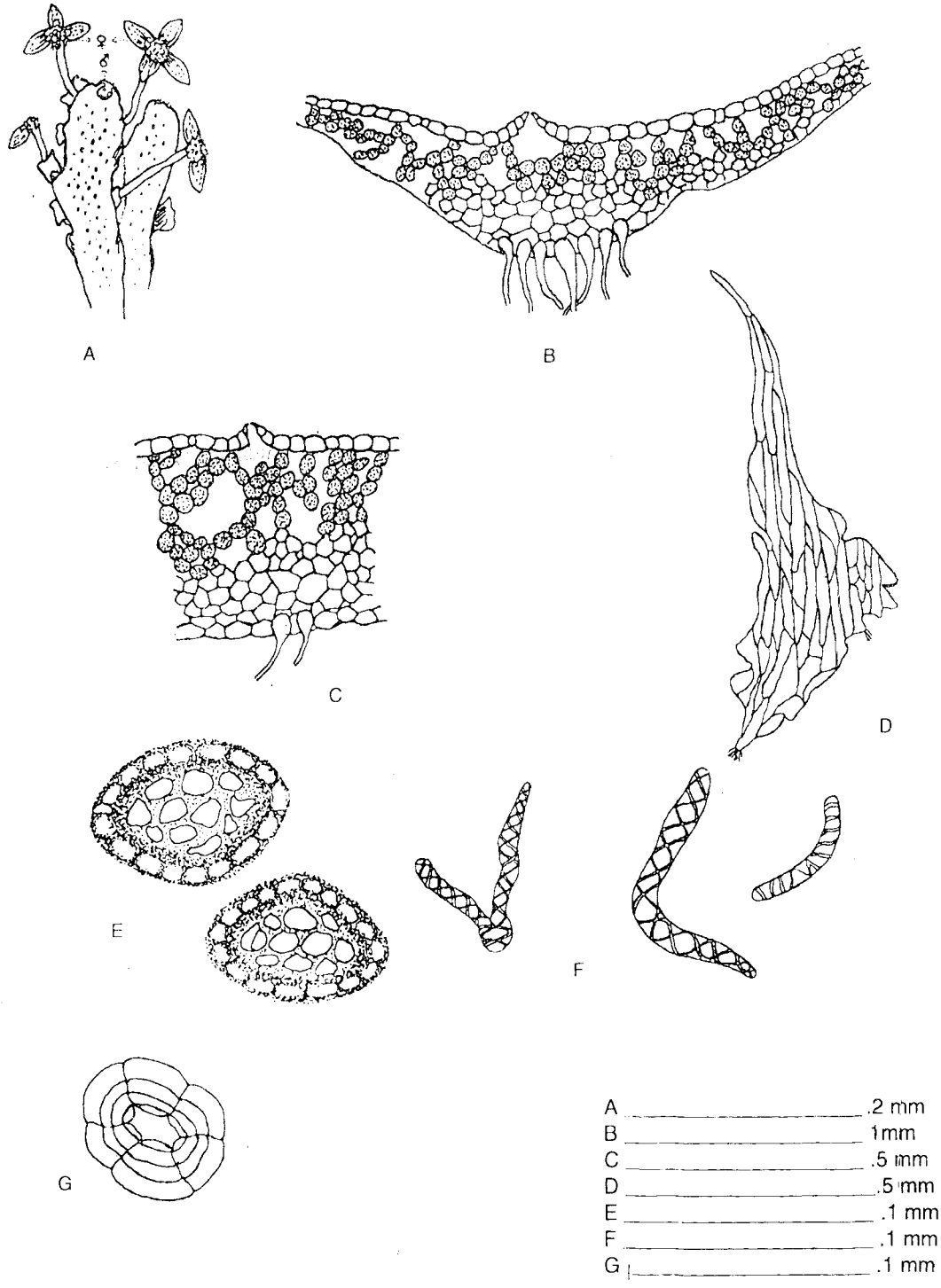

Figs. A-G : *Asterella pathankotensis* Kash. A. Male and female thallus (monoecious); B. T. S. of thallus; C. Cross section of thallus; D. Scales; E. Spores; F. Elaters; G. Air chamber.

PHOTO PLATE 1

Fig.1 Female thallus of *Asterella*

Fig.2 Male thallus of *Asterella*

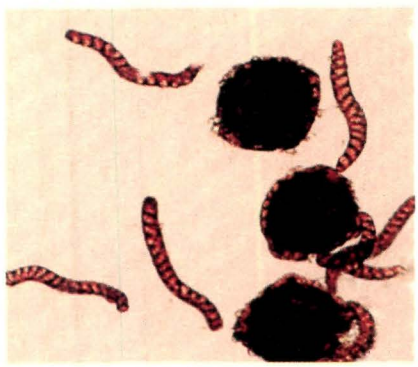

Fig. 3 Spores and Elaters of *Asterella*

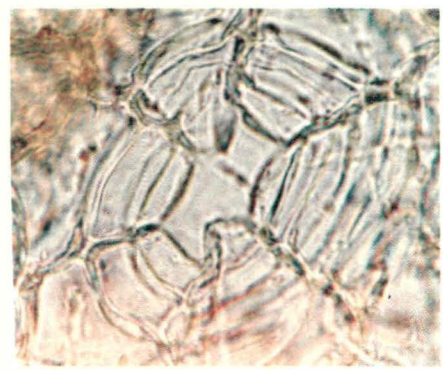

Fig.4 Air Chamber of *Asterella*

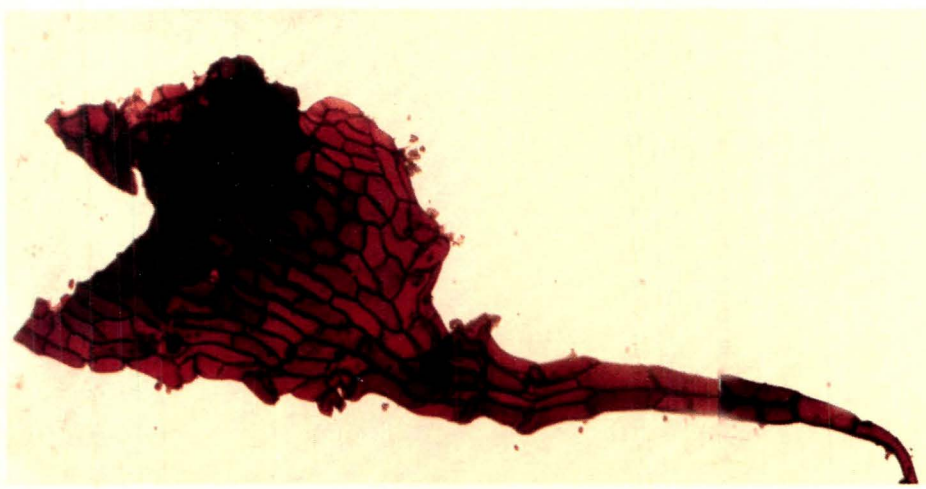

Fig. 5 Scales of *Asterella*

Photo Plate 1: 1. Female thallus of *Asterella*; 2. Male thallus of *Asterella*; 3. Spores and Elaters of *Asterella*; 4. Air Chamber of Asterella; 5. Scales of *Asterella*

empty; mid rib convex, gradually passing into the lamina. Vertical surface purple. Scales (Fig. D) overlapping, purple, is exceeding the margins, appendage long, linear, entire. Male receptacle (Fig.B) cushion-like just behind the apex on the main thallus. Female receptacles on very small ventral shoot, stalk up to 5 mm long about 1.5 mm thick paleaceous at the top. Perianth hyaline or reddish ovate.

Spores (Fig. E) brown, opaque, broadly reticulate, wing punctuate and 90 to 125 um in diameter. Elaters (Fig.F) monospiral and bispiral both are present brown up to 15.5 μm long generally less sometimes branched.

Field notes: A species of the plants chiefly grow on moist soil or on moist rocks covered with lime calcareous soil and near water channels, associated with *Plagiochasma appendiculatum*.

Locality: Kumbhalgarh Wild Life Sanctuary, Rajasthan in Viro Ka Math.

Altitude 1067 m.

Distribution: Pathankot, Lahore, Dehra Dun, Hoshiarpur, Pusa, Modinager (Meerut); Pakistan, Rajasthan.

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DIVERSITY OF *FISSIDENS* FROM BHIMASHANKAR (SAHYADRIC REGION) MAHARASHTRA, INDIA

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Abstract

In a first consolidated record of mosses of Bhimashankar Wild Life sanctuary in the Sahyadric region, four species of *fissidens* namely have been collected. F.splachnobryoides, *F.*involutus ssp *curvato-involutus* Broth, *F. bryoides* Hedw. and *F. diversifolius* Mitt. are collected and described in this note.

Key words: *Fissidens* , Bhimashankar, Sahyadric region.

INTRODUCTION

Bhimashankar Wild Life Sanctuary is one of the highest places (altitude 769m) in the Sahyadric region and is about 128 kms away from Pune. Bhimashankar region is gazetted as a reserved forest. The area is densely forested with majestic towering trees festooned with numerous perennial creepers (lianas) and is richer in fauna as compared to the rest of peninsular India. Evergreen forest comprising *Dimorphocalyx lawianus* Hook.f., *Mangifera indica* L., *Olea dioica* Roxb., *Syzygium cumini*(L.)Skeels., *Dimocarpus longon* Lour., *Holigarna grahammii* HooK.f., *Turpinia martabanica* Wall. and *Carrissa carandus* L. are present.

Due to high rainfall and humidity Bhimashankar is the richest spot of angiospermic vegetation in general and bryophytes in particular. Trees and forest-floor are densely covered with moss vegetation. The authors have collected 14 species of mosses from various parts of Bhimashankar. Study of the available literature shows that there are no reports of mosses of Sahyadric region so far (Chopra 1975; Gangulee 1980; and Dabhade1998).

MATERIAL AND METHODS

Mosses were collected from Bhimashankar Wild Life Sanctuary in the month of September, October 2003-2004. Identification was done with the help of available literature (Chopra 1975; Gangulee 1969-1980, 1985, Chaudhary and Deora, 1993, Dabhade,1998). Specimens were preserved in FAA and habit, habitats were noted at the spot. The specimens are deposited in the Herbarium of Department of Botany, M.L. Sukhadia University, Udaipur.

RESULTS AND DISCUSSION

***Fissidens splachobryoides* Broth. in schum. *et* hauterb. in Fl. Deutsch. Sehutz. Suedsee : 81(1900).**

(Fig. A1 - A5, Specimen No-3045)

Yellow-green gregarious plants. Shoots up to .8 to 1.2 cm long and 2.5 to 3.2 mm broad with leaves. Leaves crowded at apex but lax below. Leaves smaller below, curled but not much crumpled when dry, oblong-lanceolate, widely acuminate, and dorsal lamina narrower in the basal part than in the apical part. Apical parts symmetrical, uniform, smooth and simple, limbidium of 1 to 3 rows of elongated, yellowish cartilaginous calls, all round leaf and not broken at apex. Light yellow- brown costa ending far below tip. Leaf cells smooth, transparent with very thin walls, chlorophyllose with small, round chloroplasts, oval rhomboidal cells towards the base of sheathing lamini may be more elongated.

Field notes: Plants growing on the rocks.

Distribution: East Nepal, Lower Bengal, Western Himalaya, South India, Western Ghats, Mumbai, Khandala, Sri Lanka, Japan, Java and Philippines.

***Fissidens involutus* ssp *curvato - involutus* (Dix.) Gangulee comb. nov.**

Fissidens curvato- involutus Dix. in Not. R. Bot. Gard. Ednib., 19 : 279 (1938).

(Fig. B1–B5, Specimen No-3052)

Plants green, 0.7-0.9 mm. long and 0.2 mm. wide with leaves. Stems with 8-12 pairs of leaves. Leaves ovate sheathing lamini1.2 mm, lanceolate, ±1.85mm. long, 0.45

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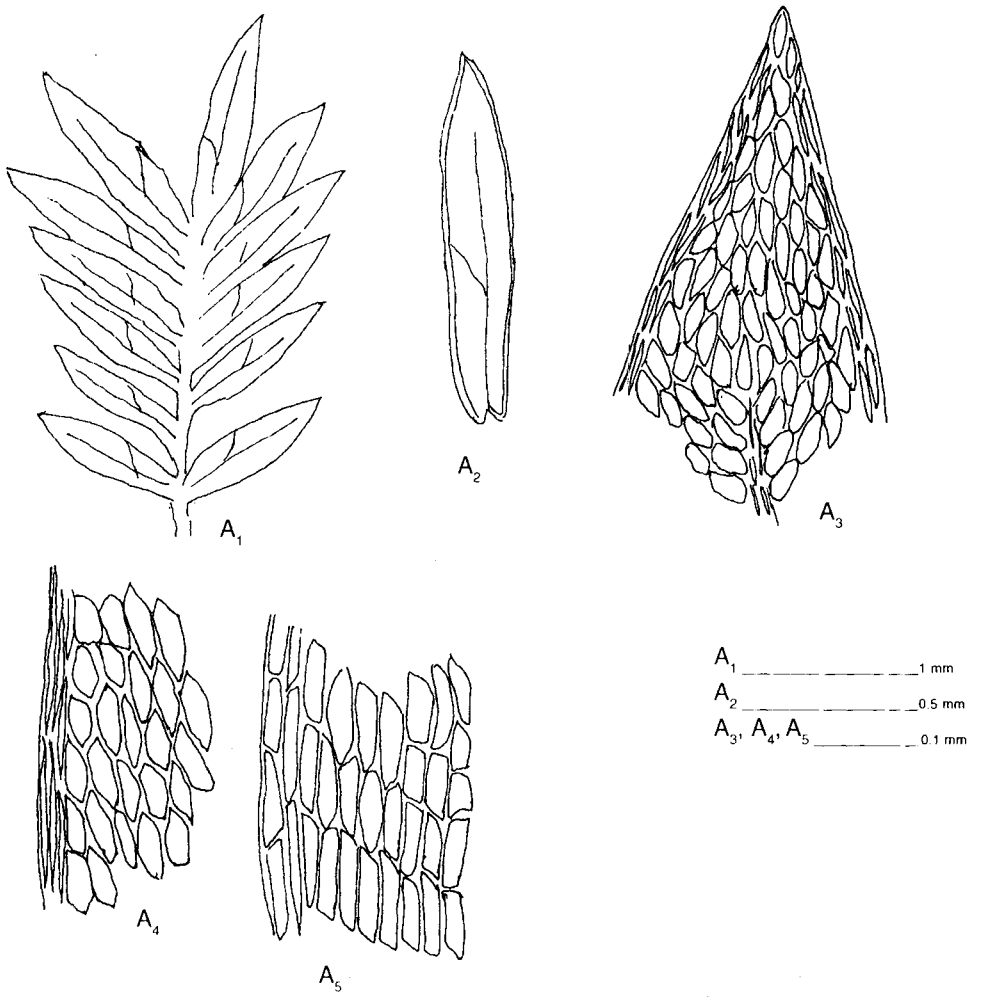

Fig. A1-A5. *: Fissidens splachobryoides* **Broth. in schum.** *et* **hauterb. in Fl. Deutsch. Sehutz. Suedsee : 81(1900).**

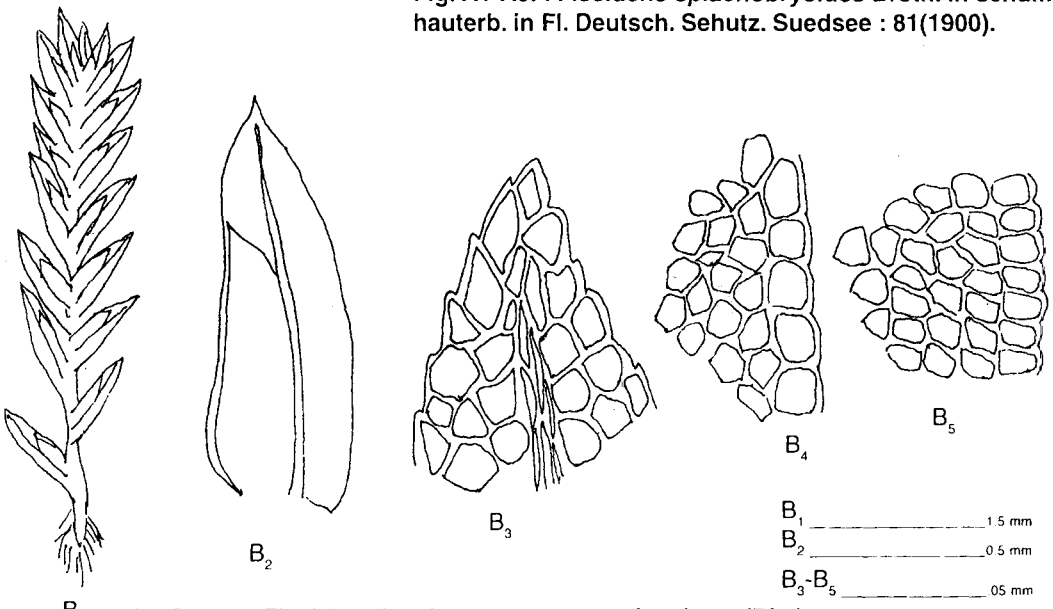

Fig. B1-B5.: *Fissidens involutus ssp curvato-involutus* **(Dix.) Gangulee Comb. nov.**

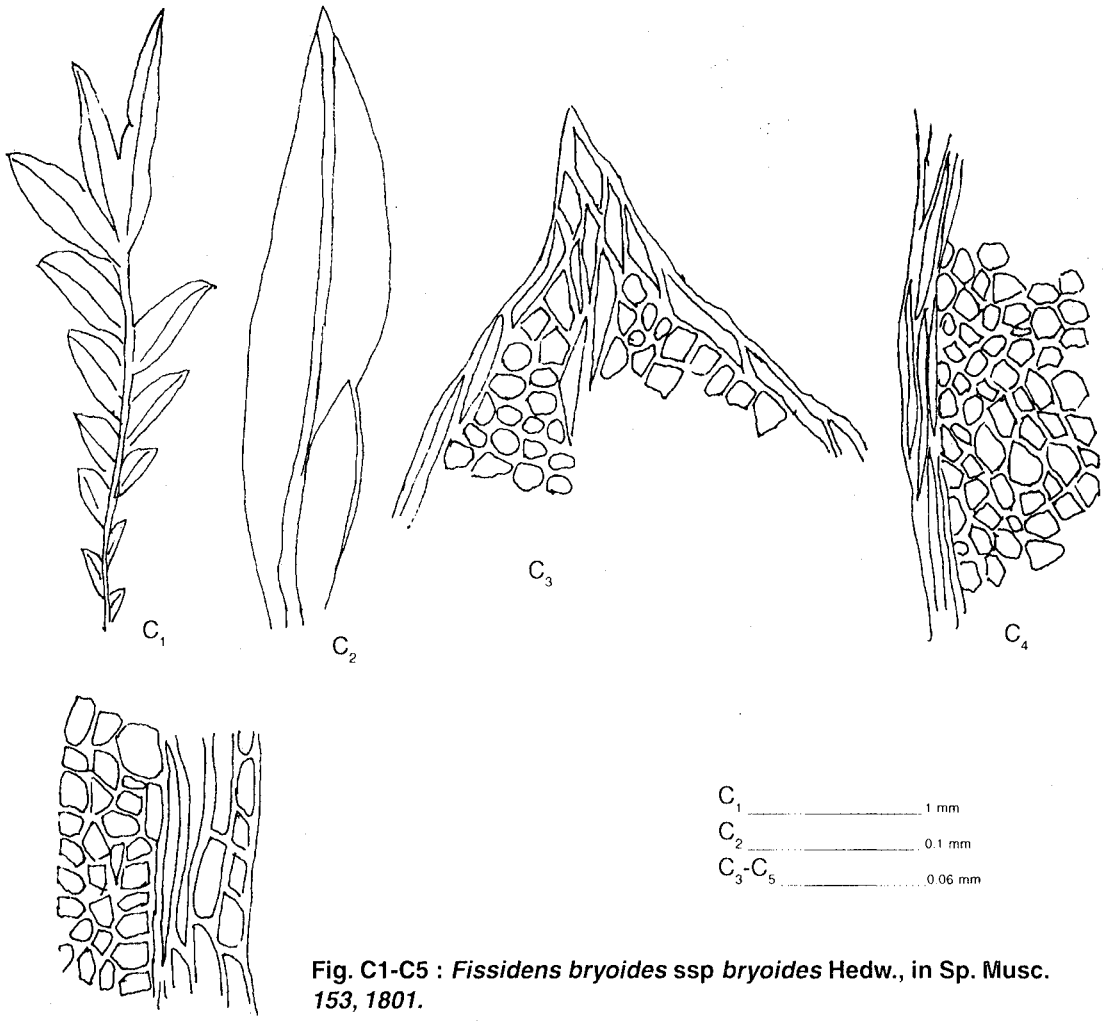

Fig. C1-C5 : *Fissidens bryoides* ssp *bryoides* Hedw., in Sp. Musc. *153, 1801.*

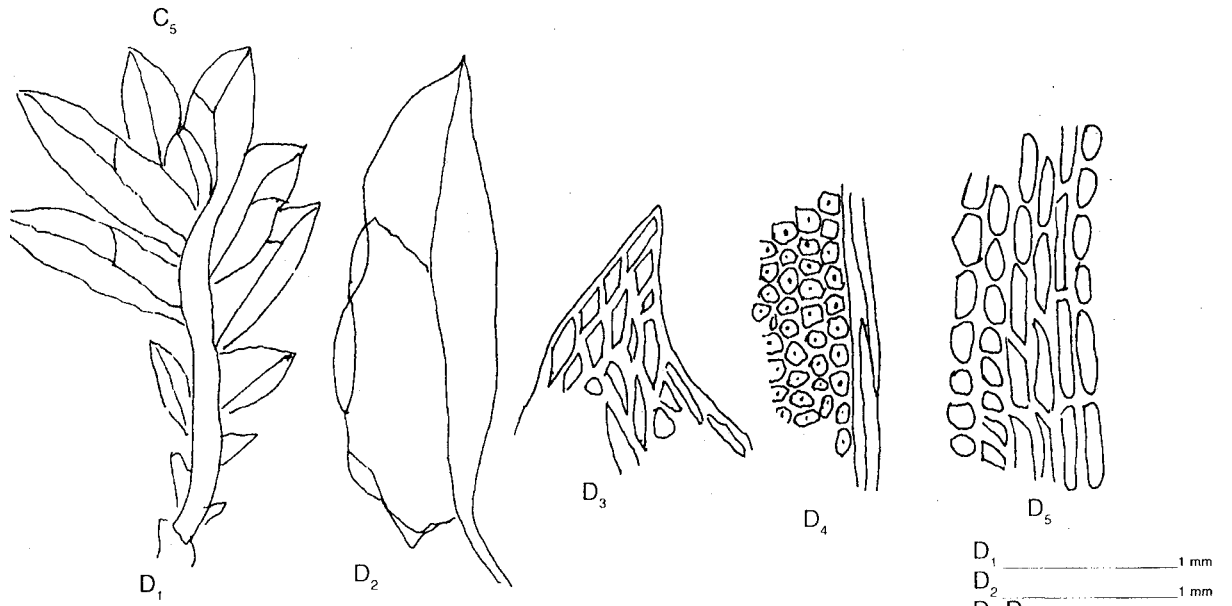

Fig. D1 - D5 : *Fissidens diversifolius* Mitt; J. Linn. in Musc.Ind. Or.: 140:1859.

mm. wide. Dorsal lamina base rounded and then decurrent with a fold. Costa is ending a few cells below apex. Leaf margins dentate due to projecting marginal cells. Leaf cells hexagonal, gradually larger towards vein. Each marginal cell with mamillose conical development on upper surface. Apical cells 3x4.77µm long, 2-3x4.77µm wide more prominently projected. Middle cells 3x4.77µm long 4x4.77µm wide and basal cells 2-3x4.77µm long, 3x4.77µm wide. Plants sterile.

Field notes : Generally plants grow on moist soil but in this exploration it is found growing epiphytically on *Dimocarpus longon* Lour.

Distribution : Darjeeling, Sikkim, Western Himalaya, Baster (M.P.), Chhota Nagpur, Rajasthan, Gujarat, East Nepal Burma, Thailand, Vietnam.

Fissidens bryoides ssp ***bryoides*** **Hedw., in Sp. Musc. 153, 1801.**

Dicranum bryoides (Hedw.) Sw. in Monthl. Rev., 34: 538, 1801.

(Fig. C1-C5, Specimen No-3038)

Plants simple, green, gregarious, 6 mm. long, leaves oblong–lanceolate, acuminate 1.0 to 2.1 mm. long and 0.4 mm. broad, up to 7 pairs, leaves (dry) not much curled or contorted, dorsal lamina narrowing down and ending at nerve base, sheathing lamina usually equal but may be slightly unequal. Leaves broadly equally but tapers down at apex. Limbidium cells elongated which is one-rowed at tip which may change to some shorter cells at the base giving rise to faint denticulations and finally joining the nerve end. On dorsal lamina the limbidium is one- rowed at tip, 2- rowed at base and may end in a few parenchymatous cells where it joints the nerve base. On the sheathing lamina the limbidium is about 4 -rowed at base and 2- rowed at apex. Costa light yellow–brown, percurrent. Cells from upper and middle part of the leaves are smooth, transparent, chlorophyllose with rounded chloroplastids, upper cells 2.5–3.5x4.77µm wide and 4–7x4.77µm long, middle cells 3.5–4x4.77µm wide and 4x4.77µm long, basal cells rounded–hexagonal, 4–5x4.77µm wide and 5x4.77µm long; cells near costa at base are rather elongated.Plants sterile.

Field notes : Plants are found to grow on black to brown coloured moist soil and also epiphytically on the bark of *Mangifera indica* Linn. and *Olea dioca* Roxb.

Distribution : Western Himalayas (Ranikhet, Shimla), South India (Western Ghats, Nilgiri, Coonoor), Rajasthan, Gujarat, Sri Lanka, Europe, Caucasus, Siberia, Japan, Taiwan, China, North and Central Africa, North and South America, Java and Philippines.

Fissidens diversifolius **Mitt; J. Linn. in Musc.Ind. Or.:140:1859**

F. debilis Wils. In Kew. J. Bot. 9:294,1857.

(Fig. D1–D5 Specimen No-3049)

Plants green, in tufts, delicate, flexuous, simple, rarely branched 3,4-9 mm long, distichous. Leaves laxly arranged on shoot, 5-8 pairs, lanceolate (moist) spreading (dry) cruld, crowded at apex and distinct below, margins entire, slightly denticulate above with acute apex, dorsal lamina narrowing down and ends at nerve base, sheathing lamina (lamina vera) cover1/2 to 3/4 of dorsal lamina. Leaf much broader near the base, narrowing down at the broadly acute apex where it is symmetrical. Costa well developed, light brown coloured ending 2 or 3 cells below the tip. Upper and middle cells hexagonal thin walled. Upper cells 20-30 x 1.7µ long and 10x 1.7µ wide, middle cells 20 x 1.7µ long and 20 x 1.7 µ wide, basal cells hexagonaly elongate, 30 x 1.7µ and 10-20 x 1.7µ wide. Plants sterile.

Field notes : Plants growing on the soil.

Distribution: Western Himalaya, Shimla, South India, Western Ghats, Nilgiri, Rajasthan, Gujarat, West Bengal, Orissa, Burma, Japan, Sri Lanka, Malaysia, Pakistan.

ACKNOWLEDGEMENTS

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MODIFIED PROTOCOL FOR ISOLATION OF AZADIRACHTIN AND QUANTITATIVE COMPARISON OF ITS CONTENT IN *IN VITRO* AND *IN VIVO* RAISED NEEM PLANTS

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Abstract

A modified protocol for the isolation of azadirachtin has been developed from leaves of neem (*Azadirachta indica*). Efforts have been made to standardize the protocol for isolation of azadirachtin from *in vivo*-growing and *in vitro*-raised plants. The quantitative yield was compared using High performance liquid chromatography (HPLC). Maximum amount of azadirachtin (4.84%) was found in total ethanolic extract from leaves of *in vivo*-growing plants which was followed by aqueous methanol layer extract (2.75%). Minimum amount of azadirachtin was obtained in petroleum ether layer, i.e. 0.075%, grown under *in vitro* conditions. However, the reasonable quantity of azadirachtin (2.38%) was obtained from total chloroform extract of leaves growing under *in vivo* conditions. The results are reproducible and have the potential for probing the extraction of more azadirachtin content in neem. However, the production and extraction have to be increased manifold to achieve the goal of large-scale production.

Key words: azadirachtin, neem, HPLC.

INTRODUCTION

Neem (*Azadirachta indica* A.Juss family Meliaceae), a tree of medicinal value is a source of insecticides and other useful secondary metabolites. It is receiving global attention due to presence of azadirachtin, and a number of bioactive chemicals in its leaves and seeds, which exhibit a great diversity in chemical nature and structure (Biswas *et al* 2002). Azadirachtin, chemically a sesquiterpenoid drastically reduces feeding by at least 40 species of insect and nematode. It is effective even at a low concentration 0.1 ppm and appears to be safe for beneficial insects, fish, animals and most crop plants (Jacobson, 1984). Neem-based biopesticides are effective against a wide spectrum of insect pests (Mordue and Blackwell, 1993). It is thus essential to enhance the produce of azadirachtin and other useful metabolites in neem. This could be achieved through tissue culture techniques, which have been widely used for multiplication of crop and ornamental plants. Unfortunately, this technique has not been used for the cloning of medicinally important plants (Heble, 1996). By standardizing protocol for clonal propagation of selected elite plants, it is possible to enhance synthesis of a particular product by 8-10 times per unit area of cultivation. Plants raised through micropropagation have identical phytochemical profiles. Therefore, plant tissue culture technology is a boon in the studies of biosynthetic pathways of secondary metabolites. Keeping the above scenario in mind, efforts have been made to standardize the protocol for isolation of azadirachtin and its quantitative comparision from *in vivo*-growing and *in vitro*-raised plants of neem in the present investigation.

MATERIAL AND METHODS

The leaves of 12-year-old neem tree growing under natural conditions were collected, washed, air-dried under shade and powdered. Two hundred grams powder of *in vivo*-grown leaves was taken in a one-litre conical flask and extracted thrice with 500ml ethanol (every time) on orbital shaker for 12 hours at room temperature. Pooled ethanol extract was evaporated below 50ºC on a flash rotary evaporator (Buchi, Switzerland) under vacuum. The concentrated ethanol extract was dried and weighed (19.614 g), out of which 40 mg was weighed in a separate vial and dissolved in HPLC grade methanol to make 10 ml solution (sample 1 for HPLC analysis).

Dried concentrated ethanol extract was dissolved in ethyl acetate. Ethyl acetate-soluble supernatant was poured into separatory funnel and washed with 4% sodium carbonate solution to obtain neutral fraction of the solution. Neutral fraction was dried over anhydrous sodium sulphate and filtered. Ethyl acetate layer was collected in round bottom flask and evaporated on a rotary evaporator under vacuum at 30ºC. Concentrated ethyl acetate layer

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was dried and weighed (9.24 g). Dry extract of ethyl acetate layer was extracted with petroleum ether (40-60 grade) three times. Pooled petroleum ether layer was poured into separatory funnel and extracted with 90% aqueous methanol 3-4 times (100 ml each) till lower layer became colourless. Petroleum ether layer (upper) was collected in round bottom flask and evaporated under reduced pressure at 30ºC. Concentrated petroleum ether extract was dried and weighed (0.08 g), 40 mg of which was dissolved in HPLC grade methanol to make 10 ml solution (sample 5). Aqueous methanol layer was evaporated after usual work-up, and the residue obtained was dried and weighed (0.210 g), 40 mg of which was dissolved in HPLC grade methanol to make 10 ml solution (sample 6). The above procedure was followed in total for the extraction of azadirachtin from leaves collected and pooled from 5-6-month-old plants raised through tissue culture in order to make a comparative study. Sample 2 was prepared by dissolving 10 mg of dried total ethanol extract and sample 4 by dissolving 40 mg residue of aqueous methanol layer in HPLC grade methanol, each obtained from *in vitro* leaves (20 g). Sample 3 was prepared by dissolving 40 mg of total leaf extract in chloroform from *in vivo*-growing plants. The quantitative yield was compared using HPLC analysis.

High-performance liquid chromatography analysis of different extracts (samples 1, 2, 3, 4, 5 and 6) obtained from powder of leaves of *in vivo*-growing and *in vitro*-raised plants was carried out at National Institute of Pharmaceutical Education and Research (NIPER), Mohali (India). The analysis of different samples was carried out on Waters 600E multisolvent delivery system using Waters, 'Nova Pak-C_{18}' (reverse phase) column and Water™ 996 (photodiode aray detector) which is a UV detector and detects at 210 nm. In each case, the known quantity of test samples (40 mg/10 ml in the case of samples 1, 3, 4, 5 and 6, and 10 mg/10 ml in the case of sample 2) was dissolved in a known volume of methanol and 10 ìl of each diluted test sample was injected on the column for HPLC run. Acetonitrile: water (40:60, v/v) was used as mobile phase for elution, with a flow rate of 1 ml/minute, at 33ºC. For qualitative and quantitative determination of azadirachtin in crude extracts, a calibration curve was plotted by running HPLC of known volume (5, 10, 15 and 20 ìl) of known concentration (0.5 mg/1,000 ìl in HPLC grade methanol) of azadirachtin (Sigma Aldrich, USA) under the same set of conditions (acetonitrile: water :: 40:60 v/v, 1 ml/minute, 33ºC). The calibration process was repeated three times to ensure accuracy of results, i.e., retention time and area. All samples were filtered through Millipore filters (0.22 ì). In general, retention times were fairly in agreement with the known standard samples, and these were checked by spiking with internal standard and by observing the increase in concentration (height of peak) of that particular compound (azadirachtin) in chromatogram. The chromatogram was recorded at 210 nm. Azadirachtin content of micropropagated plants of age 5-6 months was analyzed and compared to that obtained from *in vivo* plants (12 years old).

RESULTS AND DISCUSSION

Total yield of ethanol, ethyl acetate, petroleum ether and aqueous methanol extract was 19.614, 9.240, 0.800 and 0.210 g, respectively, as obtained from 200 g powdered leaves of *in vivo*-grown plants following the procedure given above. Similarly, 1.220 g (ethanol extract), 0.238 g (ethyl acetate extract), 0.080 g (petroleum ether extract) and 0.040 g (aqueous methanol extract) were obtained from 20g powder of leaves of *in vitro*-raised plants. 14.664 g chloroform extract was obtained from 200 g powdered leaves of *in vivo*-growing plants (Table 1). Retention time, area, average retention time and average area of azadirachtin using mobile phase acetonitrile: H_2O (40:60) on a 'Nova Pak-C_{18}' (reverse phase) column at 33ºC are shown in Table 2. Average retention time of azadirachtin (5, 10, 15 and 20 ìl) was 13.489, 13.473, 13.411 and 13.422 minutes respectively. Peak results of HPLC analysis of different extracts (test samples 1, 2, 3, 4, 5 and 6) are shown in Fig.1, 2,3,4,5,6 & (Table 3). Retention time of test samples 1, 2, 3, 4, 5 and 6 are 13.170, 13.090, 13.223, 13.223, 13.007 and 13.305, respectively, which is in coherence with retention time of standard compound and confirms the presence of azadirachtin in different test samples. Fig.7 shows that sample 5, when spiked with internal standard azadirachtin, the peak area corresponding to retention time 13.007 increased from 25.11% to 79.38%, confirming that retention time 13.007 is due to the presence of azadirachtin only. The percent amount of azadirachtin was found from a comparison to the calibration curve (Fig.8) in each sample. The amount of azadirachtin (ìg), percentage of azadirachtin in extracts, percent area, area and retention time of different test samples are shown in Table 4. Maximum amount of azadirachtin (4.84%) was found in total ethanolic extract from leaves of *in vivo*-growing plants which was followed by aqueous methanol layer extract (2.75%). Minimum amount of azadirachtin was obtained in petroleum ether layer, i.e. 0.075%, grown under *in vitro* conditions. However, the reasonable quantity of azadirachtin (2.38%) was obtained from total chloroform extract of leaves

Table 1: Amount of different extracts of leaves of *in vivo*-growing and *in vitro*-raised plants of *A. indica*.

Source	*In vivo*-growing plants (Leaf powder – 200 g)	*In vitro*-raised plants (Leaf powder – 20 g)
Ethanolic extract	19.614	1.220
Ethyl acetate extract	9.240	0.238
Petroleum ether extract	0.800	0.080
Aqueous methanol extract	0.210	0.040
Chloroform extract	14.664	–

Table 2: Retention time, area, average area and average retention time of azadirachtin using HPLC in neem.

	Injection volume (µl)	Retention time (minutes)	Area	Average Retention time (minutes)	Average area
5	13.507	1114139	13.489	1090768	
5	13.522	1152868			
5	13.440	1005297			
10	13.523	2381790	13.473	2241996	
10	13.457	2150368			
10	13.440	2193831			
15	13.372	3422730	13.411	3459683	
15	13.405	3396857			
15	13.458	3559464			
20	13.423	4498894	13.422	4475354	
20	13.440	4472412			
20	13.403	4454756			

Table 3: Test samples of leaves of *A. indica*.

Sample	Extract	Weight (g)
Test sample 1	Ethanol extract (*in vivo*)	19.614
Test sample 2	Ethanol extract (*in vitro*)	1.220
Test sample 3	Chloroform extract (*in vivo*)	14.664
Test sample 4	Aqueous methanol layer (*in vitro*)	0.040
Test sample 5	Aqueous methanol layer (*in vivo*)	0.210
Test sample 6	Petroleum-soluble layer (*in vitro*)	0.080

Table 4: Percentage of azadirachtin in leaves of *A. indica* in different samples.

Sample	Retention Time (minutes)	Area	Percent Area	Amount of Azadirachtin (ig)	Percentage of Azadirachtin In extract
Test sample 1	13.170	433794	24.52	0.387 / 8	4.840
Test sample 2	13.090	20118	8.95	0.018 / 2	0.900
Test sample 3	13.223	213637	27.88	0.191 / 8	2.380
Test sample 4	13.223	92079	16.40	0.821 / 8	1.026
Test sample 5	13.007	246308	25.11	0.220 / 8	2.750
Test sample 6	13.305	7050	10.33	0.006 / 8	0.075

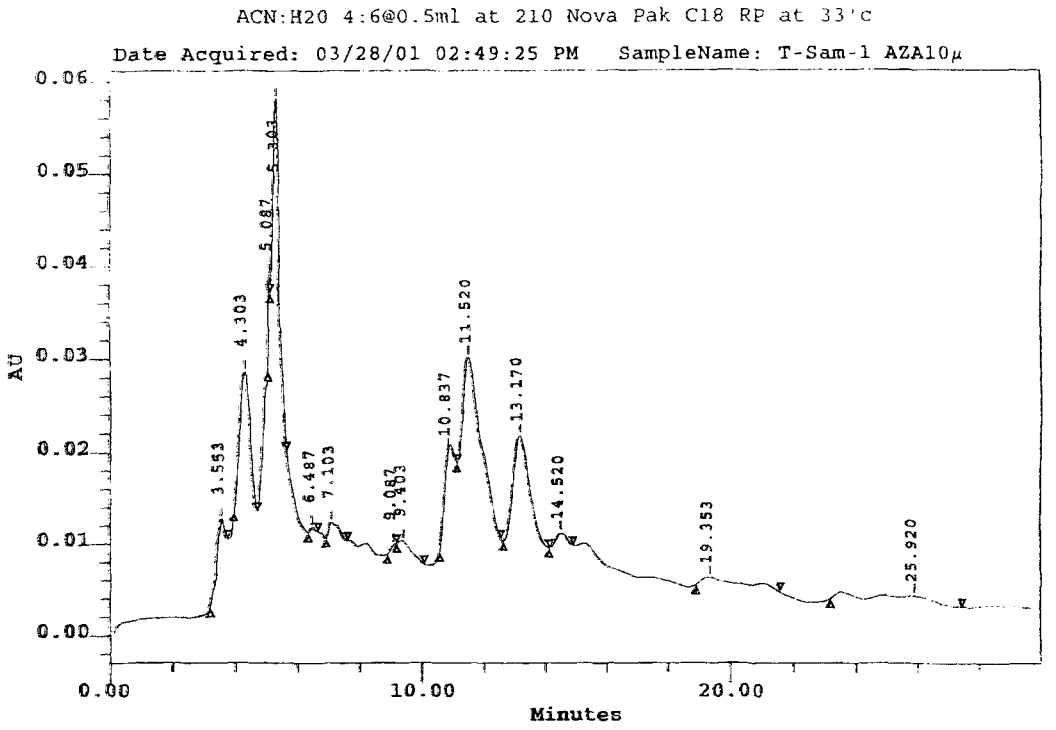

Fig. 1: HPLC Chromatogram of test sample 1 showing azadirachtin peak at 13.170 minutes.

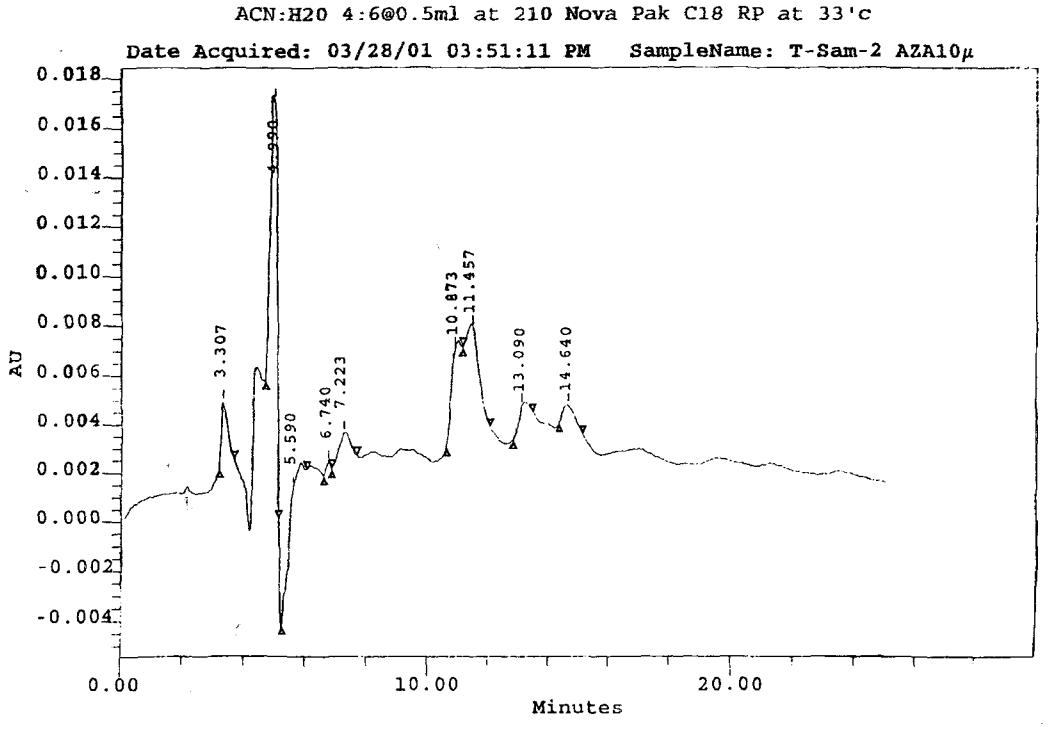

Fig. 2: HPLC Chromatogram of test sample 2 showing azadirachtin peak at 13.090 minutes.

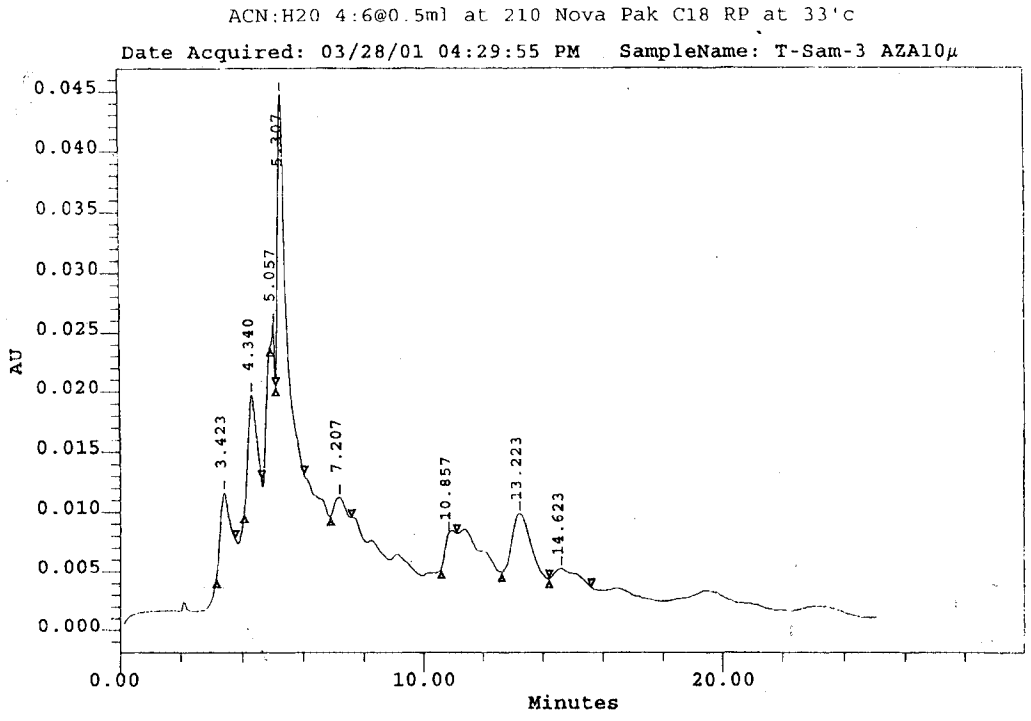

Fig. 3: HPLC Chromatogram of test sample 3 showing azadirachtin peak at 13.223 minutes.

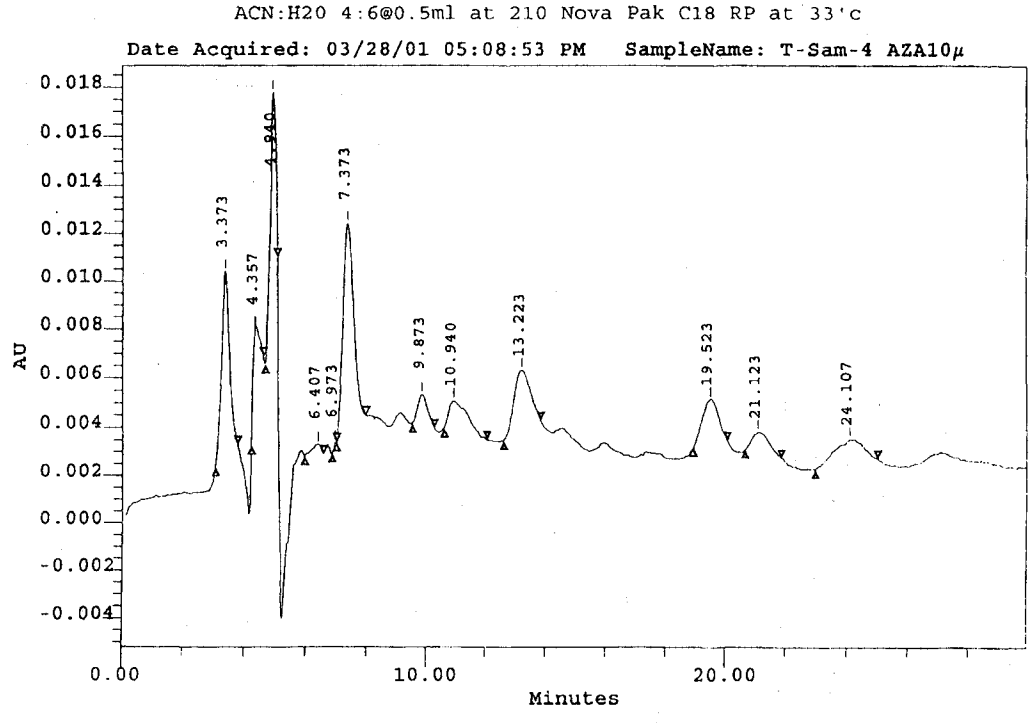

Fig. 4 : HPLC Chromatogram of test sample 4 showing azadirachtin peak at 13.223 minutes

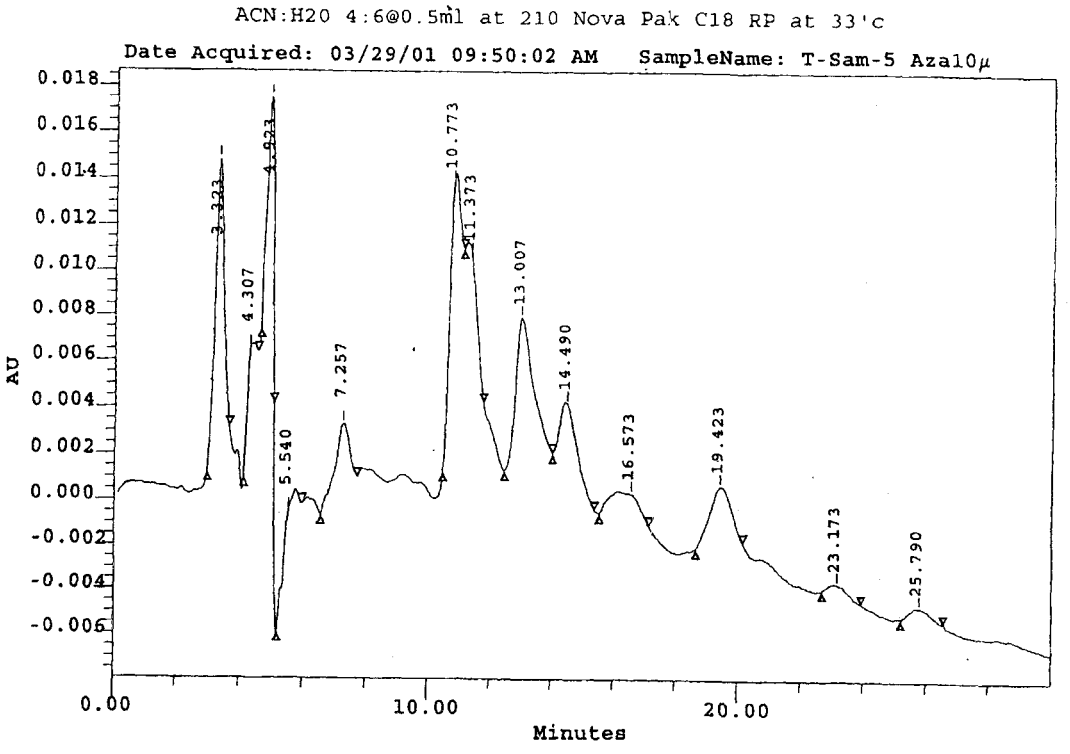

Fig. 5: HPLC Chromatogram of test sample 5 showing azadirachtin peak at 13.007 minutes.

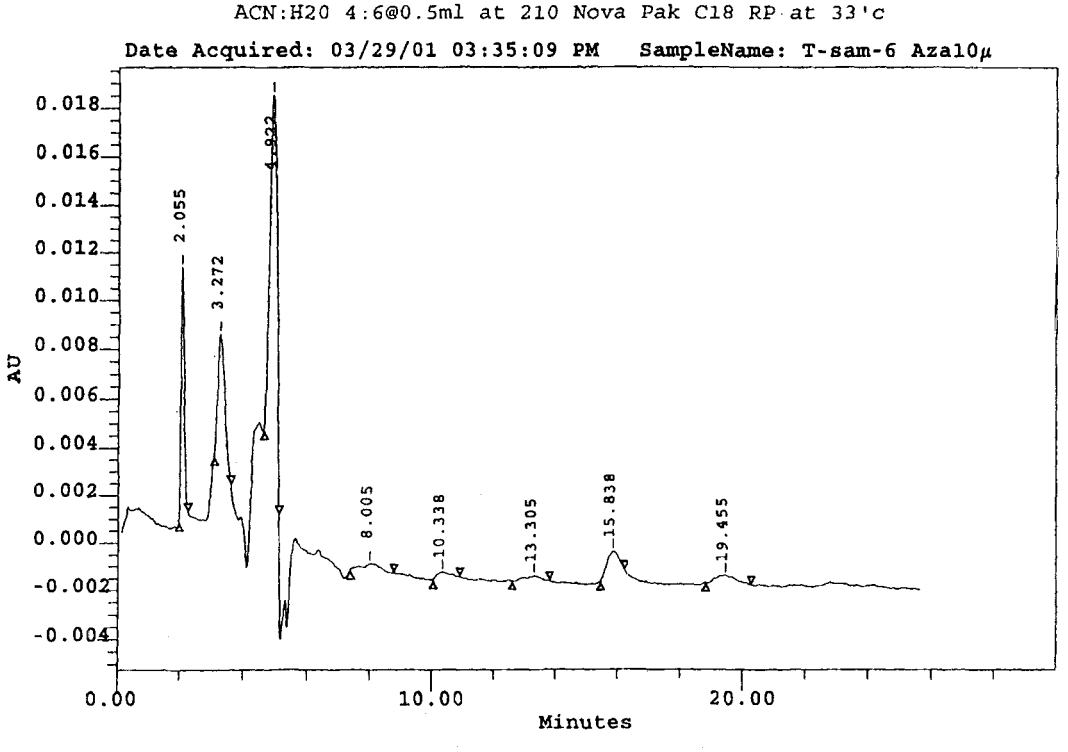

Fig. 6: HPLC Chromatogram of test sample 6 showing azadirachtin peak at 13.305 minutes.

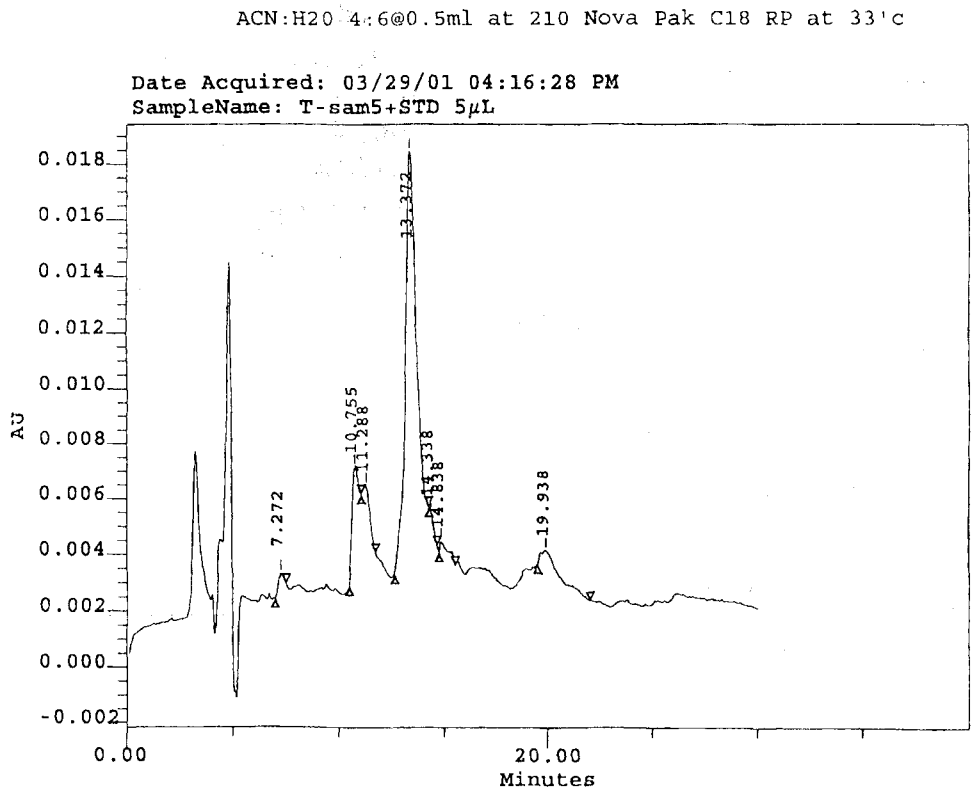

Fig. 7: HPLC Chromatogram of test sample 5 and internal standard showing azadirachtin peak at 13.372 minutes.

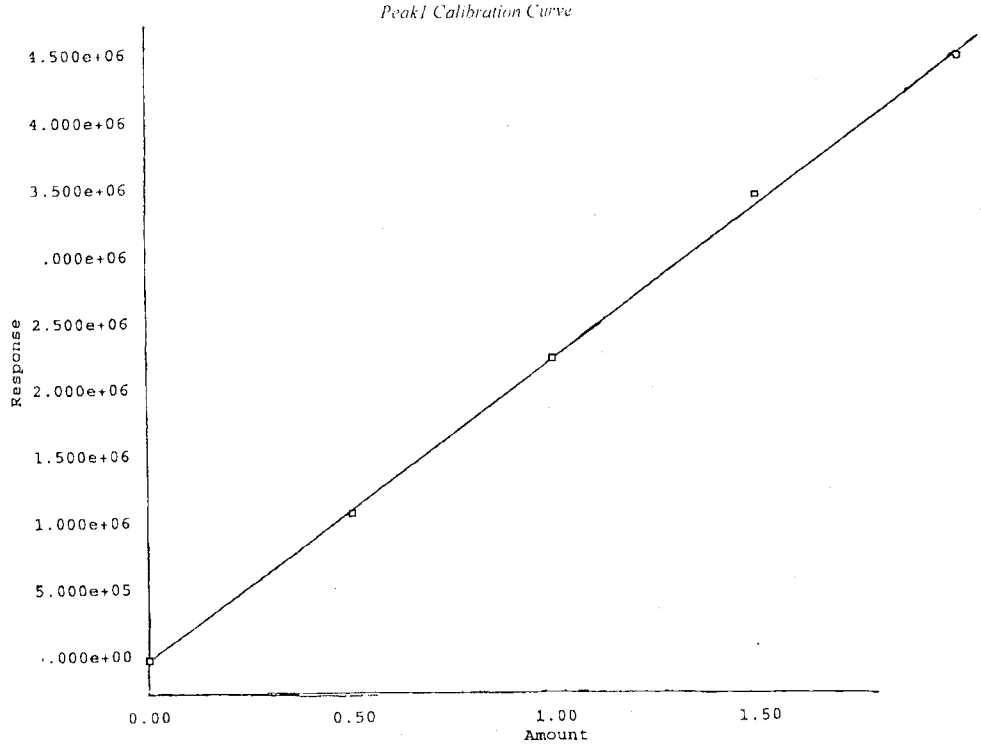

Fig. 8: Calibration curve obtained for azadirachtin.

growing under *in vivo* conditions. During the present investigation, efforts were made to compare azadirachtin content of 5-6-month-old *in vitro*-raised plants with 12-year-old *in vivo*-growing plants. Although a small amount of azadirachtin content was found in leaves of micropropagated plants, but still the results are significant, as the amount of azadirachtin depends upon a number of factors like age of plants, carbohydrate source, nature of media, cell-line age and other environmental factors. There are chances of more azadirachtin content in micropropagated plants of more age. Hence, there is a further scope of investigation to increase the amount of azadirachtin content in neem. Maximum amount of azadirachtin was found in total ethanol extract from leaves of *in vivo*-growing plants which was followed by aqueous methanol layer extract growing under *in vivo* conditions. Similarly, Siddiqui *et al.* (1991) also isolated five new triterpenoids from ethanol extract of neem fruit coatings. The extraction of azadirachtin was done using callus tissues obtained from leaf and bark by (Wewetzer, 1999) and micropropagated neem plants (Venkateswarlu and Mukhopadhyaya, 1999). The other workers have obtained azadirachtin from neem seeds using thin layer chromatography (TLC) and supercritical fluid chromatography. (Allan *et al.*, 1994; Johnson and Morgan, 1997). Azadirachtin was identified in tissue-cultured cells of neem using NMR and mass spectral data (Jasvir *et al.*, 1997; Siddiqui, 2000) in *Melia azedarach* (Fukuyama *et al.*, 2000). During the present study, azadirachtin content was compared between 5-6-month-old *in vitro*-raised plants on MS medium supplemented with sucrose and 12-year-old *in vivo*-growing plants. However, Wewetzer (1999) reported higher level of differentiation on the medium supplemented with maltose. Azadirachtin content varied from 0.5 ìg/g dry weight until 64 ìg/g dry weight depending on nutrient medium, carbohydrate source and cell-line age.

In the end, it can be concluded that neem plants are capable of producing azadirachtin content in quantities well above the limit of detection using the above, modified protocol. However, the production and extraction have to be increased manifold to achieve the goal of large-scale production.

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LEAF ANATOMICAL STUDY OF SOME INDIAN BAMBOOS AND ITS SYSTEMATIC SIGNIFICANCE

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Abstract

Leaf anatomical studies in 52 species of 16 genera of bamboos have been carried out and their role in Taxonomy has been discussed. Following leaf anatomical characters have been found to be taxonomically useful - Long cells markedly sinuous and usually papillate; bands of bulliform cells present spanning the intercostal regions of the adaxial surface of the epidermis; short cells paired or solitary; silica bodies mostly saddle-shaped; stomata more frequent on the lower epidermis and with low dome-shaped or triangular subsidiary cells; midrib complex; two sheaths surrounding the vascular bundles; chlorenchyma irregularly arranged and its cells with invaginations of the cell walls (arm cells); a palisade layer differentiated just beneath the adaxial epidermis; and fusoid cells with large fusoid cavities present in the mesophyll area.

Key words: leaf anatomy, leaf epidermis, bamboos, taxonomy.

INTRODUCTION

Bamboos are tall, arborescent grasses which occur naturally in every continent except Europe and Antarc--tica. They belong to the tribe Bambuseae, sub-family Bambusoideae of the family Poaceae. A total of 840 species and 49 genera of bamboos are found in the world (Clayton and Renvoize, 1986). In India, a total of about 128 species belonging to 18 genera are reported (Seethalakshmi and Kumar, 1998). Bamboos generally have hollow woody culms, complex rhizome and branch systems, petiolate leaf blades and prominent sheathing organs. In size, the bamboo culms range from not more than 30 cm tall in some species (*e.g. Arundinaria pygmaea*) to tall "trees" up to 35m tall and up to 30cm in diameter (*e.g. Dendrocalamus giganteus*). *Chimonobambusa densifolia* is the smallest of the Indian bamboos with culms 15-90 cm tall and 0.5-0.8mm thick.

Most bamboos flower at long intervals. Flowers in these plants are, therefore, scarcely available and one has to resort to distinguishing them by vegetative features. In bamboos, characters of vegetative morphology, anatomy, cytology and biochemistry hold a great promise from systematic point of view. Grass leaf anatomy, as revealed by features of leaf transections and the epidermal structure, has been the subject of numerous investigations and resulted in the establishment of a number of anatomical grass types in grasses, such as the festucoid, panicoid, chloridoid, bambusoid and aristidoid types (Brown, 1958), characterizing major groups of grasses corresponding to the emerging concept of subfamilies of the grass family, Poaceae.

Leaf anatomy is less well documented in bamboos than in other groups of grasses. The bambuseae has received very little attention from anatomists since the work of Metcalfe' (1960) and Jacques-Felix (1962). Other works devoted to bamboo leaf anatomy include those of Brandis (1907); Metcalfe' (1956); Wu (1958, 1960, 1962); Ghosh and Negi (1960); Calderon and Soderstrom (1980); Renvoize (1985a, 1987a); Soderstrom *et al.* (1987); Rao (1987); Soderstrom and Ellis (1988); Chauhan *et al.* (1988, 1992); Sharma *et al.* (1986-1990); Agrawal and Luxmi Chauhan (1990); Stuessy (1990); Sharma (1991); Agrawal *et al.* (1996); and Ding and Zhao (1994). Much of the work in leaf anatomy has been done on the study of the arrangement and structure of the vascular bundles (Grosser and Liese, 1971; André, 1996).

Along with the leaf cross sectional anatomy, the structure of leaf epidermis is equally important. Sharma and Kaur (1983); Gould (1986); Sharma and Kalia (1983); Sharma (1991); Agrawal and Chauhan (1990-1995); Richa and Sharma (1997, 2003) have successfully used various epidermal characters for diagnostic purposes at specific and sub specific levels in a number of grass and bamboo genera.

The present study elucidates features of leaf epidermis and leaf cross-sectional anatomy of 52 species in 16 genera of bamboos.

MATERIAL AND METHODS

Leaf material for the present study was collected and preserved from different natural localities of Darjeeling, Manipur, Shimla, Gangtok, Ooty, Kodaikanal; Bambuseta of Kerala Forest Research Institute, Peechi; Forest

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Research Institute, Dehradun; and P. N. Mehra Botanical Gardens, Panjab University, Chandigarh. The species included in the present study are - *Bambusa auriculata* Kurz, *B. balcooa* Roxb., *B. bambos* Voss, *B. binghamii* Gamble, *B. burmanica* Gamble, *B. kingiana* Gamble, *B. longispiculata* Brandis, *B. multiplex* cv. Fernleaf Young, *B. multiplex* var. *riviereorum* Maire., *B. nutans* Munro, *B. oliveriana* Gamble, *B. pallida* Munro, *B. polymorpha* Munro, *B. teres* Munro, *B. tulda* Roxb., *B. vulgaris* var. *striata* (Lindl.) Gamble, *B. vulgaris* var. *vulgaris* Schrad., *B. vulgaris* var. *wamin* McClure, *Cephalostachyum pergracile* Munro, *Dendrocalamus asper* (Schultes f.) Heyne, *D. calostachyus* (Kurz) Kurz, *D. flagellifer* Munro, *D. giganteus* Munro, *D. hamiltonii* Munro, *D. hookeri* Munro, *D. longispathus* Kurz, *D. membranaceus* Munro, *D. sericeus* Munro, *D. strictus* (Roxb.) Nees, *Dinochloa andamanica* Kurz, *D. maclellandii* (Munro) Kurz, *Gigantochloa albociliata* (Munro) Kurz, *G. atroviolacea* Widjaja, *G. atter* (Hassk.) Kurz, *G. nigrociliata* Kurz, *G. pseudoarundinacea* (Steud.) Widjaja, *G. rostrata* Wong, *Guadua angustifolia* Kunth, *Melocalamus compactiflorus* (Kurz) Benth. and Hook. f., *Melocanna baccifera* (Roxb.) Kurz, *Ochlandra scriptoria* (Dennst.) Fisch., *O. travancorica* Benth., *Oxytenanthera abyssinica* (A. Rich.) Munro, *O. bourdillonii* Gamble, *O. stocksii* Munro, *Phyllostachys aurea* A and C Riv., *Pseudosasa japonica* (Steud.) Nakai, *Shibataea kumasaca* (Steud.) Mak., *Teinostachyum wightii* Beddome, *Thamnocalamus spathiflorus* (Trin.) Munro, *Thyrsostachys oliveri* Gamble, and *T. siamensis* Gamble.

In each case, middle portion of the leaf was selected for the study. Peelings of foliar epidermis were stained in safranin (2% sol.) and mounted in 10% glycerol using the method of Metcalfe' (1960). Microtome sections of the leaves were stained in safranin-fast green combination (Johansen, 1940) and mounted in 10% glycerine jelly.

RESULTS AND DISCUSSION

A generalized description of the leaf epidermal features and leaf cross-sectional anatomy of the bamboos, based on the present study, is summarized below:

A) Leaf Epidermis: long cells markedly sinuous, rarely slightly sinuous, mostly papillate; bands of bulliform cells (isodiametric, thick-walled cells with inflated bases and varying in appearance with change in focus) present in 2-6 rows on adaxial surface spanning the intercostal regions; cross-veins present (absent in *B. nana* and *B. bambos*); short cells paired or solitary (in groups of 5 or more in *Phyllostachys aurea*); silica bodies saddle-shaped, rarely tall and narrow; microhairs slender, 2-celled and finger like, with both cells of about the same length; stomata more frequent on the lower epidermis and with low dome-shaped or triangular subsidiary cells; stomatal insertion variable, varying from 'overlapping-type' to 'overlapped-type' to 'flushed-type'; subsidiary cells triangular to low dome-shaped.

B) V. S. Lamina: leaf blades S-shaped or V-shaped or flat, asymmetric about the midrib and possess bundles of three classes; midrib distinct and complex, rarely simple; both adaxial and abaxial girders of sclerenchyma present opposite the vascular bundles; bundle sheath double, the outer being the parenchyma sheath (PS) while the inner one is the mestome sheath (MS); bulliform cells present in groups of 2-6 cells in the intercostal regions of adaxial surface; mesophyll with chlorenchyma irregularly arranged (non-radiate) and its cells with cell wall invaginations (arm cells); a palisade layer of chlorenchyma usually differentiated just below the epidermis; fusoid cells (vertical plates of thick-walled cells oriented at right angles to the long axis of the leaf) present in the mesophyll; high interveinal distances (with a* 'maximum lateral cell count' of more than 4); thick leaf blades (with a + 'maximum cells distance count' of more than 1); and starch stored principally in the mesophyll.

The presence of non-radiate mesophyll, high interveinal distances, thick leaf blades, and the presence of starch in the mesophyll suggest the presence of non-kranz leaf anatomy and hence C_3-photosynthesis (Sharma, 1991).

A perusal of leaf epidermal features provided above shows that bamboos possess a particular combination of leaf epidermal features, which are common to their constituent species. Likewise, all bamboos also possess a characteristic type of leaf cross-sectional anatomy, the special features of which are the presence of a complex midrib, fusoid cells and arm cells.

However, a review of literature reveals that these characters are not exclusive to bamboos alone. Fusoid cells have been reported in the grass genus *Homolepis* of the subfamily Panicoideae (Watson *et al.*, 1985) while these are absent in the bamboo genus *Phyllostachys* (Metcalfe', 1960). Similarly, arm cells are reported in the grass genera *Phragmitis* and *Thysanolaena* of the subfamily Arundinoideae (Watson *et al.*, 1985; Sharma *et al.*, 1987b), in the genus *Sclerodactylon* and in members of the tribe Triodieae of subfamily Chloridoideae (Watson *et al.*, 1985). Thick-walled bulliform cells spanning the intercostal regions of adaxial surface of epidermis, a characteristic feature of most bamboos, is also exhibited by grasses like *Arundo donax, Cortaderia*

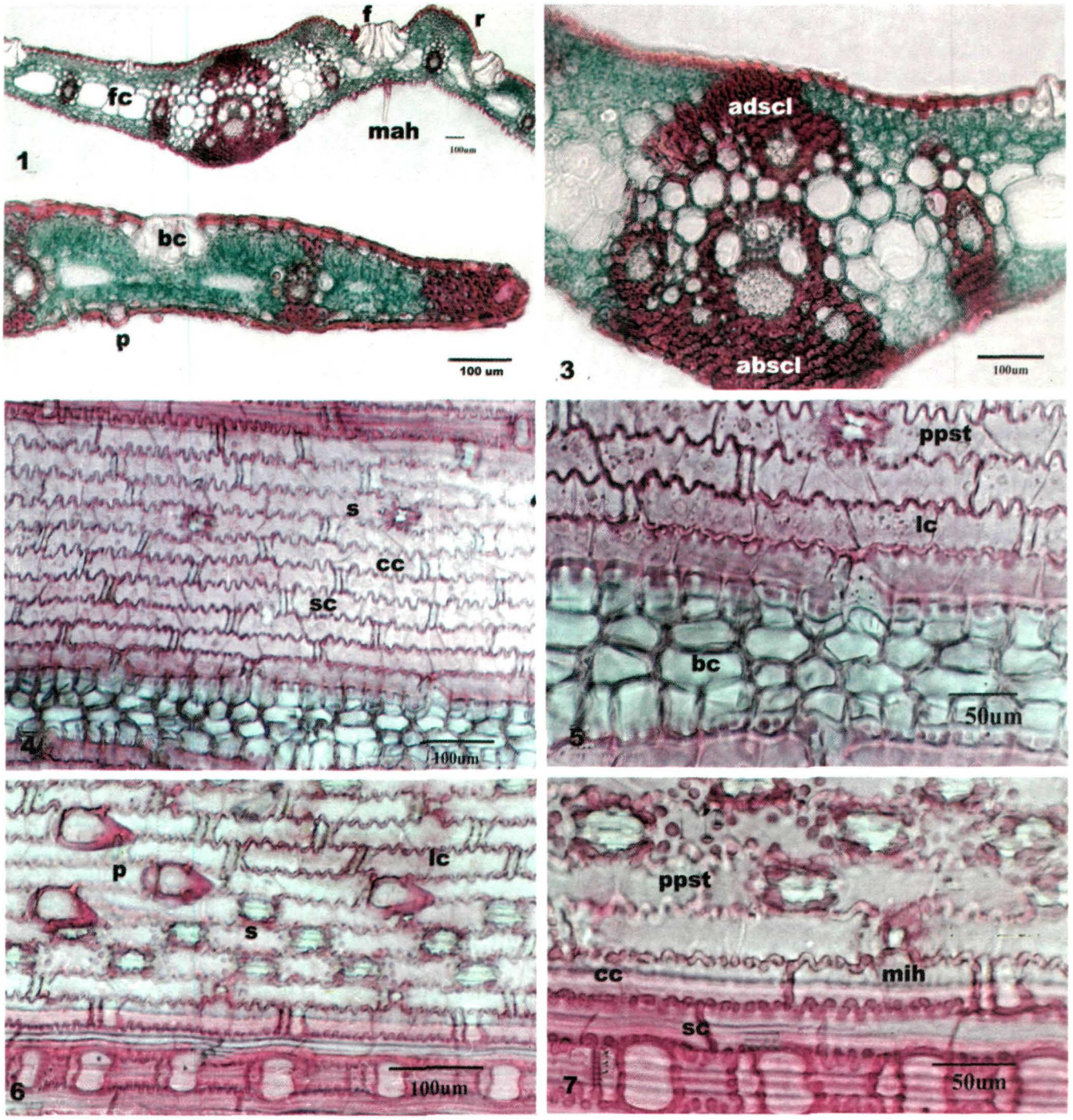

Plate: *Dinochloa maclellandii* (Munro) Kurz.

1. T.S. lamina (x10X); 2. T.S. lamina margin (x25X); 3. T.S. midrib (x25X); 4. Adaxial epidermal peeling (x20X); 5. Adaxial epidermal peeling (x40X); 6. Abaxial epidermal peeling (x20X); 7. Abaxial epidermal peeling (x40X).

(scl-sclerenchyma girder; bc-bulliform cells; fc-fusoid cavity; p-prickle; s-stomata; mah-macrohair; mih-microhair; pp-papillae; cc-cork cell; sc-silica cell).

selloana (Sharma *et al.*, 1987b) and *Desmostachya bipinnata* (Sharma *et al.*, 1987c). The taxonomically useful leaf anatomical attributes of bamboos include: long cells markedly sinuous and usually papillate; bands of bulliform cells present spanning the intercostal regions of adaxial surface of epidermis; short cells paired or solitary; silica bodies mostly saddle-shaped; stomata more frequent on the lower epidermis and with low dome-shaped or triangular subsidiary cells; midrib complex; two sheaths surrounding the vascular bundles; chlorenchyma irregularly arranged and its cells with invaginations; a palisade layer differentiated just beneath the epidermis; and fusoid cells with large fusoid cavities present in the mesophyll area. Still, however, the bamboos in general can either be distinguished by the presence of certain unique anatomical features or unique combination of these features.

The present study has helped in the identification of certain features of leaf anatomy which characterize bamboos. These features are useful in the identification of isolated bamboo leaves. The utility of leaf anatomical features in the identification of various genera and species of bamboos is currently being assessed.

* The number of chlorenchyma cells between two laterally adjacent bundle sheath cells.

+ Maximum number of cells separating any chlorenchymatous mesophyll cells from its nearest parenchymatous bundle sheath cells.

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MEIOTIC STUDIES IN SOME BARLEY (*HORDEUM VULGARE* L.) GENOTYPES

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Abstract

Four diploid genotypes *viz.* PL 426, PL 419, PL 56 and DL 70 are used in present study. Meiotic observations from young spikes were recorded for chromosome number, their distribution and chiasma frequency. Metaphase-I stage showed the presence of seven bivalents comprising of ring and rod whereas, anaphase-I stage represented (7-7) distributions of chromosomes. Univalents, trivalents, quadrivalents and probably an accessory chromosome have also been observed. Majority of the cells indicated normal chromosome configuration at metaphase and anaphase stages. Anaphase-bridges have been observed in some cells but laggards were completely absent. The mean chiasma frequency varied from 11.70 (DL 70) to 12.09 in PL 56. The number of chiasmata per bivalent is similar in PL 426 and PL 419 *i.e.* 1.71 and 1.72 in PL 56 and minimum 1.67 in DL 70. Frequency of ring and rod shaped bivalent are variable in all genotypes. The percentage of chromosomal irregularities is comparatively lower and may not be able to cause any drastic changes in genotypic potential.

Key words: *Hordeum vulgare*, meiosis, chiasma frequency.

INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the oldest, widely cultivated grain crops of the world. Productivity of the crop is mainly dependent on intrinsic and extrinsic conditions. Intrinsic conditions include the genetics of the plant stored in its seeds whereas extrinsic conditions referred to soil and weather. The principle use of the crop is brewing and distillery industry with much of its entering into the manufacturing of beer, whisky and alcohol, breakfast foods, malt, medicines and as desizing agent in textile industry (Beavan, 1945). The suitability of barley grains for malting depends on many criteria including size, shape and uniformity of the kernels. Therefore it is important to study the shape and size of the kernels (Ayoub *et al.*, 2002). Advantage of early maturity and stress tolerance make it a choice even today for stressful environment and low input agriculture. Thus, chromosomal study is helpful to estimate the stability of particular genotype of barley.

Barley crop can be successfully grown under poor soil conditions, with limited available water under rain conditions and can also be sown late where productivity of wheat declines. Barley provides the necessary diversification to Wheat-Rice system and can fit into multicropping system like Maize-Potato-Barley, Rice-Potato-Barley or Basmati-Barley combinations. The crop is likely to achieve importance in view of diversification and its use for agro-processing because it can fit very well with Basmati. Barley is an excellent plant material for genetic studies because its chromosomes are relatively larger in size and less in number. Meiotic observations from young spikes are useful to study chromosome number, their distribution and chiasma frequency. To broaden the genetic base of these genotypes as well as to transfer desirable traits from one genotype to other, it is essential for cellular genetics like searching chromosome number and behaviour and morphology of chromosomes in different genotypes. Keeping this in view, the present study was planned to understand the chromosome behaviour of some barley genotypes.

MATERIAL AND METHODS

The material consists of four barley genotypes *viz.* (i) PL 56 (ii) PL 419 (iii) PL 426 and (iv) DL 70. Seeds of these genotypes were sown in glass house. Around 40-45 plants of two genotypes *i.e.* PL 426 and PL 419 and 30 plants of another two genotypes PL 56 and DL 70 attained maturity. All agronomic practices like irrigation, weeding, hoeing *etc* were followed in order to raise a good crop.

Young spikes of barley crop were collected in the month of February between 8:30 to 10:00 a.m. and fixed in carnoys solution (3 parts ethanol: 1 part glacial acetic acid) and were tagged against their respective genotypes. For longer preservation samples were shifted to 70% ethanol (70 ml ethanol + 30 ml distilled water).

The young spike was placed on the slide. Anthers were taken out from the spikelet with the help of forceps and needles. A drop of acetocarmine was added to the anthers. The material was crushed with the help of blunt end of needle. Cover slip was placed on the crushed and stained material. Following the pressing of the cover slip with light thumb impression, gentle heating was done. Slide was observed under the microscope and chromosomes at different meiotic stages were photomicrographed.

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RESULTS

Data on configuration of chromosomes of four different genotypes are presented in Table-1. Most of the cells of PL426 showed normal seven bivalents at diakinesis/ metaphase-I. Variable number of univalent, trivalent and quadrivalent in addition to bivalents has also been observed in some pollen mother cells (Figures 1-2). The average chromosome configurations per cell at diakinesis/ metaphase-I stage are $0.014^{IV}+0.007^{III}+6.947^{II}+0.014^{I}$ in PL 426 (Table-1). A cell with a fragment of chromosome in addition to normal seven bivalents at diakinesis has also been observed.

Chromosomes in PL 419 found to be scattered at diakinesis or at the equatorial plate during metaphase-I. Besides bivalents, univalents and quadrivalents were also observed (Figures 3-4) but majority of pollen mother cells indicated seven bivalents at diakinesis/metaphase-I. Trivalents have not been found in any cells. Bivalents are either ring (open or closed) or rod shaped but the number of ring and rod bivalent vary in different cells. A cell with two univalents and six bivalents has also been observed. The average chromosome configurations per cell at diakinesis/ metaphase-I are $0.016^{IV}+6.959^{II}+0.008^{I}$.

Majority of the cells of PL56 represented seven bivalents at metaphase-I (Figures 5-7) and normal distribution of chromosome at anaphase-I . Quadrivalents (chain or ring) were present in addition to bivalents in few cells but univalents and trivalents were not found. The average chromosome configurations per cell at diakinesis/ metaphase-I are $0.017^{IV}+6.965^{II}$ (Table-1). Maximum variation in terms of ring and rod shaped bivalents has been observed in this genotype. However, large number of cells showed (5ring+2rod) chromosome configurations. In some pollen mother cells, a darkly stained fragment of chromosome was observed in addition to seven bivalents (Figures 8 -9). Bivalents of the cells in which a fragment was present, grouped into two and five or three and four.

The occurrence of seven bivalents at diakinesis/ metaphase-I was common in DL70. A cell having 5 bivalents and chain of four and ring of four chromosomes has also been observed at metaphase-I (Figure-10). Like PL56 genotype, no trivalents but quadrivalents were present. Anaphase bridges and laggards were absent. The average chromosome configurations per cell at diakinesis/metaphase-I are $0.015^{IV}+6.968^{II}$ (Table-1). Anaphase stage showed normal (7-7) distribution of chromosomes in all genotypes. Laggards were absent in all genotypes however, anaphase bridges have been observed in some cells of PL56 genotype.

CHIASMA FREQUENCY:

PL 426 - The number of chiasmata per pollen mother cell (PMC) range from 8 to 14. The mean chiasma frequency per cell is 11.97. The highest percentage of cells *i.e.* 32.30% shows 13 chiasmata, whereas 8 chiasmata are observed only in 3.07% cell. The chiasma frequency per bivalent is 1.71 (Table-2).

PL 419 – The analysis of total of 58 cells shows that the number of chiasmata per cell varies from 7 to 14. Fourteen chiasmata are observed in 17.24% cells. Maximum percentage of cells (25.86) has twelve chiasmata (5 rings + 2 rods). However, only 1.72% cells possess seven chiasmata. Chiasmata frequency per cell is 12.02 while chiasma frequency per bivalent is the same as in PL 426 *i.e.* 1.71 (Table-2).

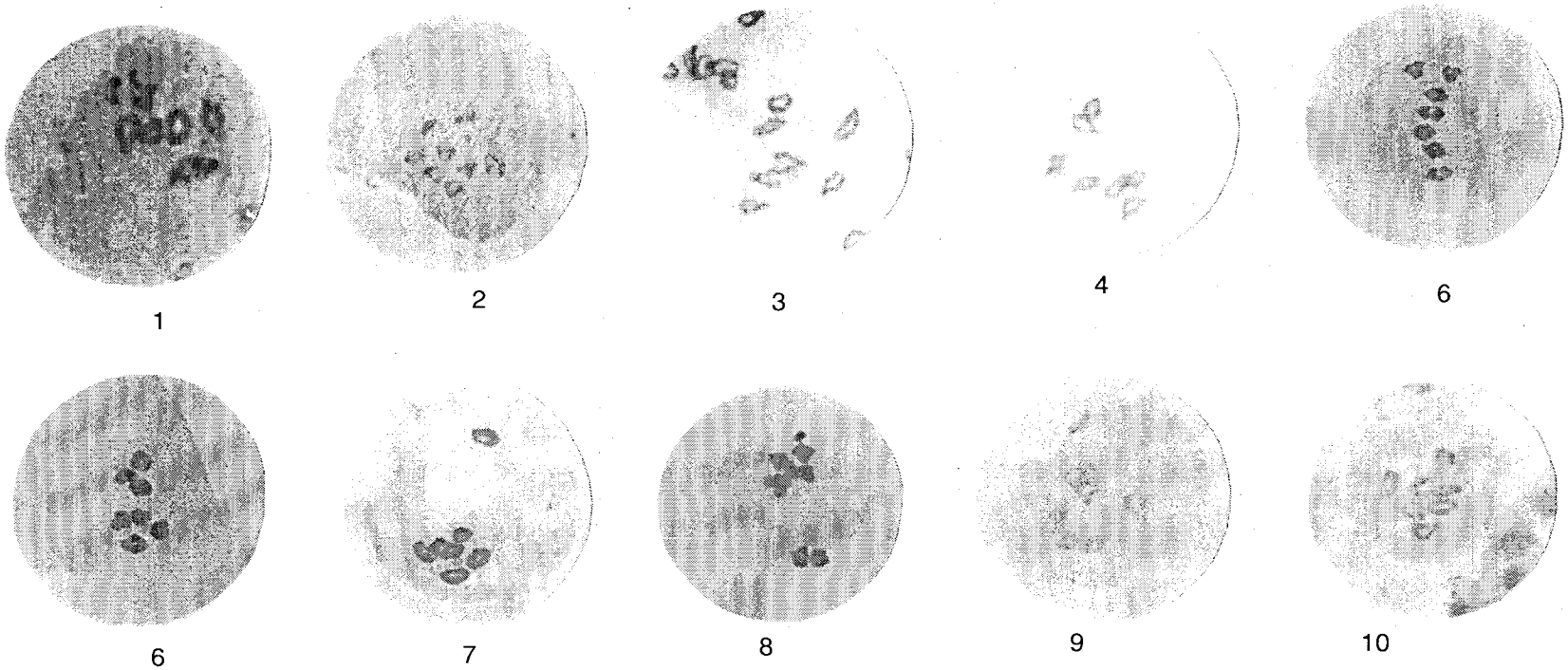
PL 56 – Seventy three cells were scored for chiasma frequency in this genotype. Number of chiasmata per cell varies from 7 to 14. Maximum mean chiasma frequency per cell is 12.09 and 1.72 chiasmata per bivalent. Only 2.73 % cells indicate seven chiasmata per cell whereas, maximum percentage of cells *i.e.* 35.61 shows 14 chiasmata per cell followed by 13 chiasmata in 20.54% cells. Eight and ten chiasmata per cell are found only in 8.21% cells. Some cells with chiasmata number 9, 11 and 12 have also been observed (Table 2).

DL 70 –Observations as carried out on 127 pollen mother cells of this genotype. The number of chiasmata per cell varies from 7 (1.57%) to 14 in (11.81%) cells. The number of chiasmata per bivalent (1.67) and chiasma frequency per cell (11.70) was lowest in this case. Maximum number of cells *i.e.* 25.19 % possesses 12 chiasmata whereas seven chiasmata were found in only 1.57% cells. Pollen mother cells with varying number of chiasmata such as: 8, 9, 10, 11, 12 and 13 have also been observed in different proportions (Table-2). The chiasma frequency per cell varies from 11.70 to 12.09 whereas; frequency per bivalent was almost similar in all genotypes.

DISCUSSION

Evidences collected from cytogenetic studies revealed that all the genotypes are diploid with chromosome number (2n=14). Cytogenetic studies, have thrown light on chromosome number, their configurations and chiasma frequency.

During meiosis, majority of the pollen mother cells showed normal seven bivalents at diakinesis and metaphase-I with equal distribution at anaphase-I. The average number of cells having normal seven bivalents varied from 6.95 to 6.97 in different genotypes. Quadrivalents were



Figs: (1) One trivalent + five bivalents + one univalent at diakinesis stage, (2) six bivalents + two univalents at metaphase, (3) chain of four + five bivalents at diakinesis, (4) ring of four + five bivalents at diakinesis, (5), (6) and (7) seven bivalents at metaphase, (8) seven bivalents + a fragment at metaphase, (9) seven bivalents + a fragment at diakinesis and (10) chain of four + five bivalents at metaphase.

Table 1: Meiotic chromosome configuration in four genotypes

Genotype PL 426

Configuration	No. of Cells	% of cells
$I^{IV} + 5^{II}$	2	1.49
$1^{III} + 5^{II} + 1^{I}$	1	0.74
$6^{II} + 2^{I}$	1	0.74
7^{II}	130	97.01

Average chromosome configuration /Cell at Diakinesis/Metaphase- $I = 0.014^{IV} + 0.007^{III} + 6.947^{II} + 0.014^{I}$

Genotype PL 419

Configuration	No. of Cells	% of cells
$I^{IV} + 5^{II}$	2	1.62
$6^{II} + 2^{I}$	1	0.81
7^{II}	120	97.56

Average chromosome configuration /Cell at Diakinesis/ Metaphase - $I = 0.016^{IV} + 6.959^{II} + 0.008^{I}$

Genotype PL 56

Configuration	No. of Cells	% of cells
$I^{IV} + 5^{II}$	2	1.71
7^{II}	115	98.29

Average chromosome configuration/Cell at Diakinesis/ Metaphase - $I = 0.017^{IV} + 6.965^{II}$

Genotype DL 70

Configuration	No. of Cells	% of cells
$I^{IV} + 5^{II}$	2	1.57
7^{II}	125	98.42

Average chromosome configuration/Cell at Diakinesis/ Metaphase - $I = 0.015^{IV} + 6.968^{II}$

Table 2: Chiasma frequencies per cell and per bivalent

Genotypes	Number of cells	Number of Chiasmata	Frequency per cell	Frequency per bivalent
PL426	130	1557	11.97	1.71
PL419	58	697	12.02	1.71
PL56	73	883	12.09	1.72
DL70	127	1486	11.70	1.67

observed in all genotypes but their average number per cell is quite low and varied from 0.014 to 0.017. Trivalents are rare and found only in one genotype *i.e.* PL 426 with its average number per cell 0.007. Univalents were observed also in a low frequency in PL 426 and PL 419 genotypes. Anaphase-bridges have been found in some cells but laggards are totally absent. The occurrence of anaphase-bridges may be due to false univalent or due to late disjunction of the largest chromosome pair (Ahmad & Chen, 2000).

A few cells had a fragment of chromosome at metaphase-I in addition to seven bivalents. These fragments were considered to be accessory chromosomes in these genotypes but their role was not clearly understood. However, the bivalents in the cells-bearing fragments were present in groups of three and four or two and five. Similar finding were also observed by Frost & Ising (1964). They found a low degree of pairing between the fragments and ordinary chromosomes. They suggested that a fragment can be originated from simple breakage followed by healing of the broken ends and such fragments might be a source of abnormalities from which accessory chromosomes can evolve.

The percentage of cells with chromosome irregularities is extensively low and may not be able to cause sterility in genotypes under investigation. The insignificant meiotic disorders result in high vitality pollen formation (Stoinova, 1994). Chiasma frequency is an indication of the degree of homology of the chromosomes and extent of hybridization (Table-2). Chiasma occurs spontaneously in the meiotic prophase when homologous chromosomes synapse during pachytene stage. Each chiasma is considered to be a result of single crossover.

Maximum chiasma frequency per cell and per bivalent is observed in PL 56 and minimum in DL 70 genotype. The average number of ring bivalents are maximum in PL 56 (5.09) and minimum in DL 70 (4.70) whereas rod bivalents varied from 1.69 (PL 56) to 2.29 (DL 70). This indicated that higher chiasma frequency is found in PL 56 genotype. Variations in chiasma frequency among four genotypes may be due to the differences in the frequency of ring and rod bivalents in them. Thus, the present study substantiated the findings of Redmann & Borgaonkar (1966).

The chiasma frequency per bivalent in all the genotypes is relatively same because all the genotypes are homozygous and stable. Thus the present study reveals a slight variation in chiasma frequency among four genotypes, which could also be influenced by the variation in date of fixation of spikes, because temperature is known to affect the chiasma frequency.

The present study corroborated the findings of Rees & Thompson (1956) and Dowrick (1957) as they suggested that variation in chiasma frequency was determined by the genotype and environmental conditions, whereas Jensen (1981) pointed out a day-to-day temperature as the major cause of variation in chiasma frequency.

Reddy *et al.* (1991) has interconnected the increase in univalent frequency and position of chiasmata with decline in chiasma frequency but in the present study, univalents are not very frequent so the position of chiasmata might be the reason for low chiasma frequency in DL 70 genotype. Variation in chiasma frequency may also be due to the environmental conditions as two out of the four genotypes were sown late. Jahan *et al.* (1992) suggested that negligible variation in chiasma frequency might occur due to the minor environmental changes.

Slight differences were observed among genotypes for cytological configuration particularly with bivalent shape, which may be due to stable homozygous constitution of the genotypes. The present study suggests that barley can be used as an ideal crop plant for studying cytogenetic analysis in relation to different environmental stresses. The chiasma frequency and extent of chromosomal configurations can be used as good indicators for studying ecologic changes in assessing effect of environmental pollutants on crop growth/ vegetation.

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POLYPOROID FUNGI OF NANDA DEVI BIOSPHERE RESERVE

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Abstract

Eighteen species of polyporoid fungi belonging to five families (Fomitopsidaceae, Ganodermataceae, Hymenochaetaceae, Polyporaceae and Steccherinaceae) and nine genera (*Daedalea, Ganoderma, Phellinus, Lenzites, Polyporus, Poria, Pycnoporus, Trametes and Irpex*) have been collected and taxonomically investigated from five localities (Reni, Lata, Juma, Suraithota and Malari) of the buffer zone (c 1800 – c 2300m) of Nanda Devi Biosphere Reserve (Uttaranchal).

Key words: polyporoid fungi, buffer zone and Nanda Devi Biosphere Reserve.

INTRODUCTION

Polyporoid fungi belonging to Basidiomycota are edible/ medicinally important, besides having important role to play in the forest ecosystem (Kirk *et al.* 2001). In spite of extreme studies conducted on taxonomy of polyporoid fungi of North Western Himalaya by Dhanda (1977), the area of Nanda Devi Biosphere Reserve remained unexplored. Till date not much is known regarding macrofungi of this region except for the works of Negi & Gadgil (1996), which pertains to lichenized fungi. The specimens cited in the text had been submitted to PAN (Herbarium, Botany Department, Panjab University, Chandigarh).

SURVEY AREA

Nanda Devi Biosphere Reserve (NDBR) was the second Biosphere Reserve to be established by the Govt. of India in 1988. It was declared as World heritage site by UNESCO in 1988. NDBR belongs to Himalayan high lands bio-geographic zonation of India. It is situated in 30°5' – 31°2' N Latitude and 79°12' -80°19' E Longitude in North Western Himalaya. It comprises of parts of Chamoli district (Garhwal Himalaya), Bageshwar and Pithoragarh districts (Kumaun Himalaya) in Uttaranchal state of India. The northern part of NDBR touches the Indo Tibetan border. It covers an area of 5,860.69 km² of which 5,148.57 km² area is under buffer zone inhabited by 47 villages, 546.34 km² area is under transition zone inhabited by 52 villages and 712.12 km² area is under core zone including the area of Valley of Flowers National Park. The core zone of the reserve is completely protected area. NDBR has a large altitudinal range (1,800 – 7,817 m amsl). The core zone of the reserve remains snow covered except from May to October. The main seasons of the area are winter, summer and rainy season. Winter extends from November to March with heavy snowfall between December to February. Summer extends from April to mid June. Rainy season extends from mid June to September. The average temperature ranges between –2.3°C to 31.7°C (Badola, 1998; Joshi *et al.* 1999 and Kala *et al.* 1998).

MATERIAL AND METHODS

The polyporoid fungi were collected from five localities (Reni, Lata, Juma, Suraithota and Malari) of the buffer zone (c 1800 – c 2300m) of Nanda Devi Biosphere Reserve in October, 2005. The field data such as texture, size, colour, smell, macroscopic features along with trophic level and form have been noted in the field after Mueller *et al.* (2004). The collected specimen were placed in polythene bags of suitable size, along with required data *viz.* collection number, details of locality, host/ substrate, approximate altitude in meters, date of collection and name of the lagator. The species have been described in detail using recent techniques. For each taxon, the following data has been recorded: name of the taxon with authority, morphological and anatomical descriptions of the taxa, various chemical tests, distribution in Nanda Devi Biosphere Reserve, PAN number, substrate, locality, approximate altitude of the locality in meters, date of collection and photograph of the specimen. The taxa described in the text have been arranged taxonomically after Kirk *et al.*(2001).

The various stains and mountants *viz.* Amann's Lactophenol, 2% Glycerine, 5% Potassium hydroxide , Melzer's Iodine , Erythrosine B in ammonia, 1% Phloxine and Cotton blue used for the taxonomic investigations were prepared after Kirk *et al.* (2001).

RESULTS

Taxonomic treatment.

Family – Fomitopsidaceae

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Daedalea stereoides Fr.

Nov. Symb. Mycol. **1** : 99, 1851

(PLATE 1, Fig. A; PLATE 4, Figs. A-E)

Basidiocarp annual, pileate, usually sessile with a narrow base, corky, 2.0-8.0 X 2.0-5.0 X 0.1-1.0 cm; basidia subclavate, thin walled, 4-sterigmate, 14.5-17.5 X 5.2-6.5 µm; basidiospores hyaline, thin walled, ellipsoid, 3.5-6.0 X 2.2-3.2 µm; cystidioles frequent, subclavate, hyaline to subhyaline, thick walled, 10.4-18.2 X 2.5-4.2 µm.

Distribution in NDBR – Reni, Juma and Suraithota.

Collection examined - PAN 28208, on gymnospermic tree, in forest, Reni, c 1850m, NDBR (U.A.), October 4, 2005, leg. Hem Chander. PAN 28261, on angiospermic tree, in forest, Juma, c 2150m, NDBR (U.A.), October 6, 2005, leg. Hem Chander. PAN 28283, on gymnospermic wood, in forest, Suraithota, c 2200m, NDBR (U.A.), October 7, 2005, leg. Hem Chander.

Remarks – The species is characterized by white pileus surface when immature, tobacco-brown when old, finely velutinate to glabrous, concentrically zonate with furrows, small raised areas composed of tufted hairs, margin even, acute; context concolorous with the pileus, upto 3 mm thick at the base; hymenial surface brown, pores daedaloid to labyrinthine towards the margin, irpicoid towards the base, 1-3 per mm, pore tubes light pink to brown, upto 6 mm long; hyphal system trimitic; generative hyphae hyaline to subhyaline, with conspicuous clamp connections, thin walled, branched, 1.5-4.5 µm wide; skeletal hyphae hyaline to pale brown, thick walled to solid, unbranched or apically forked, 1.6-6.5 µm wide; binding hyphae abundant, hyaline to pale brown, thick walled. This species is a new record for the study area.

Family – Ganodermataceae

Ganoderma lucidum (Leyss.-Curt.:Fr.) Karst.

Rev. Mycol. **3(9)** : 17, 1881

(PLATE 1, Fig. B; PLATE 4, Figs.F-J)

Basidiocarp perennial, stipitate, woody, 10-12 X 10-12 X 3-4 cm, stalk lateral, with laccate crust, upto 20 cm long; basidia hyaline, clavate, 4-sterigmate, 12.5-19.0 X 5.0-7.5 µm; basidiospores ganodermatoid, truncate, double walled, hyaline, cyanophilous, 9.2-12.2 X 6.5-9.0 µm.

Distribution in NDBR – Reni.

Collection examined - PAN 28187, on soil, in forest, Reni, c 1850m, NDBR (U.A.), October 4, 2005, leg. Hem Chander. PAN 28201, on soil, in forest, Reni, c 1850m, NDBR (U.A.), October 4, 2005, leg. Hem Chander.

Remarks- The species is characterized by shiny pileus surface with laccate crust, reddish-brown, smooth; hymenial surface cream coloured; context 2-9 mm, light brown; pores small, 4-6 per mm, brown, pores 90-245 µm in diam., pore tubes 6-7 µm long; hyphal system trimitic; generative hyphae thin walled, hyaline, with clamp connections, branched, cyanophilous, 3.0-2.0 µm in diam.; skeletal hyphae light brown, thick walled, lumen narrow, sparsely branched, 1.6-4.8 µm in diam.; binding hyphae pale-brown, thick walled, much branched, 3.2-4.8 µm in diam. This species is a new record for the study area.

Family - Hymenochaetaceae

Phellinus fastulosus (Lev.) Ryv.

Norw. J. Bot. **19(3&4)** : 234, 1972

(PLATE 1, Fig. C; PLATE 4, Figs. K-N)

Basidiocarp perennial, sessile, solitary or imbricate, rigid, applate, somewhat convex, 3.0-9.0 X 2.0-11.0 X 1.0-4.0 cm; basidia hyaline, clavate, 4-sterigmate, 8.6-12.7 X 5.5-11.2 µm; basidiospores brown to dark brown, ovoid to subglobose, smooth, thin walled, non-amyloid, 4.2-5.6 x 3.5-4.9 µm.

Distribution in NDBR – Lata and Suraithota.

Collection examined - PAN 28247, on angiospermic tree, in forest, Lata, c 1900m, NDBR (U.A.), October 5, 2005, leg. Hem Chander. PAN 28290, on angiospermic wood, in forest, Suraithota, c 2200m, NDBR (U.A.), October 7, 2005, leg. Hem Chander. PAN 28304, on gymnospermic wood, in forest, Suraithota, c 2200m, NDBR (U.A.), October 7, 2005, leg. Hem Chander.

Remarks- The species is characterized by yellowish brown to dark brown pileus surface, black in old specimens, compactly tomentose, concentrically sulcate, velvety, rimose in old parts; margin acute to blunt, rigid, entire, concolorous with pileus surface; hymenial surface yellowish brown to brown, even; pores rounded to elongated or angular, 6-8 per mm, pore tubes finely velutinate, stratified, brown, 1-3 mm deep; context yellowish brown, azonate, homogenous, xanthochronic, 1-2 mm thick, separated by a thin black line from tomentose on upper surface; hyphal system dimitic; generative hyphae thin walled, hyaline, septate, clamped, branched, 1.8-2.5 µm in diam.; skeletal hyphae yellowish brown, thick walled, rarely branched, aseptate or pseudoseptate, acyanophilous, 2.0-6.4 µm in diam. This

PLATE 1

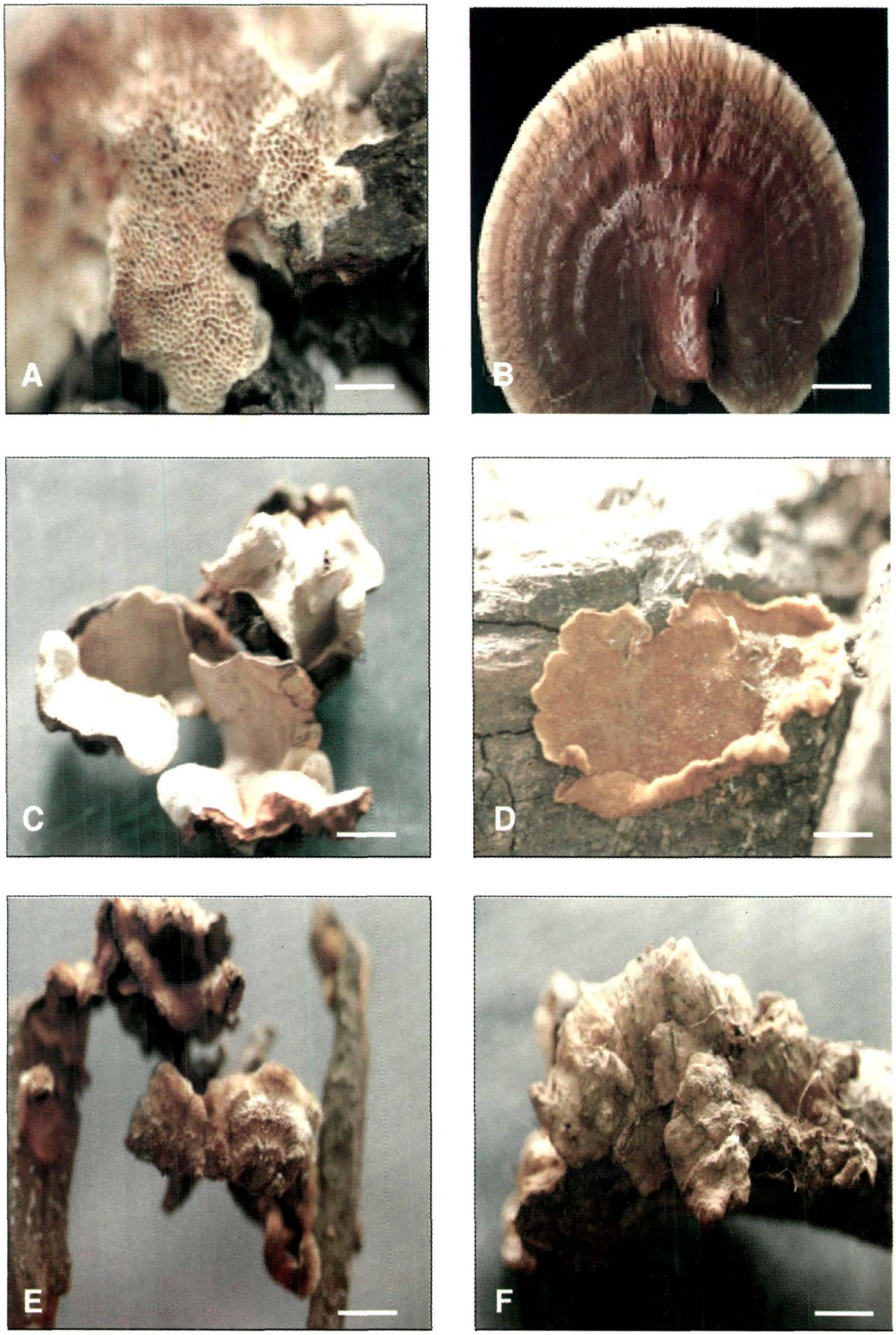

PLATE 1 (Scale = 1 cm) (Figs. A-F): (A) *Daedalea stereoides* Fr. (B) *Ganoderma lucidum* (Leyss.-Curt.:Fr.) Karst. (C) *Phellinus fastulosus* (Lev.) Ryv. (D) *Phellinus johnsonianus* (Murr.) Ryv. (E) *Phellinus merrillii* (Murr.) Ryv. (F) *Phellinus robustus* (Karst.) Bourd & Galz.

PLATE 2

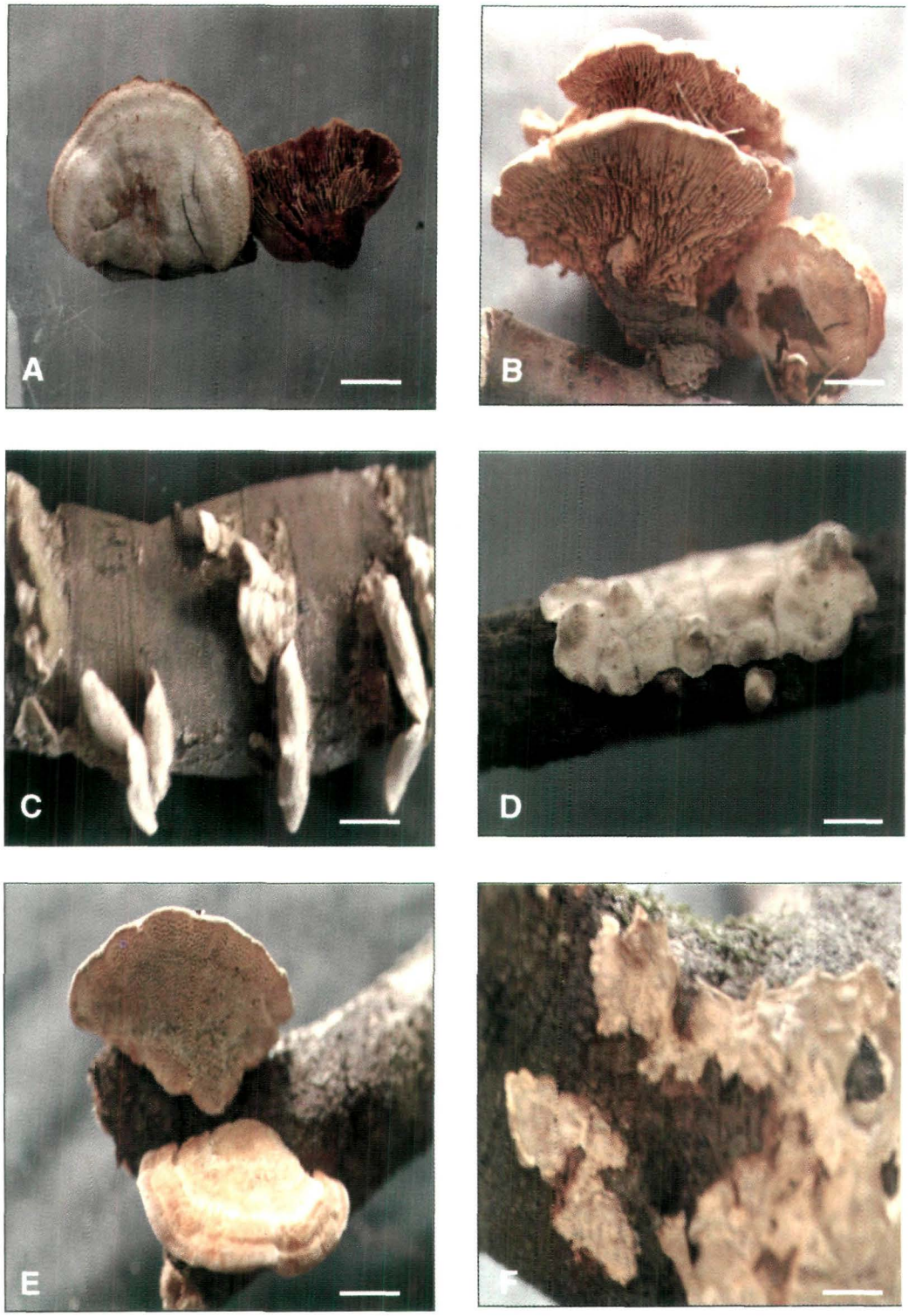

PLATE 2 (Scale = 1 cm) (Figs. A-F): (A) *Lenzites adustus* Mass. (B) *Lenzites betulina* (L.:Fr.) Fr. (C) *Polyporus biformis* Fr. (D) *Polyporus conchoides* (Mont.) Lioyd (E) *Polyporus hirsutus* Wulf. ex Fr. (F) *Poria fulviseda* Bres.

species is a new record for the study area.

Phellinus johnsonianus (Murr.) Ryv.

Bull. N.Z. Dep. Scient. Ind. Res. **164** : 238, 1965

(PLATE 1, Fig. D; PLATE 4, Figs. O-R)

Basidiocarp perennial, effused-reflexed, corky, yellowish brown, 3.0-6.0 X 6.4- 8.0 X 1.0-1.2 cm; basidia yellowish brown, clavate, 4-sterigmate, 5.6-10.9 X 4.5-7.9 µm; basidiospores pale brown, smooth, thin walled, apiculate, ellipsoid to ovoid, 3.5-9.2 X 2.8-3.0 µm.

Distribution in NDBR – Reni, Lata, Juma and Suraithota.

Collection examined - PAN 28222, on angiospermic tree, in forest, Reni, c 1850m, NDBR (U.A.), October 4, 2005, leg. Hem Chander. PAN 28246, on angiospermic tree, in forest, Lata, c 1900m, NDBR (U.A.), October 5, 2005, leg. Hem Chander. PAN 28279, on angiospermic wood, in forest, Juma, c 2150m, NDBR (U.A.), October 6, 2005, leg. Hem Chander. PAN 28297, on gymnospermic wood, in forest, Suraithota, c 2200m, NDBR (U.A.), October 7, 2005, leg. Hem Chander. PAN 28307, on angiospermic wood, in forest, Suraithota, c 2200m, NDBR (U.A.), October 7, 2005, leg. Hem Chander.

Remarks- The species is characterized by pileus surface yellowish brown to dark brown, glabrous, irregular, concentrically sulcate; margin blunt, entire, concolorous with pileus surface; hymenial surface dark brown, uneven; pores rounded, 6-7 per mm, pore tubes stratified, dark brown, 1-2 mm deep, tube openings velutinate; context yellowish brown, homogenous, xanthochronic, azonate, 0.5-1.0 mm thick; hyphal system dimitic; generative hyphae thin walled, sub-hyaline, septate, clamped, branched, 1.8-2.9 µm in diam.; skeletal hyphae pale brown, thick walled, unbranched, aseptate, 2.5-3.5 µm in diam.; Setae dark brown, thick walled with pointed apices, 16.0-18.0 X 7.0-11.2 µm. This species is a new record for the study area.

Phellinus merrillii (Murr.) Ryv.

Norw. J. Bot. **19** : 234, 1972

(PLATE 1, Fig. E; PLATE 4, Figs. S-V)

Basidiocarp perennial, sessile, attached by a small base, solitayry, hard, applanate, 7.0-8.0 X 6.0-8.0 X 2.0-3.0 cm; basidia yellowish brown, clavate, 4-sterigmate, 8.5-12.0 X 5.5-11.0 µm; basidiospores reddish brown, globose to sub-globose, thin walled, 2.0-4.0 X 3.5-6.0 µm.

Distribution in NDBR – Reni and Suraithota.

Collection examined - PAN 28189, on angiospermic tree, in forest, Reni, c 1850m, NDBR (U.A.), October 4, 2005, leg. Hem Chander. PAN 28294, on gymnospermic wood, in forest, Suraithota, c 2200m, NDBR (U.A.), October 7, 2005, leg. Hem Chander.

Remarks- The species is characterized by reddish brown, soft matted, velutinate, distinctly sulcate, faintly zonate pileus surface; margin round, velutinate, sterile; hymenial surface reddish brown; pores regular, round, 4-5 per mm, pore wall thick, pore tubes woody brown, stratified, 2-4 mm long; context brown, homogenous, xanthochronic, azonate, 0.5-1.0 mm thick; hyphal system dimitic; generative hyphae hyaline to pale brown, thin walled, septate, clamped, branched, 1.6-3.2 µm in diam.; skeletal hyphae pale brown to reddish brown, thick walled with broad lumen, rarely branched, aseptate, 1.6-3.2 µm in diam. This species is a new record for the study area.

Phellinus robustus (Karst.) Bourd & Galz.

Mym. France, p. 616, 1928

(PLATE 1, Fig. F; PLATE 4, Figs. W-Z)

Basidiocarp perennial, solitary, effused-reflexed, corky, pileus convex to ungulate, 7.0-8.0 X 10.0-15.0 X 4.0-6.0 cm; basidia yellowish brown, clavate, 4-sterigmate, 6.0-10.0 X 5.-9.0 µm; basidiospores sub-hyaline, globose, thin walled, smooth, dextrinoid, 3.8-5.9 X 6.5-8.3 µm.

Distribution in NDBR – Reni, Lata, Juma, Suraithota and Malari.

Collection examined - PAN 28209, on angiospermic tree, in forest, Reni, c 1850m, NDBR (U.A.), October 4, 2005, leg. Hem Chander. PAN 28231, on angiospermic tree, in forest, Lata, c 1900m, NDBR (U.A.), October 5, 2005, leg. Hem Chander. PAN 28232, on gymnospermic tree, in forest, Lata, c 1900m, NDBR (U.A.), October 5, 2005, leg. Hem Chander. PAN 28271, on angiospermic wood, in forest, Juma, c 2150m, NDBR (U.A.), October 6, 2005, leg. Hem Chander. PAN 28293, on gymnospermic wood, in forest, Suraithota, c 2200m, NDBR (U.A.), October 7, 2005, leg. Hem Chander. PAN 28301, on angiospermic wood, in forest, Malari, c 2300m, NDBR (U.A.), October 7, 2005, leg. Hem Chander.

Remarks- The species is characterized by yellowish brown to dark brown, glabrous, azonate pileus surface with even obtuse margin; hymenial surface dark brown, even, dull; pores rounded, 80-230 µm in diam., 4-5 per mm, pore tubes stratified, yellowish brown, 5-7 mm deep; context yellowish brown, homogenous, 5.0-8.0 mm thick; hyphal system dimitic; generative hyphae hyaline, thin walled, septate, clamped, sparsely branched, 2.0-3.0 µm

in diam.; skeletal hyphae brown, thick walled, unbranched, aseptate, acyanophilous, 2.8-5.6 µm in diam. This species is a new record for the study area.

Family – Polyporaceae

Lenzites adustus Mass.

Kew Bull. **11** : 250, 1910

(PLATE 2, Fig. A; PLATE 5, Figs. A-E)

Basidiocarp annual to perennial, dimidiate or attached by a broad base, usually singly, coriaceous when fresh, flexible when dry, 2.0-5.0 X 7.0-26.0 X 1.5-2.5 cm; margin sharp; basidia 4-sterigmate, clavate, hyaline, 11-13 X 3.5 µm; basidiospores ellipsoid-cylindrical, hyaline, thin walled, 5.0-8.0 X 2.0-3.5 µm.

Distribution in NDBR – Reni and Juma.

Collection examined - PAN 28213, on angiospermic wood, in forest, Reni, c 1850m, NDBR (U.A.), October 4, 2005, leg. Hem Chander. PAN 28269, on angiospermic tree, in forest, Juma, c 2150m, NDBR (U.A.), October 6, 2005, leg. Hem Chander.

Remarks- The species is characterized by white to cream, velutinate-glabrous, azonate to faintly concentrically zonate pileus surface with white to cream, 3-7 mm thick, corky context; hymenial surface cream coloured, lamellate, lamellae irregular towards the base, 10-12 per cm; hyphal system trimitic; generative hyphae hyaline, thin to thick walled to solid, branched, with clamp connections, 1.6-3.5 µm wide; skeletal hyphae hyaline to pale, thick walled to solid, unbranched, 3.5-5.0 µm wide; binding hyphae hyaline, thick walled to solid, with short branches, 2.5-4.0 µm wide. This species is a new record for the study area.

Lenzites betulina (L.:Fr.) Fr.

Epicr. Syst. Mycol., p. 405, 1838

(PLATE 2, Fig. B; PLATE 5, Figs. F-J)

Basidiocarp annual-perennial, dimidiate-semicircular, sessile, attached by a broad base or narrow contracted base, solitary or a few together and imbricate, 3.0-12.0 X 1.0-7.0 X 0.2-2.0 cm; margin acute, entire; basidia 4-sterigmate, clavate, hyaline, 10.0-15.0 X 3.5-5.5 µm; basidiospores ellipsoid-cylindric, hyaline, thin walled, smooth, 5.0-8.0 X 3.0-4.5 µm.

Distribution in NDBR – Reni, Lata, Juma and Malari.

Collection examined - PAN 28210, on angiospermic wood, in forest, Reni, c 1850m, NDBR (U.A.), October 4, 2005, leg. Hem Chander. PAN 28236, on angiospermic wood, in forest, Lata, c 1850m, NDBR (U.A.), October 5, 2005, leg. Hem Chander. PAN 28265, on angiospermic tree, in forest, Juma, c 2150m, NDBR (U.A.), October 6, 2005, leg. Hem Chander. PAN 28322, on angiospermic tree, in forest, Malari, c 2300m, NDBR (U.A.), October 8, 2005, leg. Hem Chander.

Remarks- The species is characterized by white to cream pileus surface, darkening on drying, 1-5 mm thick; hymenial surface white when fresh, browning on drying, lamellate, lamellae 10-15 per cm; hyphal system trimitic; generative hyphae hyaline, thin-thick walled, clamped, branched, 1.6-3.8 µm wide; skeletal hyphae hyaline, thick walled, unbranched, 3-6 µm wide; binding hyphae hyaline, thick walled, much branched, with long and short branches, 1.5-2.5 µm wide. This species is a new record for the study area.

Polyporus biformis Fr.

Ann. Mag. Hist. **3** : 392, 1839

(PLATE 2, Fig. C; PLATE 5, Figs. K-N)

Basidiocarp annual, sessile, resupinate, effused-reflexed, imbricate, soft, coriaceous when fresh, corky when dry, tomentose when fresh, with dark appressed radiating fibrils and nearly glabrous when dry, azonate to subzonate, context fibrous, white to pale yellow, 0.2-0.5 cm, angular or daedaloid, 1-2 per mm; hyphal system dimitic; generative hyphae hyaline, thin walled, branched, septate with clamp connections, 3.0-4.0 µm wide; skeletal hyphae hyaline, thick walled with lumen narrow or obliterated, rarely branched, 3-6 µm broad, abundant; basidia 4-sterigmate, clavate, hyaline, 10.0-15.0 X 3.5-5.5 µm; basidiospores cylindrical to slightly curved, apiculate, hyaline, smooth, 5.5-7.8 X 2.0-2.5 µm.

Distribution in NDBR – Reni.

Collection examined - PAN 28211, on angiospermic tree, in forest, Reni, c 1850m, NDBR (U.A.), October 4, 2005, leg. Hem Chander.

Remerks- The specimen resembles the type in characteristic features and is a new record for the study area.

Polyporus conchoides (Mont.) Lioyd

Synop. Apus, : 331, 1915

(PLATE 2, Fig. D; PLATE 5, Figs. O-R)

Basidiocarp annual, effused-reflexed, sessile, imbricate, flexible when fresh, hard on drying, 3-5 X 2-4 x 0.1 cm;

PLATE 3

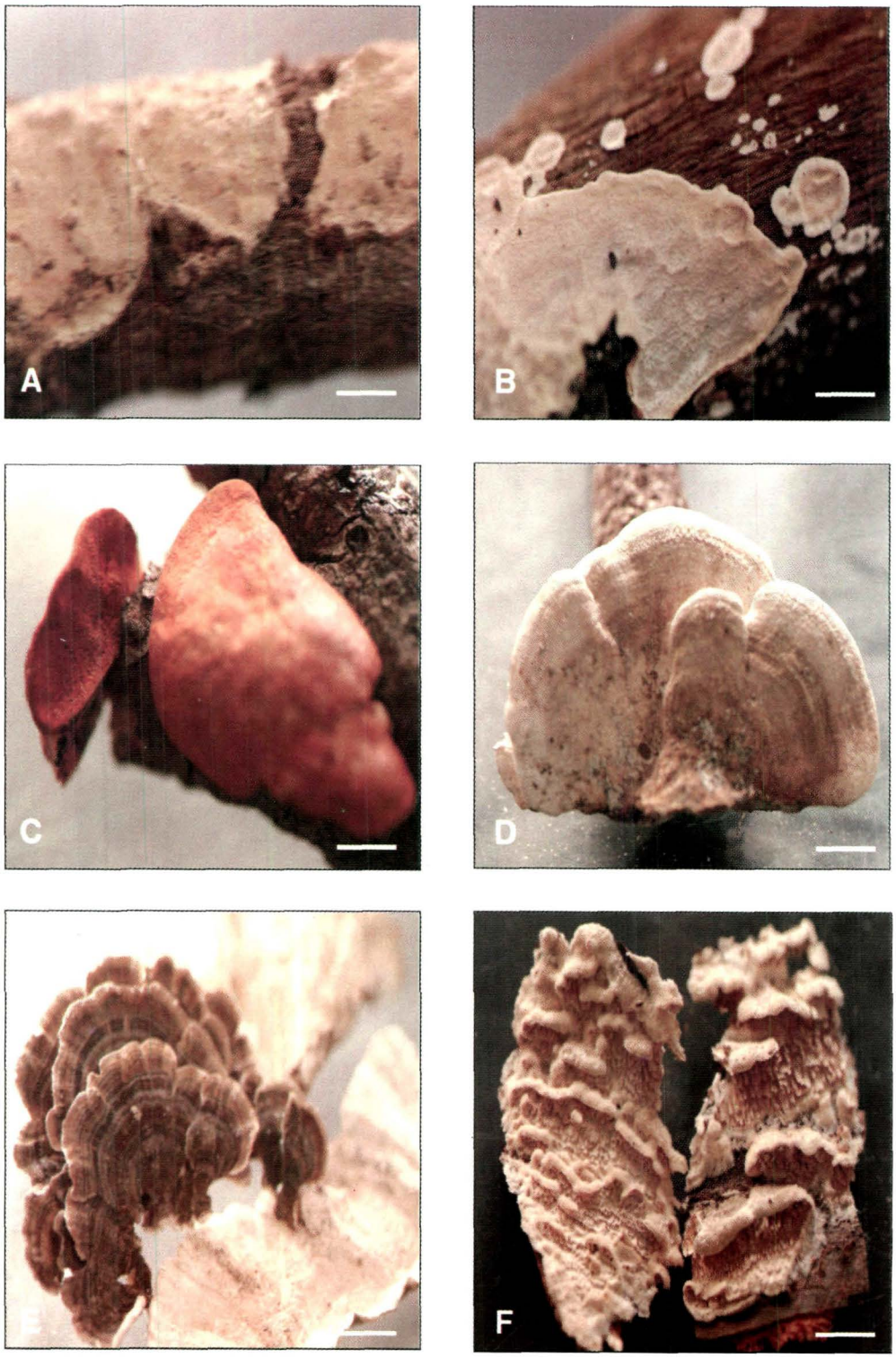

PLATE 3 (Scale = 1 cm) (Figs. A-F): (A) *Poria lenta* Overh. & Lowe. (B) *Poria leucoplaca* (Berk.) Cooke (C) *Pycnoporus coccineus* (Fr.) Bond. & Sing.(D) *Trametes gibbosa* (Pers.:Fr.) Fr. (E) *Trametes versicolor* (L.:Fr.) Pil. (F) *Irpex zonatus* Berk.

PLATE 4

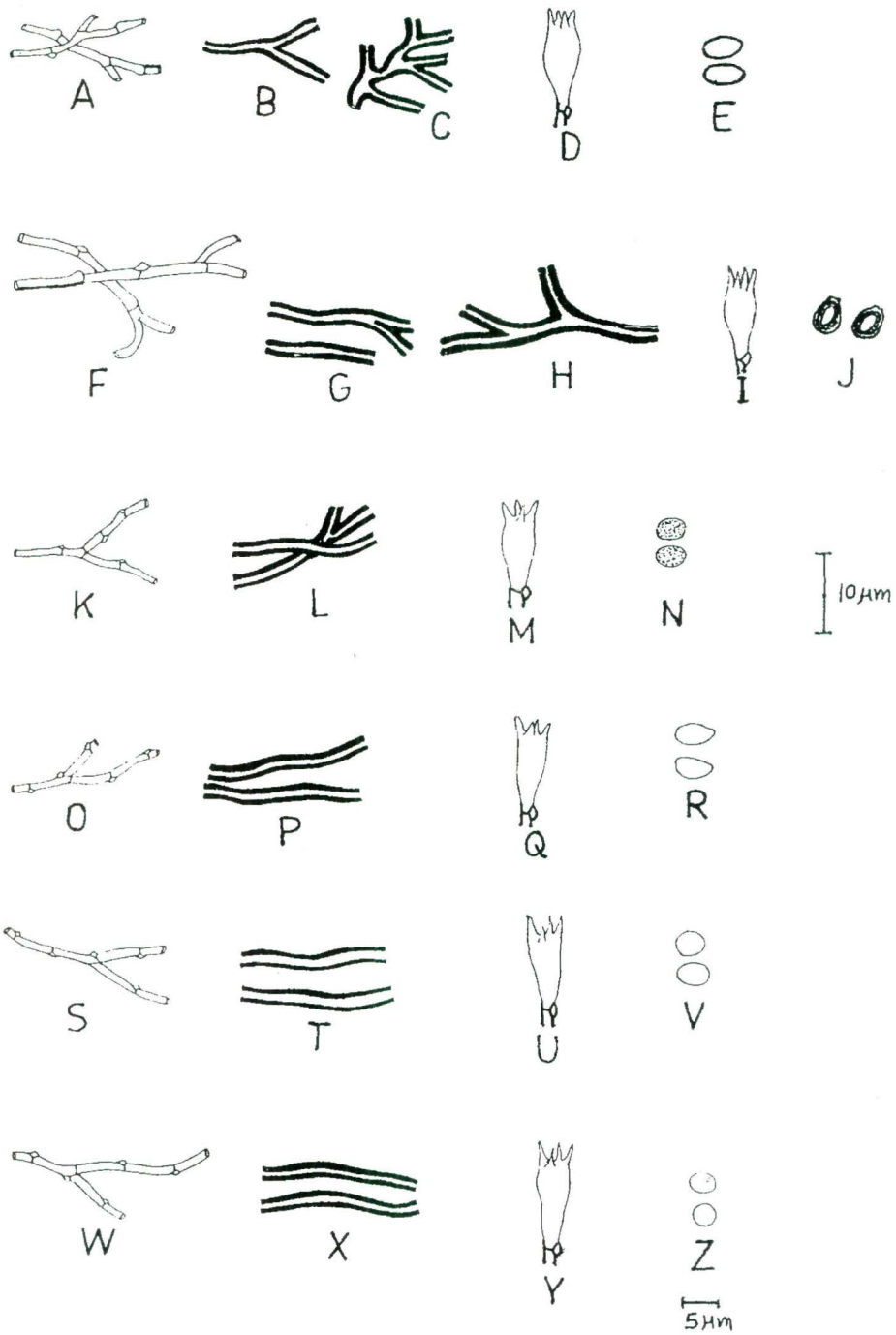

PLATE 4 : ***Daedalea stereoides*** Fr. **(Figs. A-E):** (A) Generative hypha(B) Skeletal hypha (C) Binding hypha (D) Basidium (E) Basidiospores; (**Figs. F-J**): ***Ganoderma lucidum*** (Leyss.-Curt.:Fr.) Karst. (F) Generativehyphae (G) Skeletal hyphae (H) Binding hypha (I) Basidium (J) Basidiospores; **(Figs. K-N)**: ***Phellinus fastulosus*** (Lev.) Ryv. (K) Generative hypha (L) Skeletal hyphae (M) Basidium (N) Basidiospores; **(Figs. O-R)**: ***Phellinus johnsonianus*** (Murr.) Ryv. (O) Generative hypha (P) Skeletal hyphae (Q) Basidium (R) Basidiospores; **(Figs. S-V)**: ***Phellinus merrillii*** (Murr.) Ryv. (S) Generative hypha (T) Skeletal hyphae (U) Basidium (V) Basidiospores; **(Figs. W-Z)**: ***Phellinus robustus*** (Karst.) Bourd & Galz.(W) Generative hypha (X) Skeletal hyphae (Y) Basidium (Z) Basidiospores

basidia 4-sterigmate, clavate, hyaline, 9.0-14.0 X 3.5-5.0 µm; basidiospores cylindrical, hyaline, smooth, allantoid, 4.0-5.5 X 1.0-1.2 µm.

Distribution in NDBR – Lata, Suraithota and Malari.

Collection examined - PAN 28240, on angiospermic tree, in forest, Lata, c 1900m, NDBR (U.A.), October 5, 2005, leg. Hem Chander. PAN 28257, on gymnospermic tree, in forest, Lata, c 1900m, NDBR (U.A.), October 5, 2005, leg. Hem Chander. PAN 28306, on gymnospermic tree, in forest, Suraithota, c 2200m, NDBR (U.A.), October 7, 2005, leg. Hem Chander. PAN 28323, on angiospermic wood, in forest, Malari, c 2300m, NDBR (U.A.), October 7, 2005, leg. Hem Chander.

Remarks- The species is characterized by pileus surface white when fresh turning to light brown on drying, tomentose, fibrillose, rugulose, azonate, margin thin, incurved; context white, 0.5-1.5 mm thick; hymenial surface yellowish brown, separable from context, waxy, rugulose, pores 6-8 per mm, round, pore tubes 0.1-0.3 mm long; hyphal system dimitic; generative hyphae hyaline, branched, septate with clamp connections 3-4 µm in diam.; skeletal hyphae hyaline, thick walled with narrow lumen, rarely branched, 3-6 µm in diam. This species is a new record for the study area.

Polyporus hirsutus Wulf. ex Fr.

Sys. Mycol. **1** : 367,1821

(PLATE 2, Fig. E; PLATE 5, Figs. S-V)

Basidiocarp annual, sessile or effused-reflexed, imbricate, dimidiate, applanate or fan shaped, sometimes reniform, coriaceous when fresh, hard on drying, 2-5 X 3-8 X 0.15-1.3 cm; basidia 4-sterigmate, clavate, 11.0-15.0 X 4.0-5.5 µm; basidiospores cylindrical-straight to slightly curved, hyaline, smooth, 4.5-6.0 X 1.5-2.4 µm, hyphal pegs present.

Distribution in NDBR – Reni, Lata, Juma, Suraithota and Malari.

Collection examined - PAN 28196, on angiospermic wood, in forest, Reni, c 1850m, NDBR (U.A.), October 4, 2005, leg. Hem Chander. PAN 28204, on angiospermic wood, in forest, Reni, c 1850m, NDBR (U.A.), October 4, 2005, leg. Hem Chander. PAN 28235, on gymnospermic wood, in forest, Lata, c 1900m, NDBR (U.A.), October 5, 2005, leg. Hem Chander. PAN 28253, on gymnospermic tree, in forest, Lata, c 1900m, NDBR (U.A.), October 5, 2005, leg. Hem Chander. PAN 28254, on angiospermic wood, in forest, Lata, c 1900m, NDBR (U.A.), October 5, 2005, leg. Hem Chander. PAN 28256, on gymnospermic tree, in forest, Lata, c 1900m, NDBR (U.A.), October 5, 2005, leg. Hem Chander. PAN 28275, on gymnospermic tree, in forest, Juma, c 2150m, NDBR (U.A.), October 6, 2005, leg. Hem Chander. PAN 28278, on gymnospermic tree, in forest, Lata, c 1900m, NDBR (U.A.), October 5, 2005, leg. Hem Chander. PAN 28291, on angiospermic wood, in forest, Suraithota, c 2200m, NDBR (U.A.), October 7, 2005, leg. Hem Chander. PAN 28325, on gymnospermic wood, in forest, Malari, c 2300m, NDBR (U.A.), October 8, 2005, leg. Hem Chander. PAN 28329, on angiospemic wood, in forest, Malari, c 2300m, NDBR (U.A.), October 8, 2005, leg. Hem Chander.

Remarks- The species is characterized by pileus surface yellowish to yellowish brown, sometimes greyish, coarsely hirsute to velvety tomentose, concentrically zonate, margin even, sometimes incurved on drying; context white to light yellow, floccose, 0.1-1.0 cm, thick at the base; hymenial surface white when fresh, light yellow to yellowish brown on drying; pores regular, 2-3 per mm, pore tubes 2-3 mm long; hyphal system dimitic; generative hyphae hyaline, thin walled, branched, septate with clamp connections, 1.5-3.0 µm in diam.; skeletal hyphae arboriform, hyaline, thick walled, much branched, aseptate, 2.0-3.0 µm in diam. This species is a new record for the study area.

Poria fulviseda Bres.

Accad. Rover. Agiati. Atti. **3** : 84, 1897

(PLATE 2, Fig. F; PLATE 5, Figs. W-Z)

Basidiocarp annual to perennial, resupinate, soft when fresh becoming brittle on drying, 6 X 3 cm, 4-6 mm thick; margin fibrillose to rhizomorphic, adnate, concolorous; basidia clavate, hyaline, 4-sterigmate, 12.0-15.0 X 5.0-8.0 µm; basidiospores ellipsoid to ovoid, minutely apiculate, truncate at the apex, thin walled, dextrinoid, acyanophilous, 3.5-5.0 X 2.8-4.2 µm.

Distribution in NDBR – Reni, Lata, Juma, Suraithota and Malari.

Collection examined - PAN 28219, on angiospermic tree, in forest, Reni, c 1850m, NDBR (U.A.), October 4, 2005, leg. Hem Chander. PAN 28252, on gymnospermic tree, in forest, Lata, c 1900m, NDBR (U.A.), October 5, 2005, leg. Hem Chander. PAN 28259, on gymnospermic tree, in forest, Lata, c 1900m, NDBR (U.A.), October 5, 2005, leg. Hem Chander. PAN 28280, on gymnospermic tree, in forest, Juma, c 2150m, NDBR (U.A.), October 6, 2005, leg. Hem Chander. PAN 28300, on angiospermic tree, in forest, Suraithota, c 2200m, NDBR (U.A.), October 7, 2005, leg. Hem Chander. PAN 28318, on angiospermic

PLATE 5

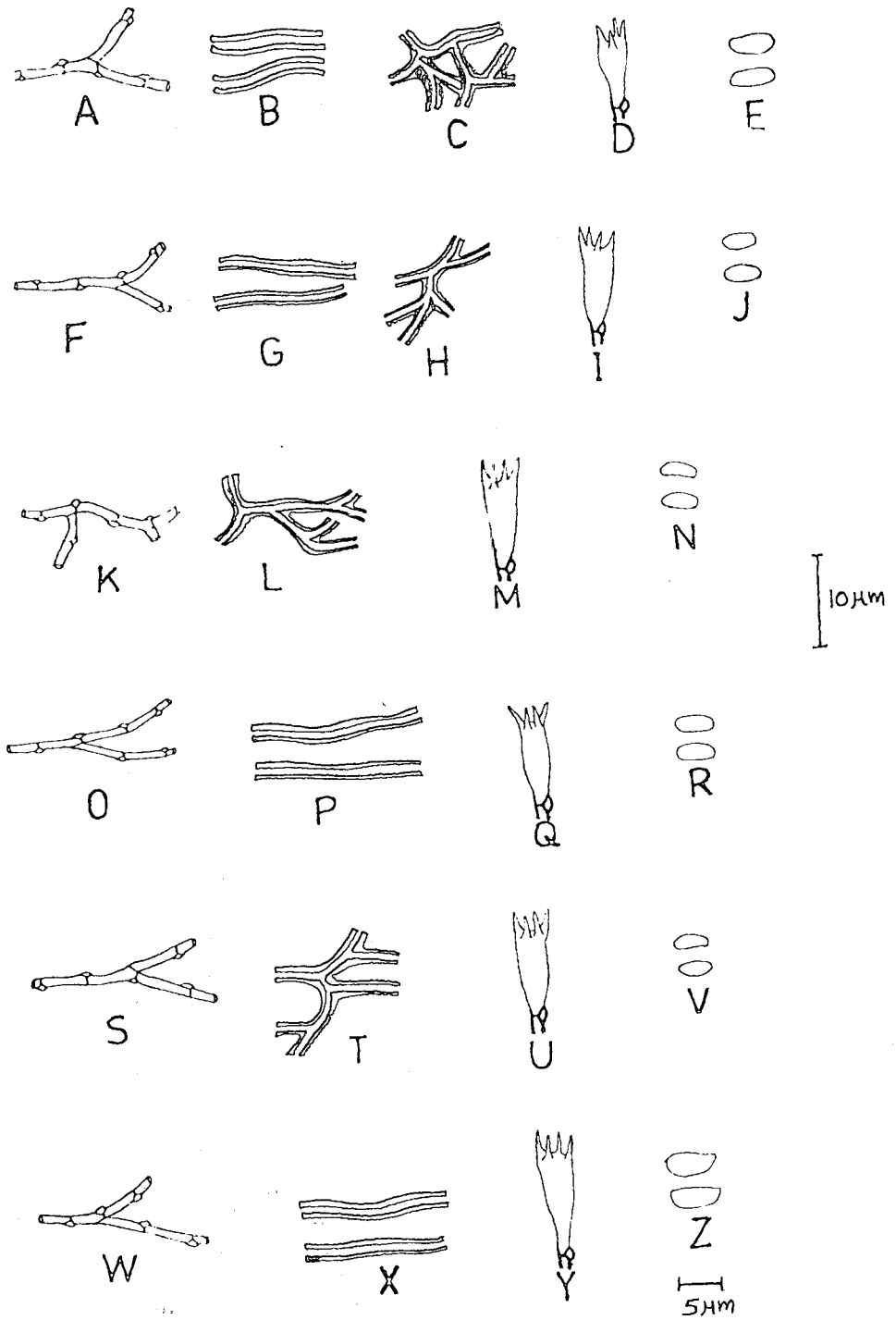

PLATE 5 (Figs. A-E): ***Lenzites adustus*** Mass. (A) Generative hypha (B) Skeletal hyphae (C) Binding hypha (D) Basidium (E) Basidiospores **(Figs. F-J)*Lenzites betulina*** (L.:Fr.) Fr. (F) Generative hypha (G) Skeletal hyphae (H) Binding hypha (I) Basidium (J) Basidiospores; **(Figs. K-N)*Polyporus biformis*** Fr. (K) Generative hypha (L) Skeletal hypha (M) Basidium (N) Basidiospores; **(Figs. O-R)** ***Polyporus conchoides*** (Mont.) Lioyd (O) Generative hypha (P) Skeletal hyphae (Q) Basidium (R) Basidiospores; **(Figs. S-V)*Polyporus hirsutus*** Wulf. ex Fr. (S) Generative hypha (T) Skeletal hypha (U) Basidium (V) Basidiospores; **(Figs. W-Z)** ***Poria fulviseda*** Bres. (W) Generative hypha (X) Skeletal hyphae (Y) Basidium (Z) Basidiospores

tree, in forest, Malari, c 2300m, NDBR (U.A.), October 7, 2005, leg. Hem Chander.

Remarks- The species is characterized by hymenial surface yellowish brown when fresh and yellowish on drying, smooth, glaucous, pores stratose, pore opening angular to oval, 75-150 µm in diam., 6-8 per mm; dissepiments 25-75 µm thick, even; context 5 mm wide, creamish yellow, composed of compactly arranged hyphae; hyphal system dimitic; generative hyphae hyaline, acyanophilous, non-dextrinoid, branched, septate with clamp connections, thin walled, 1.5-2.9 µm in diam.; skeletal hyphae cyanophilous, thick walled, aseptate, sparsely branched, 1.5-3.5 µm in diam. This species is a new record for the study area.

Poria lenta Overh. & Lowe.

Mycologia **38** : 210, 1946

(PLATE 3, Fig. A; PLATE 6, Figs. A-D)

Basidiocarp annual to perennial, resupinate to effused, 8 X 3 cm, 2-3 mm thick; adnate to separable; margin white; cystidia absent; basidia clavate, hyaline, 2-4 sterigmate, 12.5-14.0 X 5.0-7.5 µm; basidiospores hyaline to sub-hyaline, ellipsoid to obovoid, apiculate, apiculus small, some uniguttulate, non-amyloid, 4-5.5X2.5-3.0 µm.

Distribution in NDBR – Lata.

Collection examined - PAN 28228, on gymnospermic tree, in forest, Lata, c 1900m, NDBR (U.A.), October 5, 2005, leg. Hem Chander. PAN 28230, on angiospermic tree, in forest, Lata, c 1900m, NDBR (U.A.), October 5, 2005, leg. Hem Chander.

Remarks- The species is characterized by hymenial surface white to creamish when fresh becoming creamish yellow on drying, uneven; pores rounded to angular, 407-949 µm in diam., 1-2 per mm, pore tubes 3.0-5.0 mm deep, non-stratified; dissepiments 68-163 µm thick, equal, composed of compact parallel hyphae, finely velutinate; context 3-5 mm wide, white, soft; hyphal system dimitic; generative hyphae hyaline, cyanophilous, branched, septate with clamp connections, thin walled, 2.9-6.0 µm in diam.; skeletal hyphae, acyanophilous, thick walled, aseptate, sparsely branched, 2.1-6.5 µm in diam. This species is a new record for the study area.

Poria leucoplaca (Berk.) Cooke

Grevilea **14** : 113, 1886

(PLATE 3, Fig. B; PLATE 6, Figs. E-H)

Basidiocarp annual, resupinate, inseparable, coriaceous, 9.0 X 3.5 cm, 0.5-2 mm thick; margin white, 0.5-1.0 mm thick; basidia clavate, hyaline, 4-sterigmate, 12.0-14.0 X 5.5-7.0 µm; basidiospores hyaline, oblong-ellipsoid, smooth, apiculate, non-amyloid, 9.0-12.0 X 4.5-5.5 µm.

Distribution in NDBR – Reni, Lata and Suraithota.

Collection examined - PAN 28198, on angiospermic tree, in forest, Reni, c 1850m, NDBR (U.A.), October 4, 2005, leg. Hem Chander. PAN 28212, on gymnospermic tree, in forest, Reni, c 1850m, NDBR (U.A.), October 4, 2005, leg. Hem Chander. PAN 28220, on angiospermic tree, in forest, Reni, c 1850m, NDBR (U.A.), October 4, 2005, leg. Hem Chander. PAN 28260, on gymnospermic tree, in forest, Lata, c 1900m, NDBR (U.A.), October 5, 2005, leg. Hem Chander. PAN 28281, on angiospermic tree, in forest, Suraithota, c 2200m, NDBR (U.A.), October 7, 2005, leg. Hem Chander.

Remarks- The species is characterized by hymenial surface deep creamish to creamish brown, dull, pores angular to oval, 200-300 µm in diam., often oblique, 2-3 per mm, pore opening velutinate, pore tubes 0.5-1 mm deep, non-stratified; dissepiments 50-175 µm thick, composed of compact agglutinate hyphae; context 0.5-1 mm wide, creamish, homogenous, azonate; hyphal system dimitic; generative hyphae hyaline, branched, septate with clamp connections, thin walled, 2.9-6.0 µm in diam.; skeletal hyphae, hyaline, thick walled, aseptate, branched, 2-4 µm in diam. This species is a new record for the study area.

Pycnoporus coccineus (Fr.) Bond. & Sing.

Ann. Mycol. 39, **59**, 1941

(PLATE 3, Fig. C; PLATE 6, Figs. I-M)

Basidiocarp annual, sessile, broadly attached or effused-reflexed, dimidiate, 5 x 12 X 1 cm; basidia 4-sterigmate, clavate, hyaline, 8.0-12.8 X 4.8-6.4 µm; basidiospores hyaline, thin walled, short cylindric, smooth, slightly curved, 4.0-5.2 X 2.0-2.2 µm; hyphal pegs present.

Distribution in NDBR – Suraithota.

Collection examined - PAN 28285, on gymnospermic wood, in forest, Suraithota, c 2200m, NDBR (U.A.), October 7, 2005, leg. Hem Chander.

Remarks- The species is characterized by azonate pileus surface, soft velvety and chamois like, orange; context 4.0-7.5 mm thick, zonate with alternative whitish and orange zones; hymenial surface orange red, pores 6-8 per mm, pore tubes 1.5-2.5 µm long; hyphal system trimitic; generative hyphae hyaline, thin walled, clamped,

PLATE 6

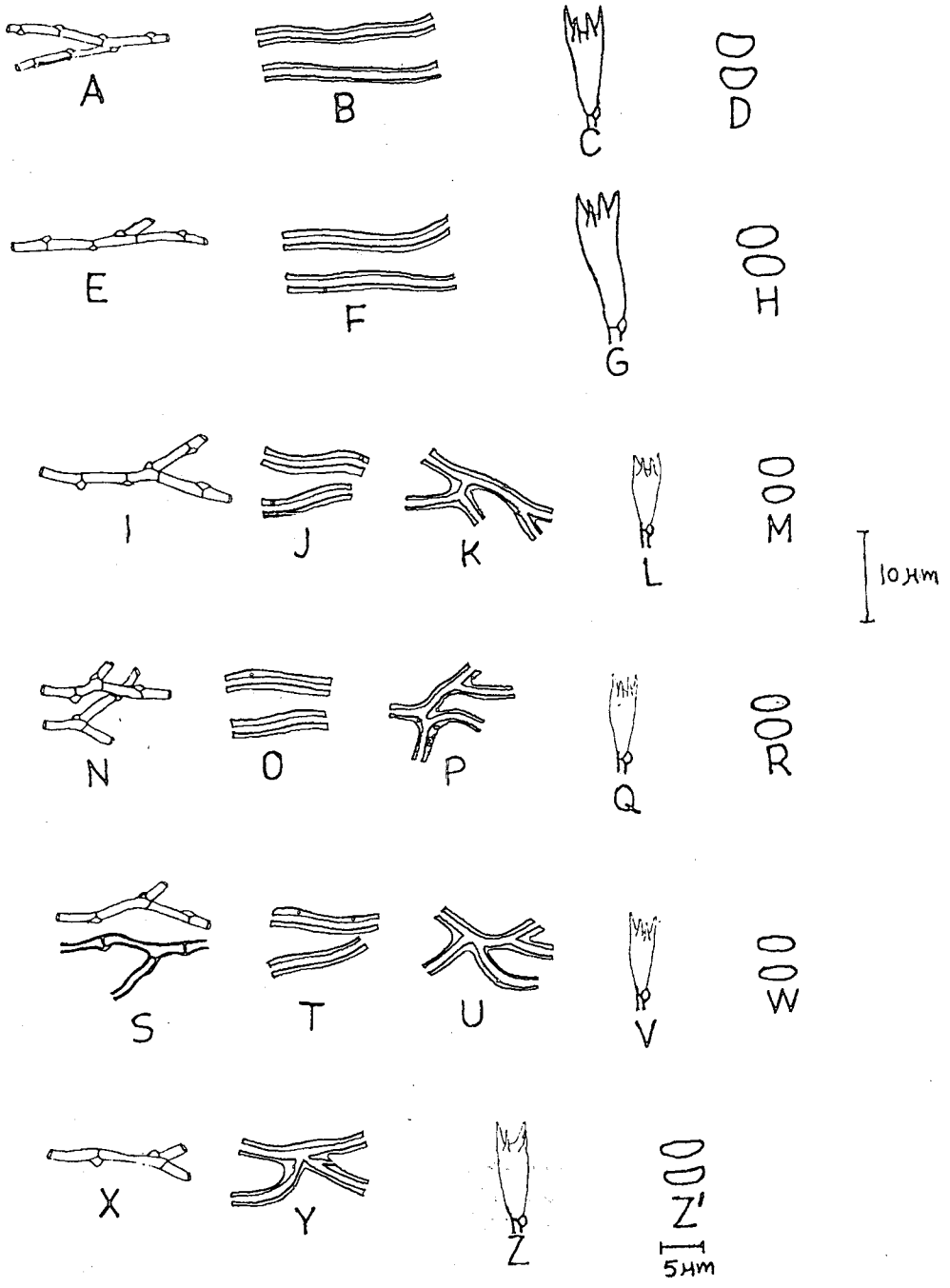

PLATE 6 (Figs. W-Z): ***Poria lenta*** Overh. & Lowe (A) Generative hypha (B) Skeletal hyphae (C) Basidium (D) Basidiospores **(Figs. E-H)** ***Poria leucoplaca*** (Berk.) Cooke (E) Generative hypha (F) Skeletal hyphae (G) Basidium (H) Basidiospores; **(Figs. I-M):** ***Pycnoporus coccineus*** (Fr.) Bond. & Sing. (I) Generative hypha (J) Skeletal hyphae (K) Binding hypha (L) Basidium (M) Basidiospores; **(Figs. N-R):** ***Trametes gibbosa*** (Pers.:Fr.) Fr. (N) Generative hypha (O) Skeletal hyphae(P) Binding hypha (Q) Basidium (R) Basidiospores; **(Figs. S-W) :*Trametes versicolor*** (L.:Fr.) Pil. (S) Generative hypha (T) Skeletal hyphae(U) Binding hypha (V) Basidium (W) Basidiospores; **(Figs. X-Z'):** ***Irpex zonatus*** Berk.(X) Generative hypha (Y) Skeletal hypha (Z) Basidium(Z') Basidiospores

2.5-3.5 µm wide; skeletal hyphae hyaline, thick walled, covered with orange granules, 3.5-6.5 µm wide; binding hyphae hyaline, thick walled to solid, much branched, 3.5-4.5 µm wide. This species is a new record for the study area.

Trametes gibbosa (Pers.:Fr.) Fr.

Epicr. Syst. Mycol., p 492, 1838

(PLATE 3, Fig. D; PLATE 6, Figs. N-R)

Basidiocarp annual, sessile, applanate, single, 8-12 X 5-12 X 1-2 cm; basidia 4-sterigmate, clavate, 14-16 X 4-5 µm; basidiospores hyaline, thin walled, subcylindrical, 3.5-4.5 X 2.0-2.5 µm.

Distribution in NDBR – Reni, Lata, Suraithota and Malari.

Collection examined - PAN 28190, on angiospermic wood, in forest, Reni, c 1850m, NDBR (U.A.), October 4, 2005, leg. Hem Chander. PAN 28192, on angiospermic wood, in forest, Reni, c 1850m, NDBR (U.A.), October 4, 2005, leg. Hem Chander. PAN 28255, on gymnospermic wood, in forest, Lata, c 1900m, NDBR (U.A.), October 5, 2005, leg. Hem Chander. PAN 28286, on gymnospermic wood, in forest, Suraithota, c 2200m, NDBR (U.A.), October 7, 2005, leg. Hem Chander. PAN 28310, on angiospermic wood, in forest, Malari, c 2300m, NDBR (U.A.), October 8, 2005, leg. Hem Chander.

Remarks- The species is characterized by pileus surface white when fresh, turning yellowish on drying, pubescent to tomentose, soft, delicately zonate, margin even, thick; context white, soft when fresh, corky on drying, 0.5-1.5 cm, rounded near the margin, pore tubes light brown, 0.3-0.9 cm long; hyphal system trimitic; generative hyphae hyaline, thin walled, clamped, branched, 2-3 µm wide; skeletal hyphae hyaline, thick walled, flexuous, unbranched, 3.5-6.5 µm wide; binding hyphae hyaline, thick walled, branched, flexuous, 2-3 µm wide. This species is a new record for the study area.

Trametes versicolor (L.:Fr.) Pil.

Atl. Champ. Eur. **3** : 261, 1939

(PLATE 3, Fig. E; PLATE 6, Figs. S-W)

Basidiocarp annual, sessile or effused-reflexed, thin, substipitate, usually imbricate, dimidiate to applanate, coriaceous when fresh, rigid on drying, 2.0-6.0 X 3.0-8.0 X 0.1-0.5 cm; basidia 4-sterigmate, clavate, 10.0-15.0 X 3.5-4.5 µm; basidiospores hyaline, subcylindric, thin walled, smooth, slightly curved on one side, 5.0-6.0 X 1.5-2.0 µm; hyphal pegs present.

Distribution in NDBR – Reni, Lata, Juma, Suraithota and Malari.

Collection examined - PAN 28197, on angiospermic wood, in forest, Reni, c 1850m, NDBR (U.A.), October 4, 2005, leg. Hem Chander. PAN 28199, on gymnospermic wood, in forest, Reni, c 1850m, NDBR (U.A.), October 4, 2005, leg. Hem Chander. PAN 28207, on angiospermic wood, in forest, Reni, c 1850m, NDBR (U.A.), October 4, 2005, leg. Hem Chander. PAN 28217, on angiospermic wood, in forest, Reni, c 1850m, NDBR (U.A.), October 4, 2005, leg. Hem Chander. PAN 28218, on angiospermic wood, in forest, Reni, c 1850m, NDBR (U.A.), October 4, 2005, leg. Hem Chander. PAN 28234, on gymnospermic wood, in forest, Lata, c 1900m, NDBR (U.A.), October 5, 2005, leg. Hem Chander. PAN 28245, on gymnospermic wood, in forest, Lata, c 1900m, NDBR (U.A.), October 5, 2005, leg. Hem Chander. PAN 28250, on gymnospermic wood, in forest, Lata, c 1900m, NDBR (U.A.), October 5, 2005, leg. Hem Chander. PAN 28268, on gymnospermic wood, in forest, Juma, c 2150m, NDBR (U.A.), October 6, 2005, leg. Hem Chander. PAN 28287, on gymnospermic wood, in forest, Suraithota, c 2200m, NDBR (U.A.), October 7, 2005, leg. Hem Chander.PAN 28288, on gymnospermic wood, in forest, Suraithota, c 2200m, NDBR (U.A.), October 7, 2005, leg. Hem Chander. PAN 28303, on gymnospermic wood, in forest, Suraithota, c 2200m, NDBR (U.A.), October 7, 2005, leg. Hem Chander. PAN 28309, on angiospermic wood, in forest, Malari, c 2300m, NDBR (U.A.), October 8, 2005, leg. Hem Chander. PAN 28321, on angiospermic wood, in forest, Malari, c 2300m, NDBR (U.A.), October 8, 2005, leg. Hem Chander. PAN 28326, on angiospermic wood, in forest, Malari, c 2300m, NDBR (U.A.), October 8, 2005, leg. Hem Chander. PAN 28330, on angiospermic wood, in forest, Malari, c 2300m, NDBR (U.A.), October 8, 2005, leg. Hem Chander.

Remarks- The species is characterized by pileus surface of various shades of yellowish, yellow brown, greyish brown, concentrically zonate with multicoloured zones, velvety tomentose, becoming glabrous at places and sometimes on entire surface, margin thin, incurved on drying; context white to pale, pores circular, 3-4 per mm, pore tubes 0.5-2.0 mm long; hyphal system trimitic; generative hyphae hyaline, thin walled, clamped, branched, 2.2-3.5 µm wide, sometimes thick walled, pale brown, 2.2-3.0 µm wide and occur in closely compact condition in brown zones on the pileus surface; skeletal hyphae hyaline, thick walled, unbranched, straight, aseptate, 3.5-8.0 µm wide; binding hyphae hyaline, thick walled, branched, 2.2-3.2 µm wide. This species is anew record for the study area.

Family – Steccherinaceae

Irpex zonatus Berk.

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(PLATE 3, Fig. F; PLATE 6, Figs. X-Z')

Basidiocarp annual, effused-reflexed to pileate, sessile, imbricate, coriaceous when fresh becoming corky on drying; basidia 4-sterigmate, clavate, thin walled, 8-12 x 3.0-3.5 µm; basidiospores hyaline, thin walled, cylindric-ellipsoid, 4.0-5.5 X 2.0-2.5 µm.

Distribution in NDBR – Reni, Lata and Malari.

Collection examined - PAN 28194, on angiospermic wood, in forest, Reni, c 1850m, NDBR (U.A.), October 4, 2005, leg. Hem Chander. PAN 28206, on gymnospermic wood, in forest, Reni, c 1850m, NDBR (U.A.), October 4, 2005, leg. Hem Chander. PAN 28258, on gymnospermic wood, in forest, Lata, c 1900m, NDBR (U.A.), October 5, 2005, leg. Hem Chander. PAN 28324, on angiospermic wood, in forest, Malari, c 2300m, NDBR (U.A.), October 8, 2005, leg. Hem Chander.

Remarks- The species is characterized by pileus 1.0-4.5 X 0.5-1.5 X 0.3-0.6 cm, dimidiate to flabelliform, margin entire, acute, pileus surface grey, glabrous, zonate, zones very faint; pore surface grey coloured, irpiciform; context 1-2 mm thick, homogenous; hyphal system dimitic; generative hyphae 2-3 µm wide, hyphae, thin walled, septate, branched, with clamp connections; skeletal hyphae 2.5-4.0 µm wide, thick walled, wall 1.0-1.5 µm thick, unbranched, aseptate, lumen narrow; pores 350-426 µm in diam., oval to irregular in shape, pore tubes 0.4-0.5 µm long, 1-2 pores per mm. This species is a new record for the study area.

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TOXICITY OF ARSENIC IN PLANTS: EFFECTS AND ADAPTIVE MECHANISMS

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Abstract

Arsenic, a naturally occurring element in earth's crust is present in different background levels. Its concentration is increasing in the environment due to anthropogenic activities of various kinds. Arsenic toxicity and consequent health risks to all forms of life, has generated interests among researchers to study various aspects of arsenic toxicity. This paper, presents recent developments in the field of arsenic uptake, phytotoxicity, translocation, metabolism, detoxification and strategies to reclaim the contaminated soils.

Key words : adaptation, arsenic, phytoremediation, toxcity.

INTRODUCTION

Arsenic is a natural element present in the atmosphere as well as in the aquatic and terrestrial environment. It occurs in both organic and inorganic forms in soils. Inorganic arsenic species, arsenite and arsenate, are the major forms of arsenic intoxication, and plants readily take up these anions (Schmöger *et al.*, 2000). The natural forms of arsenic in soils are oxysalts and sulfur containing minerals. Arsenate (As V) composes 90% of total oxysalts in aerobic soils. Arsenite (AsIII) is 60-85% of the total oxy salts in water saturated anaerobic soils. Arsenate is more water-soluble and mobile than arsenite. Natural background levels of arsenic are 5-6 mg/Kg dry soil. However, natural anomalies and anthropogenic activities of various kinds such as pesticide use, mining or irrigation by contaminated ground water results in strong enrichment of the area (Bleeker *et al.*, 2003). Meharg & Rahman (2003) reported high levels of arsenic in soils irrigated with arsenic contaminated water. Rice grain collected in districts of Bangladesh with high arsenic levels in paddy soils had 10-fold higher than the normal level of 0.2mg/kg arsenic (Meharg & Rahman, 2003). Studies of Xie & Huang (1998) and Abedin *et al.* (2002a,b) have shown that arsenic levels in the grain increased with increasing arsenic in irrigation water/soil. Elevation of arsenic levels in the soil is of concern with respect to plant uptake and subsequent entry into the wildlife and human food chains. Infact their concentration tend to rise at each trophic level in a food chain through the process of biomagnification. Arsenic has no role to play in plant metabolic activities and thus is altogether non-essential.

Health Risks: Arsenic is ranked one of the most hazardous natural elements to human health. The primary route of exposure to high levels of arsenic is typically through occupational hazards, or near hazardous waste sites or areas with high natural levels. Arsenic has also been shown up in drinking water, especially in well water. Long-term exposure to arsenic in drinking water has been linked to cancer of the bladder, lungs, skin, kidney, nasal passages, liver and prostate. Exposures to low levels of arsenic can cause nausea and vomiting, decreased production of red and white blood cells, abnormal heart rhythm, damage to blood vessels (Schmöger *et al.*, 2000).

Phyto-toxicity: The term phyto-toxicity has been defined as the phenomenon whereby a potentially harmful substance has accumulated in the plant tissue to a level affecting optimal growth and development of the plant (Beckett and Davis, 1988). Inorganic arsenic species are generally highly toxic to plants. Arsenate (As V) and arsenite (As III) are the inorganic phytoavailable forms of arsenic in soil solution. Arsenate is the dominant form of arsenic available to plants. Studies on arsenate toxicity have shown that plant species, which are not tolerant to arsenic show toxic symptoms such as inhibition of root growth, root discoloration and plasmolysis, wilting and necrosis of leaf tips and leaf margins and a decrease in photosynthetic activity (Bondada and Ma, 2003). Arsenite is also highly toxic to plants as it reacts with sulfhydryl (-SH) bonds of enzymes and tissue proteins leading to inhibition of cellular function and death (Ullrich-Eberius *et al.*, 1989). Exposure to inorganic arsenic species results in generation of reactive oxygen species (ROS) (Hartley-Whitaker *et al.*, 2001a). These authors (Hartley-Whitaker *et al.*, 2001a) also hypothesized that arsenate is converted to arsenite through the process of reduction in plants, and leads to the synthesis of enzymatic antioxidants such as superoxide dismutase (SOD),

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catalase and glutathione-S-transferase and non enzymatic antioxidants, for example glutathione and ascorbate (Alscher, 1989; Hartley-Whitaker *et al.*, 2001a). In addition to glutathione's role as an antioxidant, it is also a precursor of phytochelatins (PCs) ([γ-Glutamate-Cysteine]$_n$–Glycine), which are synthesized upon exposure to inorganic arsenic. Synthesis of PCs can therefore result in glutathione depletion, reducing the amount of antioxidants available for quenching ROS, thus, leading to oxidative stress (Hartley-Whitaker *et al.*, 2001b).

Organic arsenic species are generally considered to be less toxic than inorganic species. The various factors, which affect availability and consequent phytotoxicity of inorganic and organic arsenic species, are pH, cation exchange capacity (CEC), redox potential, soil texture, clay content and organic matter content. The redox state and pH of soil has a major influence on arsenic speciation and solubility. Soil parameters influence the toxicity of arsenic species due to altered availability.

Uptake: Arsenic is released into the environment in both inorganic and organic forms. Arsenate and Arsenite are the inorganic, phytoavailable forms of arsenic in soil solution. However, microbes, which can methylate and demethylate arsenic species in soils, may transform inorganic arsenic species to organic species and vice versa. Generally only small levels of organic arsenic species have been detected in plant tissues; however, it is unclear whether these species are product of transformation in plants or whether they are simply taken up from soil as such (Mehrag and Hartley-Whitaker, 2002). Arsenic is highly phytotoxic, with toxicity threshold concentrations in plant tissues normally varying from 5 to100-mg/kg dry wt. (Caille *et al.*, 2005). Arsenate is the dominant form of plant available arsenic in aerobic soils. The uptake of As (V) by plants has been studied extensively. Arsenate is the chemical analog of phosphate and studies suggest that plants take up arsenate via the phosphate transport systems (Mehrag and Hartley-Whitaker, 2002; Quaghebeur and Rengel, 2004). Once inside the cell it competes with phosphate, replacing phosphate in ATP to form unstable ADP-As complex. In anaerobic soils arsenate is readily reduced to arsenite. Recently it has been shown that in paddy rice, arsenite is taken up at rates of influx, following Michael-Menton kinetics (Abedin *et al.*, 2002a). Wysocki *et al.* (2001) undertook the first mechanistic study of arsenite uptake by microorganism (*Saccharomyces cerevisae*). They found transport of arsenite was facilitated by a glycerol-transporting channel, which belongs to the major intrinsic protein (MIP) family. MIPs transport water and solutes, and are ubiquitous in bacteria, fungi, plants and animals. These MIPs are also considered to be aquaporins. Mehrag and Jardine (2003) also investigated the mechanism of arsenic uptake into paddy rice roots. They have shown that glycerol competes with arsenite for uptake into rice roots. Both antimonite and glycerol inhibited arsenite uptake into rice roots, thereby suggesting that arsenite is transported across the plasmamembrane via MIPs /aquaporins.

Translocation and Metabolism: Raab *et al.* (2005) found the presence of unbound As (V) and As (III) and absence of As-phytochelatin (As-PC) complexes in the xylem sap of sunflower in arsenate environment. Similar results were reported in Indian mustard (*Brassica juncea*) by Pickering *et al.* (2000) in which they identified unbound As (V) and As (III) species in the xylem sap. Raab *et al.* (2005) found only PC_2 and glutathione (GSH) as main thiol species in sunflower sap, and PC_3 was absent. They concluded that PC_2 and GSH can undergo long distance transport in *Helianthus annuus*. Gong *et al.* (2003) also reported the same result for transgenic *Arabidopsis* plants. Wang *et al.* (2002) and Zhao *et al.* (2002) reported As (III) as the main As species loaded in xylem of hyperaccumulating fern *Pteris vittata*. This lead Raab *et al.* (2005) to postulate that As (V) and As (III) are the main species of arsenic that are translocated from roots to shoots via the xylem and not the AsIII-PC complex. Studies of Pickering *et al.* (2000) and Raab *et al.* (2005) showed that a major fraction of the arsenate taken up by plants was retained in roots and was further reduced to arsenite by endogenous arsenate reductase. Quaghebeur and Rengel (2004) suggested that As (III) is the main As species translocated from roots to shoots in *Arabidopsis thaliana*. These preliminary studies suggest that plants may have As (III) transporter in roots that translocate As (III) from roots to shoot tissues.

In As-nontolerant sunflower (*H. annuus*), Raab *et al.* (2005) showed the formation of 14 different As species, some form complexes with arsenite (AsIII-PC_3, GS-AsIII-PC_2, AsIII-$(PC_2)_2$) and newly identified Monomethylarsenic-PC_2(MAIII-PC_2) in response to arsenic exposure. Hartley-Whitaker *et al.* (2001b) demonstrated the presence of PC_2 in an As-tolerant clone and PC_3 in nontolerant clone of arsenic tolerant non-hyperaccumulator *Holocus lanatus*. As tolerance in *H. lanatus* was due to suppression of the high-affinity phosphate/arsenate uptake system (Mehrag and Mecnair, 1992), which decreases the arsenate influx into plant roots (Mehrag and Hartley-Whitaker, 2002). Zhao

et al. (2003) reported PC_2 and PC_3 as major phytochelatins species in response to arsenic exposures in As hyperaccumulater *P. vittata*. *P. vittata* differs from *H. lanatus* and sunflower, in that a large amount of As is translocated and stored in above-ground tissues and less As is retained in the rhizome (Wang *et al.*, 2002). Most of the As translocated in the frond was in the form of unbound AsIII. Raab *et al.* (2005) also reported monomethyl As species complex with PC_2 (MAIII–PC_2) in sunflower. Metabolism of As (V) to organic As species such as dimethylarsinic acid (DMA) and monomethylarsonic acid (MMA) has also been observed in phytoplankton, macrophages and at low concentration in some terrestrial plant species. These methylated forms of As are then metabolized to organophospholipids and arseno-sugars such as arsenobetaine (Mehrag and Hartley-Whitaker, 2002). Plants contain all the enzymes required for the methylation of arsenic. In plants they are involved in necessary enzymatic processes such as polyamine and ethylene synthesis. These findings indicate the existence of an alternative mechanism of As detoxification in plants that require further investigation. The presence of a range of arsenic species in arsenic resistance plants suggests, that metabolism of arsenic is linked to resistance (Mehrag and Hartley-Whitaker, 2002).

Till date, there is no confirmed report about the occurrence of metabolism of organic arsenic species in plants. This aspect needs further investigations, as arsenic speciation can significantly affect toxicity.

Tolerance and Detoxification: The mechanisms of As detoxification have been well characterized in bacteria and yeast, which commonly achieve tolerance to As by the reduction of As (V) to As (III) by arsenate reductase enzymes, and then the exclusion of toxic oxyanions As(III) from the cell by inducible and selective transporters (Mukhopadhyay *et al.*, 2002; Rosen, 2002). However, no such functional arsenate reductase and As (III) transporters have been identified in plants and thus there is no evidence to suggest that plant use these same mechanisms. Recently in addition to ferns, several plants with increased arsenic tolerance have been identified. These are *Andropogon scoparius* (Rocovich & West, 1975), *Agrostis castellana*, *A. delicatula* (De Koe and Jacques, 1993), *A. capillaris, Deschampsia cespitosa* (Meharg and Macnair, 1991), *H. lanatus* (Macnair and Cumbes, 1987), *S. vulgaris* (Paliouris and Hutchinson, 1991), *Plantago lanceolata* (Pollard, 1980), and *C. vulgaris* (Sharples *et al.*, 2000b).

Inorganic As species are generally highly phytotoxic. Arsenate is a phosphate analogue and can disrupt phosphate metabolism, whereas arsenite reacts with sulfydryl groups of enzymes and tissue proteins, leading to inhibition of cellular function and death (Meharg & Hartley-Whitaker, 2002). In As non-hyperaccumulating plant species, two mechanisms of As tolerance have been identified: (i) tolerant plants have a decreased uptake of arsenate due to a suppression of the high-affinity phosphate uptake system (Meharg and Macnair, 1991, 1992; Meharg and Hartley-Whitaker, 2002) and (ii) arsenate taken up by plants is detoxified by reducing As(V) to As(III) (Pickering *et al.*, 2000), which is subsequently detoxified via forming complexes with Thiol-reactive peptides such as γ-Glutamylcysteine (γ-EC), glutathione (GSH) and phytochelatins (PCs) (Pickering *et al.*, 2000; Hartley-Whitaker *et al.*, 2001; Wang *et al.*, 2002; Schmöger *et al.*, 2000). Phytochelatins are simple γ-glutamyl peptides containing glutamate, cysteine and glycine with general formula $(\gamma\text{-Glu-Cys})_n\text{-Gly}$ (n=2-11). These peptides are synthesized from glutathione by the action of PC synthase. Recently the gene encoding PC synthase has been cloned by several laboratories (Cobbett, 2000). PC synthase is activated by heavy metal ions, these metal ions are chelated by PCs formed thus terminating the reaction. Since the immobilized metals are less toxic than the free ions, PCs are considered to be part of detoxifying mechanism of higher plants. This concept is supported by various studies, which are:-

a) *Arabidopsis* and *Schizosaccharomyces pombe* mutants lacking PC synthase activity display an enhanced sensitivity toward arsenate (Ha *et al.* 1999)

b) Exposure to As induces a large response in the synthesis and accumulation of PCs in a number of plant species (Sneller *et al.*, 1999; Schmöger *et al.*, 2000)

c) As-tolerant populations of *Holcus lanatus* were found to accumulate more PCs than nontolerant ones under the same degree of As stress (Hartley-Whitaker *et al.*, 2001; Hartley-Whitaker *et al.*, 2002)

d) Inhibition of PC synthesis by a treatment with L-buthionine-sulphoxime (BSO), a potent inhibitor of γ-glutamylcysteine synthetase, leads to hypersensitivity to As in both As nontolerant and tolerant plants (Schmöger *et al.*, 2000; Hartley-Whitaker *et al.*, 2002; Schat *et al.*, 2002).

e) An *Arabidopsis thaliana* mutant lacking functional

PC synthase was significantly more sensitive to As toxicity than the wild type (Ha *et al.*, 1999).

f) X-ray absorption spectroscopy reveals that the majority of As in the leaves of *Brassica juncea* and *A. thaliana* was coordinated to thiols (Pickering *et al.*, 2000; Dhankher *et al.*, 2002). Moreover, intact PCs-As complexes have been isolated from plant tissues (Sneller *et al.*, 1999; Schmöger *et al.*, 2000, Raab *et al.*, 2005).

Mycorrhizal fungi also provide arsenic resistance to their host plant. Plants growing on arsenic contaminated soils found to have mycorrhizal association (Meharg & Cairney, 1999; Sharples *et al.*, 2000a,b). Gonzalez-Chavez (2000) reported that infection of both tolerant and non-tolerant plants by mycorrhizal fungi from arsenic contaminated sites enhanced their resistance to arsenate. However, the mechanism by which these fungi influence arsenate resistance is yet to be studied.

Hyperaccumulation and bioremediation: Hyperaccumulation refers to uptake, sequestration and accumulation of exceptional concentrations of an element in above ground parts of a plant under field conditions (Pollard, 2000), while bioremediation is the use of plants and trees to clean up the contaminated soils and water. The pollutant can be stored in the plant (phytoextraction), volatized by the plant (phytovolatization) or metabolized by the plant (phytodegradation) (Salt *et al.*, 1998).

Hyperaccumulator plants accumulate high concentrations of metals without showing any sign of toxicity. It is a rare phenomenon in terrestrial higher plants. Till date nearly 400 taxa of hyperaccumulator species have been identified, most of them are nickel (Ni) hyperaccumulators (Baker *et al.*, 2000). Arsenic hyperaccumulation was first reported by Ma *et al.* (2001) in fern *Pteris vittata*. This fern can accumulate up to 22,630mgAs/kg in the shoot. Visoottiviseth *et al.* (2002) has identified another As hyperaccumulator fern, *Pityrogramma calomelanos* from Thailand. Zhao *et al.* (2002) have identified three new As hyperaccumulator species in the genus *Pteris* including *P. cretica, P. longifolia* and *P. umbrosa. Pteris vittata* and *Pityrogramma calomelanos* have relatively large biomass and have remarkable ability to extract and accumulate As in above ground parts. Thus, they can offer the possibility of phytoremediation of As-contaminated soils. Hyperaccumulation is considered to be an adaptive mechanism to cope up with contaminated environment. Long term exposures to high arsenic concentrations provide selection pressure on existing genotypes of a population, and survival of the resistant genotype (Mehrag and Hartley-Whitaker, 2002). Generally, As tolerant plants have decreased arsenate uptake through suppressed high affinity phosphate transporters, but *P. vittata* does not employ same mechanism (Mehrag and Macnair, 1992). Zhao *et al.* (2003) found that As (III) was the predominant species of As in the shoots of *P. vittata*, whereas in the roots As was present mainly as As (V). On the basis of difference between roots and shoots in As speciation they suggested that leaves may be the main location of arsenate reduction, although this has to be established in *P. vittata*. Generally, Arsenite has a high affinity to thiols and form PC complexes. But in *P. vittata*, concentrations of PCs formed were considerably lower, and PCs were of shorter chain length. They suggested that *P. vittata* has a limited capacity to accumulate PCs in response to As exposure. They concluded that although arsenate exposure induces PC synthesis in *P. vittata*, the role of PCs in the detoxification of As appears to be limited.

Genetics: The genetics of arsenic uptake and accumulation has not been extensively studied in plants. Naturally occurring resistance to high soil arsenic observed in some species has been shown to be caused by the reduction of phosphate as well as arsenate uptake (Meharg & Hartley-Whitaker, 2002) (as mentioned above, arsenate is an analogue of phosphate and enters the plant through phosphate transporters). Studies on the grass *Holcus lanatus* have indicated the presence of a single major gene conferring tolerance to arsenate (Macnair *et al.*, 1992). This gene has been shown to have a pleiotropic effect on phosphate uptake and use, where resistant plants had reduced phosphate uptake by the roots, poorer P use efficiency, lower vegetative growth and earlier and more abundant flowering compared to non-tolerants (Wright *et al.*, 2000). This *H. lanatus* gene is postulated to be a transcription factor regulating the expression of phosphate transport proteins (Meharg & Macnair, 1992). Dasgupta *et al.*, (2004) screened 108 recombinant inbred lines of the Bala × Azucena mapping population revealing the presence of a major gene, *AsTol*, which mapped between markers RZ516 and RG213 on chromosome 6. In addition to the naturally occurring genes observed in rice and *H. lanatus*, an arsenate tolerance gene has also been identified in *Arabidopsis* by the generation of an induced mutant (Lee *et al.*, 2003). Fern *P. vittata* exhibits an extraordinary tolerance for arsenic, so its genome mapping studies could provide further information in relation to As tolerance.

Conclusions and future perspective

Since arsenic is highly toxic to all organisms including

plants, there is a need to develop efficient strategies for cleaning As-polluted soil and water, and also to reduce uptake of As in food crops to minimize the risk of As toxicity. Phytoremediation, a plant-based technology, however, holds great promise for the purification of contaminated soil and water. For example, the natural As-hyperaccumulating fern *P. vittata* can be used to clean contaminated soils but the mechanism of As hyperaccumulation is still not understood completely. This fern is restricted in growth to the tropics of the southern hemisphere and may not be highly effective in temperate climatic conditions. The uptake and hyperaccumulation capacity of plants can be significantly enhanced by genetic engineering. This requires thorough understanding of the basic molecular and biochemical mechanisms of As uptake and detoxification in plants. Mechanism of arsenic metabolism and arsenic speciation in plants also require further investigations as these are linked to resistance. Knowledge of the basic processes of metal transport, accumulation and tolerance in both plants and other organisms could help in designing the strategies for the manipulation of these traits in transgenic plants with the aim of maximizing the capacity of plants for phytoremediation. So, further studies should focus on transgenic plants having high-biomass, fast-growing non-food plants for soil remediation and aquatic plants for water remediation. For food crops, there is a need to develop strategies for lesser As uptake to reduce its toxicity to plants as well as consumers.

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A PRELIMINARY STUDY ON THE DIPTERAN FLOWER VISITORS OF JESSORE SLOTH BEAR AND BALARAM-AMBAJI WILD LIFE SANCTUARIES, NORTH GUJARAT

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Abstract

Enumeration of 15 species of dipterans flower visitors belonging to 13 genera under 5 families from Balaram- Ambaji wildlife and Jessore Sloth Bear sanctuaries, North Gujarat along with their diagnostics, visited plant species and foraging activity.

Key words : flower visitors, pollinators, Jessore Sloth-Bear Sanctuary, Balaram-Ambaji Wildlife Sanctuary, North Gujarat.

INTRODUCTION

Knowledge on insect flower visitors is required as they perform vital role in pollination. Flies that visit flowers regularly are potential pollinators. Moreover, with their suctorial or lapping mouthparts they are considered to be the primitive pollinators (Kevan and Baker, 1983).

In contrast to large number of publications on bees, butterflies & moths pollinator species, very little attention has been given to the flies. To study the myiophily in different groups of plants in different ecosystems, it is first and foremost duty to identify the fly species.

Keeping in view, a study was undertaken to identify dipteran flower visiting species in two protected sanctuaries of North Gujarat that also come under the Indian part of Thar Desert. The study was undertaken in two different seasons (Pre- & Post monsoon) of the year 2000. Mitra & Parui (2002) reported for the first time a list of flower visitors of these sanctuaries. The present communication reports the detailed study of 15 species of flower visitors of order Diptera belonging to 13 genera under 5 families from these sanctuaries along with their diagnostics, visited plant species and foraging activity.

MATERIAL AND METHODS

The study was conducted in the Jessore Sloth Bear Sanctuary (180.66 sq. km.) and Balaram-Ambaji Wildlife sanctuary (542.08 sq. km.). Geographically, both the sanctuaries lie in the north and northeastern parts of Gujarat state at the southern most tip of the Aravalis and spread within the latitudes of 24° 20'N to 20°31'N and longitudes 72°23' to 72° 37'E. The study was undertaken in two different seasons (pre- and post-monsoon) in the year 2000. Fly visitors were collected by the insect net, and were got identified from the Diptera section, Zoological Survey of India, Kolkata.

RESULTS

FAMILY STRATIOMYIDAE

1. ***Adoxomyia heminopla*** (Wiedemann)

1819. ***Clitellaria heminopla*** Wiedemann, *Zool. Mag.*, 1(3): 30.

Material examined: 3♂, Sambalpani, 10.viii.2000; 1♂, Khapha, BAWS, 13.viii. 2000, coll. P.Pauri.

Diagnosis: Black, antennal segments I and II black, III reddish brown to yellow; thorax without definite hair stripes, but with a dark median stripe surrounded by 4 spots arranged in a square; abdomen generally black; all femora and tibiae anteriorly and posteriorly brownish orange, rest black; wings grey, yellowish brown at centre.

Visiting Plant species: *Zizyphus* sp. (Family Rhamnaceae) and *Nerium indicum* Mill.(Family Apocynaceae).

Remarks: This species visits *Nerium* more than *Zizyphus*. Mostly visit the flowers of upper canopy. During surveys, this species was encountered twice and its activity was observed only during early morning hours i.e. between 6 a.m to 7 a.m.

FAMILY BOMBYLIIDAE

2. ***Toxophora javana*** Wiedemann

1821. ***Toxophora javana*** Wiedemann, *Dipt .exot.,:* 179.

Material examined: 1♀, near Ghata village, 1♀, Iswania hills, BAWS, 13.viii.2000, coll. P.Pauri.

Dignosis: Antennae very long, prominent, horizontal, approximate at the base; vertex very small, black with a

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pair of strong ocellar bristle; thorax black, humped with a coating of very small, oblong, brownish scales lying flat on the surface; abdomen black, long, narrow, with a layer of small brownish scales as on the thorax; three posterior cells.

Visiting Plant species: *Cassia auriculata* Linnaeus (Caesalpiniaceae).

Remarks: This species was found active throughout the day.

3. ***Bombylius maculatus*** Fabricius

1775. ***Bombylius maculatus*** Fabricius, *Syst.Ent.*, Pp. 803.

Material examined: 1♂,1♀, Iswania hill, BAWS, 13.viii.2000, coll. P. Pauri.

Diagnosis: Head in male black; frons with elongate white or yellowish-white scales; antennae black; thorax black, a pair of faint bluish well-separated narrow stripes visible on the denuded surface; abdomen black, black hairs surround the tip of the abdomen; wings clear, costal cell yellowish, whole base of wing as far as humeral cross-vein dark brown.

Visiting Plant species: *Sorghum vulgare* Pers. (Family Gramineae).

Remarks: A good number of specimens were observed to visit the flowers of *Sorghum* throughout the day.

4. ***Petrorossia albofulva*** (Walker)

1852. ***Anthrax albofulva*** Walker, *Insecta Saundersiana*, 1:182.

Material examined: 1♀, 5♂, Balundera village, JSBS, 5.ii.2000, coll. P.Pauri.

Diagnosis: This species can be recognized by its second longitudinal vein which is normally with a distinct curve, followed by a distinct downward one just before tip, anterior cross vein at one third of discal cell, placed some distance beyond tip of praefurca

Visiting Plant species: *Solanum melongena* Linnaeus (Family Solanaceae), and *Capsicum* sp. (Family Solanaceae).

Remarks: This species is very fast mover and was observed only in the month of February in the agricultural field of JSBS (Table-1). A good number of specimens were found to visit on the flowers of Chilly and Brinjal.

5. ***Petrorossia nigrofemorata*** (Brunetti)

1909. ***Argyramoeba nigrofemorata*** Brunetti, *Rec. Indian Mus.*, 3:219.

Material examined: 2♀, 3 ♂, Balundera village, JSBS, 5. ii.2000, coll. P.Pauri.

Diagnosis: Blackish grey species with soft white hairs; thorax with a long and a short spiny yellow bristle in front of each wing; femora black with base and apex reddish yellow, tibiae uniformly reddish yellow.

Visiting Plant species: *Coriandrum sativum* Linnaeus (Family Umbelliferae).

Remarks: This species was found as flower visitors in all the methi fields of JSBS and active throughout the day.

6. ***Argyramoeba duvaucelli*** (Macquart)

1840. ***Anthrax duvaucelii*** Macquart, *Dipt. exot.*, 2 (1): 63.

Material examined: 1♂, 2♀ Sambalpani, BAWS.10.viii.2000, coll. P.Pauri.

Diagnosis: Black species; anterior part of thorax with a dense fringe of narrow long grayish white scales legs black with coxae long silk white haired; wing almost clear with small suffusion at base of second vein, at fork of third vein, on outer cross vein and at inner side of discal cell.

Visiting Plant species: *Cassia tora* Linnaeus (Family Fabaceae).

Remarks: The species was found active mostly in morning.

7. ***Thyridanthrax (Exhyalanthrax) absalon*** (Wiedemann)

1828. ***Anthrax absalon*** Wiedemann, *Analecta Ent.*, :24.

Material examined: 1♂, 2♀ Chitrasani, 7. ii.2000, coll.P.Parui ; 2♂, 2♀ Virampur, 10.viii.2000, coll. P.Pauri ; 1♂, 2♀, Sambalpani, BAWS.10.viii. 2000, coll. P.Pauri.

Diagnosis: A medium-sized species with porrect antennae, second antennal joint cup-shaped, third segment onion-shaped; thorax quadrate; abdomen flat, oblong; wings brown with hyaline spots.

Visiting Plant species: *Cassia tora* Linnaeus (Family Fabaceae), *Lycopersicon esculentum* Mill. (Family Solanaceae) and *Solanum melongena* Linnaeus (Family Solanaceae).

Remarks: The species was found as flower visitors of

Solanaceae (Chilli and Tomato) in February and on the flowers of Fabaceae (*Cassia tora*) in August.

8. ***Exoprosopa (Exoprosopa) insulata*** (Walker)

1852. ***Anthrax insulata*** Walker, *Insecta Saundersiana*, 1:172.

Material examined: 6♂, 2 ♀ Chitrasani, BAWS, 7. ii.2000, coll. P.Pauri.

Diagnosis: Body brownish yellow with shining black abdomen and spotted wings; lateral portion of thorax with long black bristly hairs but dorsum with short black pubescence and minute yellow scales; wing with a broad oblique dark brown band extending from costa to middle of axillary cell embracing basal third of discal cell.

Visiting Plant species: *Cassia tora* Linnaeus (Family Fabaceae), *Ipomoea* sp. (Family Convolvulaceae), *Lycopersicon esculentum* Mill (Family Solanaceae) and *Solanum melongena* Linnaeus (Family Solanaceae).

Remarks: This species was found active on flowers only between 06.00 – 09.00 hours.

9. ***Ligyra aurantiaca*** (Guérin-Méneville)

1844. ***Anthrax aurantiaca*** Guérin-Méneville, *Icon, du regne anim, Dipt.*, 3 : 539.

Material examined: 2♂, Balaram nursery, BAWS, 17.viii.2000, coll. P.Pauri.

Diagnosis: Body black with bright yellow pubescence, lower abdomen with patch of silvery white scales, wings dark brown with violet iridescence.

Visiting Plant species: *Tephrosia purpurea* Pers.(Family Papilionaceae).

Remarks: This species was spotted only once in BAWS in the month of August.

10. ***Ligyra oenomaus*** (Rondani)

1875 ***Hyperalonia oenomaus*** Rondani, *Annali Mus. Civ Stor.nat. Giacomo Doria*, 7 : 453.

Material examined: 2♂, Balaram nursery, BAWS, 17.viii.2000, coll. P.Pauri.

Diagnosis: Body black, covered with orange-yellow pubescence, abdomen with yellowish white spots, clear lightly pictured wing.

Visiting Plant species: *Tephrosia purpurea* Pers. (Family Papilionaceae).

Remarks: This species was spotted only once in BAWS in the month of August.

FAMILY SYRPHIDAE

11. ***Asarkina (Asarkina) ericetorum*** (Fabricius)

1781. ***Syrphus ericetorum*** Fabricius, *Spec. Insect.*, 2:425.

Material examined: 1♂, Kapasiya village, JSBS, 13.viii.2000, coll. P.Pauri; 1♀, Vara village JSBS, 11.viii.2000, coll. P.Pauri.

Diagnosis: Yellowish species; frons yellow, vertex black but both with black pubescence; thorax and scutellum covered with bright yellow pubescence; abdomen orange yellow with all the segments black banded at posterior margin; legs yellow.

Visiting Plant species: *Cassia tora* Linnaeus (Family Fabaceae) and *Commelina* sp (Family Commelinaceae).

Remarks: During surveys, this species was found active in the morning only.

12. ***Ischiodon scutellaris*** (Fabricius)

1805. ***Scaeva scutellaris*** Fabricius, *Syst Antliat.*: 252.

Material examined: 1♂, Machkoda, BAWS, 8.ii.2000, coll.P.Pauri; 2 ♀, Ganapipli, BAWS, 9.ii.2000, coll. P.Pauri.

Diagnosis: Blackish species with frons and face bright sulphur yellow; thorax shining black with side margins bright yellow from anterior margin of wing; abdomen black with a pair of yellow spots on second segment, a yellow band each on third and fourth, fifth segment with greater part orange; legs yellow with a broad subapical black ring on hind femora.

Visiting Plant species: *Cassia tora* Linnaeus (Family Fabaceae) and *Anogeissus pendula* Linnaeus (Family Combretaceae).

Remarks: Like other syrphid species, this species was found active in the morning only.

FAMILY TEPHRITIDAE

13. ***Euphranta (Staurella) crux*** (Fabricius)

1794. ***Musca crux*** Fabricius , *Ent. Syst.*, 4: 358.

Material examined: 2♂, 2♀ Gahta village, BAWS, 13.viii.2000, coll. P.Pauri; 1♂, Ganesh Temple, JSBS, 7.viii.2000, coll. P.Pauri.

Diagnosis: Antennal arista with long hairs on upper side;

bristles of thorax black, dorsum with yellow pubescence, metanotum dark reddish brown, very shining in the middle; scutellum light brown; abdomen with black pubescence and black bristles; wing tip with a cross shaped double band between 4th and 5th veins, and apical band between 2nd and 3rd and 3rd and 4th veins.

Visiting Plant species: *Lantana camara* Linnaeus (Verbanaceae) and *Tectona grandis* Linnaeus (Verbanaceae).

Remarks: This species was found active throughout the day.

FAMILY CALLIPHORIDAE

14. ***Chrysomya megacephala*** (Fabricius)

1794. ***Musca megacephala*** Fabricius, *Syst. Ent.*, 4: 317.

Material examined: 3♀, Khapha, BAWS, 11.viii.2000, coll. P. Pauri 10♂, 2♀, Rupwas, BAWS, 9.viii. 2000, coll.P. Pauri.

Diagnosis: Blue or green coloured fly with upper eye facets greatly enlarged, larger than lower third in male; wing hyaline, slightly dark at base; legs black.

Visiting Plant species: *Holarrhena antidysenterica* Wall. (Family Apocyanaceae), *Lantana camara* Linnaeus (Family Verbanaceae) and *Tectona grandis* Linnaeus (Family Verbanaceae).

Remarks: A large number of examples were found to sit on the white flowers of *Holarrhena antidysenterica* in the early morning at Rupwas Village.

15. ***Stomorhina discolor*** (Fabricius)

1794. ***Musca discolor*** Fabricius, *Ent. Syst.*, 4: 320.

Material examined: 2♂, Machkoda, BAWS, 8.ii.2000, coll.P. Pauri; 11♂, 5♀, Khapha, 13.viii. 2000, coll. P. Pauri.

Diagnosis: Thorax green, abdomen blackish; face, epistome and anterior half of gena shining black; anterior and middle femora black, rest of legs otherwise coloured.

Visiting Plant species: *Zizyphus* sp. (Family Rhamnaceae) and *Anogeissus pendula* Wall. (Family Combretaceae).

Remarks: A large number of specimens were found to hover on the flowers of *Anogeissus* in the dusk.

Altogether 15 dipteran species were recorded as flower visitors from Balaram-Ambaji wildlife & Jessore Sloth Bear sanctuary of North Gujarat. Maximum number of flower visitors (12 species) has been collected from the Balaram-Ambaji wildlife sanctuary, whereas maximum number of species was reported in the month of August (11species). During this survey, it has been found that, the bee-flies (9 species) are the predominant flower visiting groups of these sanctuaries.

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CYTOGENETIC ANALYSIS OF ABNORMAL ANIMALS OF TWO INDIAN GOAT BREEDS

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Abstract

The cytogenetic investigations were carried out on 05 abnormal animals belonging to Jhakrana and Black Bengal breeds of Indian goats. The animals having reproductive problems did not reveal any numerical deviation from the normal chromosome complement. However, the chromosomes showed structural abnormalities as gaps, breaks and centromeric breaks in autosomes. The Jhakrana female animal with a leg deformity showed no gross chromosomal abnormalities. It revealed a normal 60, XX female chromosome constitution.

Key words: goat, breed, abnormal, chromosomes, leg deformity.

INTRODUCTION

Cytogenetic studies contribute an array of information independent from morphological biochemical, behavioral and other characters that are used for phylogenetic analysis. As with biochemical data, cytogenetic information can reveal differences and similarities that may not be obvious at the morphological level. Chromosomes can be studied as a morphological manifestation of the genetic compliment in terms of their microscopically visible size, shape number and behavior during meiosis and mitosis. Chromosomal studies have led to an understanding of the basic structure of the genetic complement of most of the domestic and other useful and known species. There are various theories on speciation based on chromosomal model (Wurster and Benirschke, 1968; Hsu and Mead, 1969; Yadav, 2002a).

Chromosomal abnormalities are an important cause of reproductive disturbances in domestic animals as they have often been associated with low fertility (Gustavsson, 1980; Long, 1985; Rangel-Figueiredo and Iannuzzi, 1993) or sterility (Iannuzzi *et al.*, 2000, 2001). Hence Karyotype analysis in domestic animals, especially breeding animals, is very important, as chromosomal abnormalities which cause serious losses of potential productivity (Gustavsson, 1980) may be easily passed to offspring, especially when artificial insemination is used. Chromosome aberrations fall under two types: structural involving alteration in the structure due to translocation, deletion, duplication, substitution, insertion, centric fusion, isochromosomes, etc. and numerical involving a variations in the normal number of chromosomes (Yadav, 2002b). Numerical chromosome aberrations are responsible mainly for phenotypic malformation where as structural aberration affect fertility (Berardino *et al.*, 1983)

Autosomal numeric chromosomal abnormalities are phenotypically visible and easily eliminated by breeders during the normal breeding selection. Structural abnormalities and numeric abnormalities involving autosomes are generally tolerated by the species (as the carriers tend to show normal body conformation), however, the sex chromosome abnormalities are often associated with low fertility or sterility, especially in females (Gustavsson, 1980; Long, 1985; Rangel-Figueiredo and Iannuzzi, 1993; Iannuzzi *et al.*, 2000, 2001). The present study was conducted on some of the abnormal animals belonging to two different Indian breeds.

MATERIAL AND METHODS

The cytogenetic investigations were carried out on 05 abnormal goats belonging to Jhakrana and Black Bengal. The details of abnormal individuals are given in Table 1. The normal fertile animals of these breeds were taken as control.

Blood samples were collected from the jugular vein of the animals in 10 ml capacity vaccutainer tubes containing sodium heparin. Aseptic precautions were taken at the time of blood collection. Heparinized whole blood was stored in the refrigerator at 5ºC till use (2-3 days), but usually it was used on the same afternoon or next day. Short-term whole blood lymphocyte culture technique

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Table 1: Details of animals with morphological and reproductive abnormalities.

Sr. No.	Breed Type	Phenotypic sex	Observed defect	No. of Animals
1.	Jhakrana	F	Sterile	02
2.	Jhakrana	F	Leg defect (twisted)	01
3.	Black Bengal	F	Sterile	02

Table 2: Frequency of abnormal cells

Type of animal studied	Sterile Jhakrana female	Sterile Jhakrana female	Sterile Black Bengal female	Sterile Black Bengal female	Jhakrana female (control)	Black Bengal female (control)	Jhakrana female with leg deformity
No. of cells studied	100	100	100	100	50	50	100
No. of abnormal cells	16	17	13	14	2	1	0

described by Yadav and Balakrishnan (1985) was followed for the preparation of mitotic chromosomes. Aliquots of 0.5 ml of peripheral blood in 6 ml of TC-199 medium supplemented with adult cattle serum (20%), pokeweed mitogen (0.20mg/100ml), streptomycin (100µg/6ml) and penicillin (100 units/6ml) were incubated at 37.5°C ± 0.5°C for 72 hours. The cultures were treated with colchicine (0.5 µg/ml) for 45 minutes before harvesting. The cells were treated with hypotonic solution of 0.075 M potassium chloride for 10 minutes at 37°C and then fixed in methanol and acetic acid (3: 10). Slides were prepared by air-drying method and stained with 2% Giemsa solution for 25 minutes and subsequently rinsed thoroughly in distilled water. Extra moisture was removed by putting the slides in the folds of a filter paper and then kept in an incubator for 24 hours for drying before mounting.

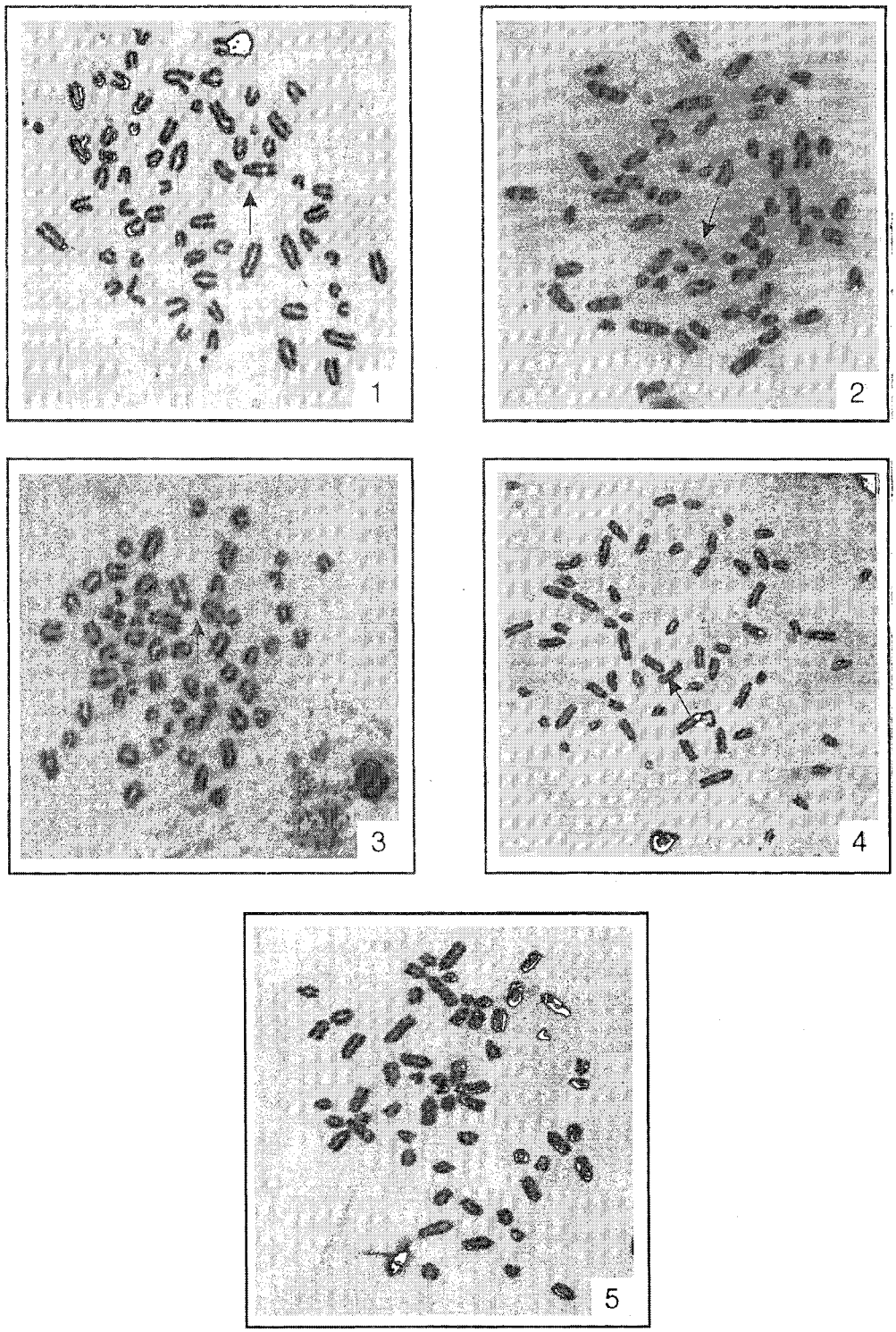
Slides were screened on Leica microscope (German). Normally 40-50 well spread metaphase plates were screened per normal animal (control). In case of abnormal animals, at least 100 plates were recorded. Selected plates were photographed by using Leica camera on Kodak black and white (ASA 125) film. A green panchromatic filter was used for black and white photography

RESULTS AND DISCUSSION

In the present investigation, the animals having reproductive problems did not reveal any numerical deviation from the normal chromosome complement. However, the chromosome showed structural abnormalities as gaps, breaks and centromeric breaks in autosomes (Figs 1-4). The frequency of abnormal cells has been tabulated (Table 2). In abnormal animals, 15 cells (an average of 4 animals) were found to be abnormal out of 100 cells studied. In normal animals (control) only 1 or 2 cells were found to be abnormal. It was found that frequency of abnormal cells was significantly high in sterile animals ($P < 0.05$). The Jhakrana female animal with leg deformity showed no gross chromosomal abnormalities. It revealed a normal 60, XX female chromosome constitution (Fig. 5).

Chromosomal abnormalities are an important cause of reproductive disturbances in domestic animals as they have often been associated with low fertility [Gustavsson, 1980; Long, 1985; Rangel-Figueredo and Iannuzzi, 1993] or sterility [Iannuzzi *et al.*, 2000, 2001]. In the present study also, goats with reproductive problems showed structural chromosomal abnormalities such as gaps and breaks as reported by Bhatia and Shanker (1991).

Leg deformity was suggested to be an autosomal recessive character (Leipold, 1972; Ojo *et al.*, 1975). Hamori (1983) had reported leg deformities in cattle, goat and sheep. He suggested that a mutant allele when expressed in the phenotype produces deformed legs. Alternative explanation to the phenotype includes the effect of teratogenic agents or even the participation of lethal factors (Hamori, 1983). Yadav *et al.* (1995) also reported such a case and suggested a mutant effect of recessive gene or action of teratogens during foetal



Figs.1-5: Abnormal chromosomes of two Indian goat breeds: I-2. Sterile Black Bengal female goats with autosomal constriction/ gap and isolocous centromeric gap; 3-4. Sterile Jhakrana female goats with autosomal unilocus gap and break; 5. Jhakrana female goat (with leg deformity) with normal chromosome constitution of 60,XX.

development of the animal. A similar explanation for leg deformity seems to be responsible in the present case too.

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CHROMOSOME ANALYSIS OF THREE INDIAN GOAT BREEDS BY R BANDING

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Abstract

Differential banding patterns were obtained on metaphase chromosomes of three breeds of goat with RB- FPG banding technique (R banding method using 5-bromo-deoxyuridine (BrdU), the fluorescent 33258 Hoechst dye, followed by blue-black light exposure and Giemsa staining). R banded pattern of chromosomes resembled the standard pattern in its essential characteristics. However, most of the chromosomes showed lesser number of bands. The RB-FPG method demonstrated the phenomenon of X chromosome inactivation, as late replicating X chromosome was without bands except a thin pair of bands in some preparations. All the autosomes revealed light and dark alternate band patterns similar in both homologues. There was no difference in R banding pattern among breeds.

Key words: goat breed, RB-FPG banding, X chromosome inactivation.

INTRODUCTION

Goat is the most prolific ruminant among all domesticated ruminant under tropical and subtropical conditions (Naude and Hofmeyr, 1981). It is resourceful and efficient ruminant producing meat, milk, skin and hair (Mussman, 1982). Goat has been described as poor man's cow, a befitting description of their immense contribution to the poor people's economy. Thus there is an utmost need on accurate knowledge of their various traits not only at phenotypic level but also at genetic architecture. Different types of genetic markers have been used in genome analysis. These include morphological markers, cytogenetic markers, protein markers and more recently the DNA markers.

Cytogenetic studies contribute an array of information independent from morphological biochemical, behavioral and other characters that are used for phylogenetic analysis. Chromosomal studies have led to an understanding of the basic structure of the genetic complement of most of the domestic and other useful and known species (Wurster and Benirschke, 1968).

Technical precision of banding patterns are tools to the definite identification of chromosomes and their structural details and made comparisons among individuals (normal and abnormal) and species more meaningful (Gibson, 1986). Banding techniques improved the possibilities of diagnosing chromosome variations and have also made possible to link some of the chromosomal abnormalities to production and reproductions traits of farm animals (Gustavsson, 1980). Banding patterns give more information on chromosome anomalies by specifying the location of anomaly (i.e. gap, break, deletion, translocation etc.) or the morphological feature (i.e. secondary constriction etc.) (Seabright, 1972). A particular chromosome or its part responsible for the abnormal trait can be detected by comparing the banded chromosomes of the abnormal animals with the normal.

Among various banding techniques, R-banding technique is quite important in cytogenetic studies. This technique reveals alternate dark and light bands along the length of chromosomes. It is known as reverse banding as band pattern obtained is reverse of G-banding patterns. The centromeres and telomeres of the chromosomes of most of the bovines are R-positive i.e. these show a dark R-band while these regions show light bands in G-banding. So any alterations in the chromosomes involving these regions, which are quite commonly encountered in nature, can easily be detected with the help of R-banding technique.

In the present study, differential banding patterns were obtained on metaphase chromosomes of three breeds of goats with RB-FPG banding technique (R banding method using 5-bromo-deoxyuridine (BrdU), the fluorescent 33258 Hoechst dye, followed by blue-black light exposure and Giemsa staining)

MATERIAL AND METHODS

Investigations were carried out on ten males and ten females of each of three breeds of goats viz. Black Bengal,

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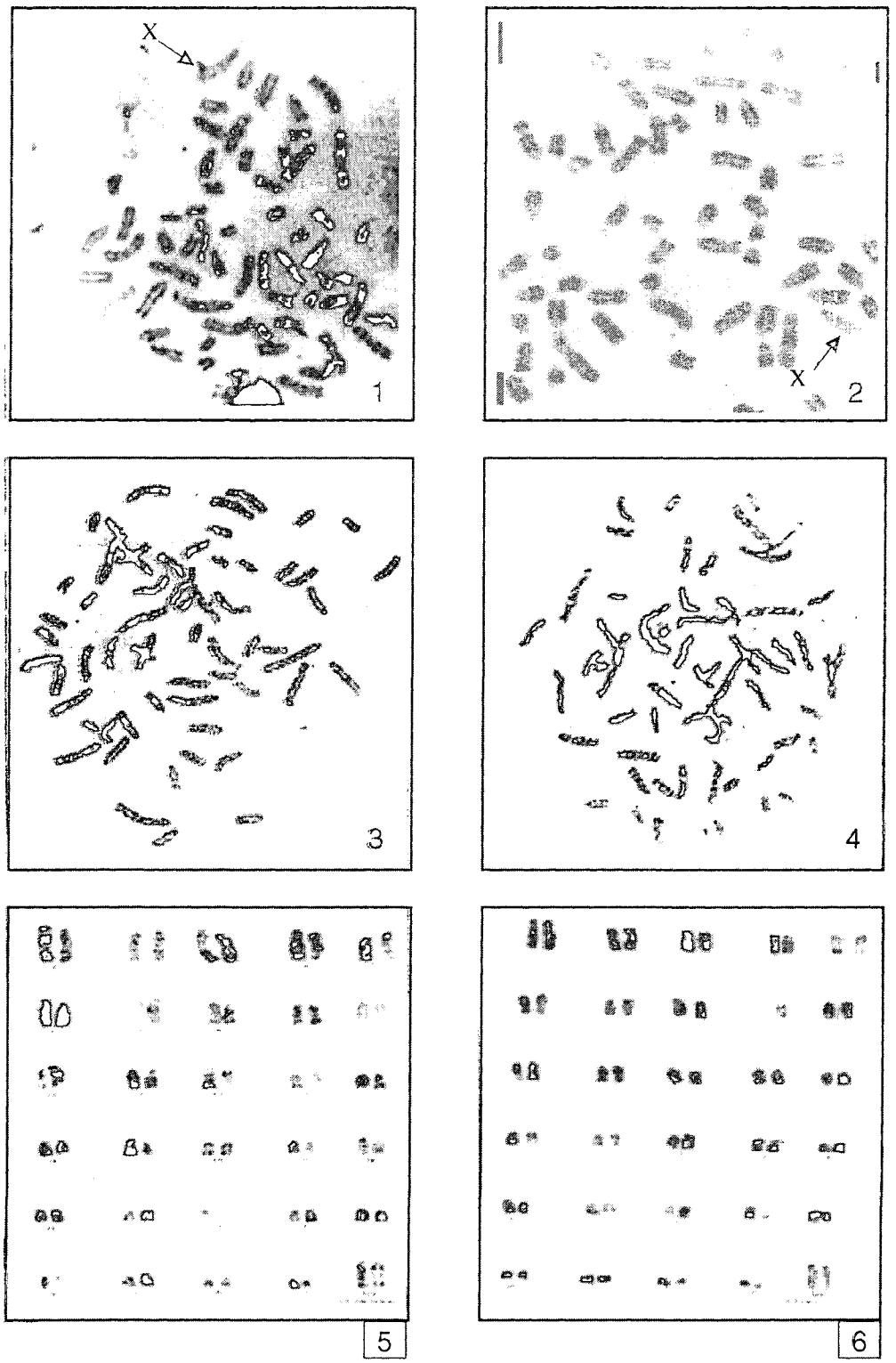

Figs. 1-6: R banding pattern of Chromosomes of three Indian Goat breeds: (1) Female Black Bengal goat, (2) Female Jamnapari goat, (3) Female Jhakhrana goat, (4) Male Black Bengal goat, (5) R banded karyotype of Black Bengal goat, (6) R banded karyotype of Jhakhrana goat.

Jamunapari and Jhakrana. All the three breeds were maintained in organized herd at Central Institute for Research on Goats (CIRG), Makhdoom, Mathura (UP). In case of Black Bengal and Jhakhrana breeds, sampling was also done from their native breeding tract in West Bengal and Rajasthan respectively. Blood samples were collected from the Jugular vein of the animals in 10 ml capacity vaccutainer tubes containing sodium heparin. Aseptic precautions were taken at the time of blood collection. Heparinized whole blood was stored in the refrigerator at 5ºC till use (2-3 days), usually same afternoon or next day. The blood samples collected were brought to the laboratory in an icebox keeping the samples and ice apart, taking precautions that the cells did not come in direct contact with ice or freeze. Short-term whole blood lymphocyte culture technique described by Yadav and Balakrishnan (1985) was followed for the preparation of mitotic chromosomes. Aliqots of 0.5 ml of peripheral blood in 6 ml of TC-199 medium supplemented with adult cattle serum (20%), pokeweed mitogen (0.20mg\100ml), streptomycin (100µg/6ml) and penicillin (100 units\6ml) were incubated at 37.5°C ± 0.5°C for 72 hours. The cultures were treated with colchicines (0.5 µg/ml) for 45 minutes before harvesting. The cells were treated with hypotonic solution of 0.075 M potassium chloride for 10 minutes at 37°C and then fixed in methanol and acetic acid (3: 10). Slides were prepared by air-drying method.

Procedure of R-banding:

RB-FPG technique (R-banding technique by fluorescence photolysis Giemsa methods after BrdU incorporation) was followed (Perry and Wolf, 1974 modified by Romagnano and Richer, 1985). It involves the incorporation of 5' Bromo 2' Deoxyuridine (BrdU) added at the rate of 0.1 ml (conc.120 mg / 10 ml of 0.9% normal saline solution) per culture, 7-8 hours prior to harvesting. Subsequently, cultures were harvested in usual manner and slides were prepared by air-drying method. Slides were treated in bis-benzimide (Hoechst 33258, Sigma) dye solution for 15 minutes and rinsed with in distilled water. The slides were mounted with cover glass in 2X SSC and were placed under a fluorescent blue-black tube for two hours. The slides were then rinsed with distilled water and stained with 2% Giemsa solution for 5 minutes.

Slides were screened by on Leica microscope (German). Normally 40-50 well spread metaphase plates were screened per animal. Plates with excellent spread and chromosome morphology were selected for photography.

Selected plates were photographed by using Leica Camera on Kodak black and white (ASA 125). A green panchromatic filter was used for black and white photography.

Chromosomes were cut from 6" X 4" size enlarged photographs and pasted on specially printed sheets.

RESULTS AND DISCUSSION

Differential banding patterns were obtained on metaphase chromosomes of three breeds of goat with RB- FPG banding technique (R banding method using 5-bromo-deoxyuridine (BrdU), the fluorescent 33258 Hoechst dye, followed by blue-black light exposure and Giemsa staining) (Figs.1-4). R banded karyotypes of Black Bengal and Jhakrana goats have been presented in Figs 5 and 6. R banded pattern of chromosomes resembled the standard pattern (ISCNDA 1989) in its essential characteristics. However, most of the chromosomes showed lesser number of bands. This may be due to low resolution of bands and high condensation of chromosomes. There was no difference in R banding pattern among breeds.

The RB-FPG method demonstrated the phenomenon of X chromosome inactivation, as late replicating X chromosome was without bands except a thin pair of bands in some preparations. All the autosomes revealed light and dark alternate band patterns similar in both homologues.

The explanation for the RB-FPG patterns has been given by Romagnano and Richer (1984) is that BrdU gets incorporated in most of the regions of the late replicating X chromosome followed by the attachment of a light sensitive chemical. Hoechst 33258 binds to the chromosome regions having BrdU. Subsequently upon exposure to blue black light the regions of the chromosomes to which Hoechst 33258 is attached get destroyed. During staining with Giemsa these regions do not take the stain and appear pale. The early replicating X chromosome show differential banding patterns similar to autosomes. It is assumed that early replicating X chromosome might have replicated and could have incorporated BrdU just like other autosomes, which showed an alternate light and dark R-band pattern.

The RB-FPG banding patterns of the two X chromosomes differed in all the three breeds of goats. One X chromosome showed dark and light bands as revealed by all the autosomes, the second X chromosome appeared totally bandless or showed one to few dark bands. The results are similar to those found in different mammals by earlier workers (Schempp and Muller, 1982;

Romagnano and Richer, 1984; Drouin *et al.*, 1988; Drouin and Richer, 1989; Mukherjee, 1995; Vats, 1996). One or few band as seen in late replicating X chromosome could be explained due to asynchrony in S phase of mitotic cycle. The cells which stayed for the maximum duration got incorporated more BrdU and hence result in pale X chromosome upon treatment with Hoechst 33258 and subsequent photolysis. The gradual decrease in duration of S phase could be responsible for increase in the number of bands in X chromosome.

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RAPD ANALYSIS OF FEW CHAROPHYTE TAXA FOR THEIR MOLECULAR CHARACTERIZATION

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Abstract

Charophytes, being morphologically divergent taxonomic puzzle, have been characterized using RAPD as molecular marker for their characterization on the basis of DNA amplification. Six charophyte taxa namely *Nitella furcata subsp. flagellifera; N furata subsp mucronata f wrightii; Chara corallina var wallichii (female); Chara corallina (dioecious)*; *Chara braunii f. braunii* and *Chara fibrosa f. tylacantha.* have been subjected to the genomic DNA isolation, purification and amplification by using two oligonucleotide random primers namely OPD-08 and OPF-14 for the first time in India. The PCR products (amplicons) were subjected to agarose gel electrophoresis using 100 bp DNA ladder and Lambda DNA Eco R I/Hind III double digest markers for estimation of their molecular sizes which shall be used for molecular characterization of Charophyta.

Keywords: charophyta, Molecular characterization, RAPD, genomic DNA.

INTRODUCTION

Charophyta, being a highly divergent group of macrophytic green algae, could not be arranged systematically despite sincere attempts by Pal *et al.* (1962) and Wood and Imahori (1965). The classification of charophyte taxa by using meagre morphological characters as taxonomic criteria created confusion due to the lack of evidence that these morphological characters have stable molecular basis or not. Though the genomic DNA has been isolated from various algal taxa for molecular characterization (Ho *et al.*, 1995; Alberto *et al.*, 1997; Satoh *et al.*, 1997 and Hu and Zhou, 2001) but in Charophyta, attempts have only been made by using 18S rDNA sequences (Meiers *et al.*, 2004) and rbcL sequences (Karol *et al.*, 2004; McCourt *et al.*, 2004).

The perusal of literature clearly reveals that there is no report on molecular characterization of Indian charophytes. Therefore, the present investigation is new and will lead to a convincing out put for charophycean phylogeny and interrelationship. This paper embodies DNA isolation and RAPD amplification of six charophyte taxa namely *Nitella furcata subsp. flagellifera; N furata subsp mucronata f wrightii; Chara corallina var wallichii (female); Chara corallina (dioecious)*; *Chara braunii f. braunii* and *Chara fibrosa f. tylacantha.* against two oligonucleotide random primers for the first time from India. The investigations are preliminary and need further expansion on other charophyte taxa with more oligonucleotide primers before characterizing charophycean taxa in toto.

MATERIAL AND METHODS

Taxa under investigation were collected from fresh water ponds at Fatehganj on Rampur road and Bhuta on Bisalpur road during November-December, 2004 and were made epiphyte free. Taxa were identified as *Nitella furcata subsp. flagellifera* (C-1); *N furata subsp mucronata f wrightii* (C-2); *Chara corallina var wallichii (female)* (C-3); *Chara corallina (dioecious)* (C-4); *Chara braunii f. braunii* (C-5) and *Chara fibrosa f. tylacantha.*(C-6) by using the monographs of Pal *et al* (1962) and Wood and Imahori (1965). Plants were kept in dark to decolorize and the filter dried specimens were used to isolate genomic DNA following the protocol given by Bhatnagar *et al.* (2005) for charophyte taxa.

Genomic DNA was purified and subjected to qualitative and quantitative analysis before proceeding for RAPD amplification by using optimized PCR protocol for Indian Charophyta (*cf.* Abrol, 2004). Random oligonucleotide primers were selected from operon series as per G-C ratio and were procured from Bangalore Genei Pvt. Ltd. The primers used were OPD-08 and OPF-14. The amplified DNA samples were run on agarose gel along with 100bp DNA ladder marker (M_1) and Lambda DNA-Eco R I /Hind III double digest (M_2) to determine the molecular sizes of PCR products by using a computer programme given by Schaffer and Sederoff (1981).

RESULTS AND DISCUSSION

The PCR products, obtained by using two oligonucleotide random primers OPD-08 and OPF-14 in six charophyte taxa were subjected to agarose gel electrophoresis. The molecular sizes and electrophoretic banding patterns in all amplified taxa with two random primers reveal that all

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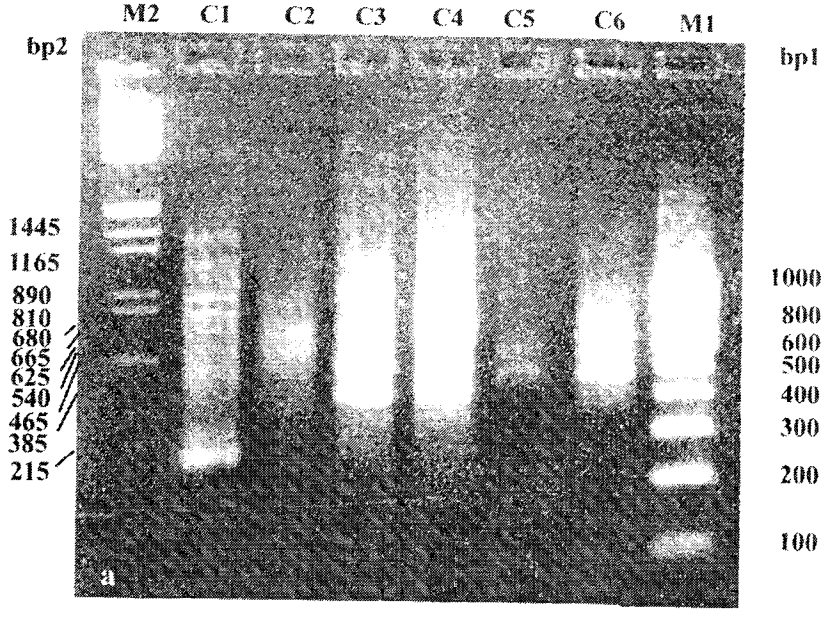

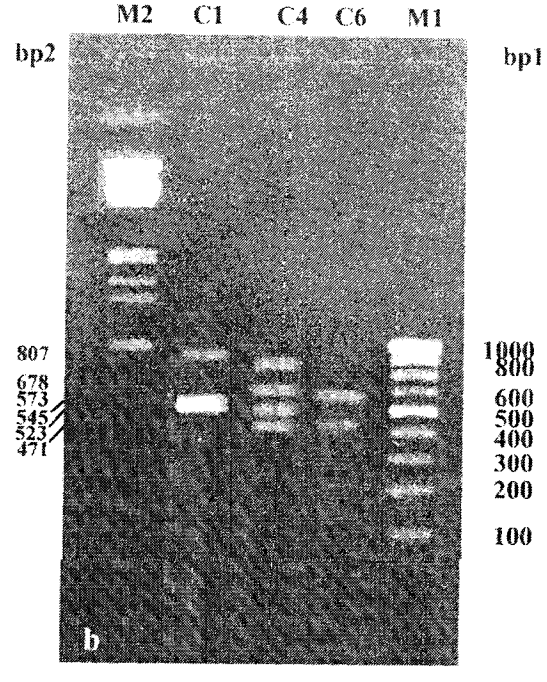

Photoplate 1(a and b): RAPD-PCR amplification patterns of individual DNA samples of charophyte taxa (C1 - C6) with primers OPD-08 and OPF-14 respectively. Markers are 100 bp DNA and (M1) and lambda DNA *Eco* RI/*Hind* III double digest (M2). Molecular sizes of markers and amplicons are donoted as bp1 and bp2, respectively.

the six investigated charophyte taxa (C-1 to C-6) showed amplification with primer OPD-08 whereas only three taxa (C-1, C-4 and C-6) amplified with primer OPF-14. The molecular sizes and banding patterns of all the amplicons as compared to the molecular markers M_1 and M_2 have been presented in Photoplate 1 (a & b).

The gel with primer OPD-08 revealed 11 scorable bands having molecular sizes Ranging from 1445 to 215 bp (1445, 1165, 890, 810, 680, 665, 625, 540, 465, 385 and 215). On the other hand, only three taxa viz. *Nitella furcata subsp. flagellifera* (C-1); *Chara corallina (dioecious)* (C-4) and *Chara fibrosa f. tylacantha*.(C-6) showed amplification with primer OPF-14. The banding pattern with OPF-14 shows six scorable bands having the molecular sizes ranging from 807 to 471 bp (807, 678, 573, 545, 523 and 471).

Williams *et al.* (1990) emphasized that the monomorphic bands represent the highly conserved region of primer binding sites in the genome. while the presence of polymorphic bands might be due to nucleotide changes in DNA sequence at primer binding sites. Being highly divergent, the charophyta taxa show higher degree of polymorphism at molecular level, which may be helpful in exploring phylogenetic relationships in between them after exhaustive investigations on more taxa and primers.

Though the attempts have been made by using 18S rDNA sequences (Meiers *et al.*, 2004) and rbcL sequences (Karol *et al.*, 2004; McCourt *et al.*, 2004) in charophytes but there is absolutely no work on molecular characterization of Indian Charophyta by using genomic DNA. Present investigations on RAPD amplification with two primers and six charophyte taxa show polymorphic banding pattern which indicate greater degree of biodiversity within this group. Though definite conclusion regarding molecular characterization need similar investigations on other charophyte taxa (Abrol, 2004) but the present observations will be instrumental for molecular characterization of this macrophytic algal group.

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BACTERIOPHAGES- AN ALTERNATIVE THERAPY FOR INFECTIONS

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Abstract

The concept of phage therapy to treat bacterial infections was borne with the discovery of the bacteriophages (viruses that eat bacteria) almost a century ago. After an enthusiastic beginning, problems with inappropriate use and uncontrolled studies along with overshadowing discovery of antibiotics caused the cessation of phage therapy research especially in West. Only a few institutions in Eastern Europe continued to study and use phages as therapeutic agents for human infections. Now, however, the alarming rise in antibiotic resistant pathogens is spurring a resurgence of interest in phages and is leading to the initiation of controlled experiments in animal models. Promising results from few of these recent studies have confirmed them to be highly effective in treating many different type of bacterial infections. So, may be the time has come that we realize the potential therapeutic value of bacteriophages as an alternative to antibiotics in both veterinary and human medicine and also as a tool to kill food borne pathogens.

Key words : bacteriophages, phage therapy, antibiotic resistance, recent studies.

INTRODUCTION

The discovery of antibiotics is considered to be one of the most important achievements in the history of medicine. It enabled the physicians to treat successfully various bacterial infections infecting man, but the worldwide emergence of multidrug resistant bacteria so called 'superbugs' has become a major problem in modern medicine, especially because of the concomitant rise in immunosuppressed patients. It was known from earlier times that when natural pathogens are exposed to natural antibiotics, they adapt themselves and acquire resistance. Clearly, this phenomenon of antibiotic resistance superbugs is a man created scientific misadventure that can be blamed on massive use, misuse and overuse of antibiotics. The concern that humankind is entering the 'postbiotic era' has become very real and the development of alternative anti infection modalities has become the need of the hour (Sulakvelidze, 2001). One intriguing approach is to use Bacteriophages (viruses that eat bacteria) to eliminate specific bacterial pathogens (Sulakvelidze and Morris, 2001). Presence of 'Superbugs' excites the reproductive machinery of bacteriophages into action. In a short time, while a superbug multiplies by dividing into two, the phage produces 200 offspring for every superbug killed; thus making the superbug victim of bacteriophage. Until 1940, phage therapy was widely practiced and researched but often with contradictory results, mainly because the phage biology was poorly understood. Now, interest in phage therapy has been rekindled after its initial failure due to the need to re-examine our potential to cure human, animal and plant infections with antibiotics alone. In this article, we briefly describe the past history of bacteriophages, early clinical studies with phages, reasons of their abandonment especially in West, recent trials suggesting their utility and the future prospects for treatment and research in humans.

DISCOVERY

The discovery of bacteriophages is controversial (Duckworth, 1976; Summers, 2001) but the first observation seems to be by Ernest Hankin, a British bacteriologist, who reported in 1896, the presence of marked antibacterial activity (against *Vibrio cholerae*) in the waters of Ganges and Yamuna rivers in India (Stone, 2002). Two years later, Russian bacteriologist Gamaleya, observed similar phenomenon while working with *Bacillus subtilis* (Samsygina, 1984). However, it was Frederick Twort (1915), a medically trained bacteriologist from England, who explored the subject and gave the hypothesis that it may be, among the other possibilities, a virus. Twort could not pursue his findings further and it was Felix d'Herelle (1917), a French – Canadian microbiologist working at the Pasteur Institute in Paris, who 'officially' discovered the bacteriophages and gave clear description of 'the bacteriophage phenomenon' and 'plaque assays'. He also proposed the name 'Bacteriophage' (bacteria + Phagein – to eat or devour, in Greek) and was meant to imply that phages 'eat' or 'devour' bacteria (Sulakvelidze, 2001).

EARLY PHAGE THERAPY TRIALS

D'Herelle observed what he called an 'invisible microbe' that was present in the bacteria free filtrates of stool

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samples from dysentery patients. His initial motivation for investigation was that he suspected some filterable virus as a cofactor in the pathogenecity of dysentery, but as he investigated this idea, he found that the phage titers usually were low or absent at the beginning of the illness, increased dramatically as the illness progressed, and were highest as recovery was occurring. A natural extension of d'Herelle's concept of phage as the agent of recovery in natural infectious disease was his attempt to introduce phages as therapeutic agents. D'Herelle (1926) first tested the therapeutic utility of phages by carrying out various studies in field with avian typhosis (*Salmonella gallinarum*) and in the laboratory with *Shigella dysenteriae* infection of rabbits. The early studies on the use of phages to control epidemics of avian typhosis seem rather reasonable. He inoculated numerous flocks of chickens on farms in several widely separated regions of France where the epidemic was severe. The overall results suggested that phage treated flocks had fewer deaths, the duration of the epidemic was shorter, and the second rounds of infection were prevented. D'Herelle results were confirmed for the same disease in Holland by Kramer (d'Herelle, 1926).

Phage therapy was also evaluated in field trials against bovine hemorrhagic septicemia in Indochina. In this disease too, phages specifically used against causative bacterium (now called *Pasteurella multocida*) were successful in treating the disease, usually a highly fatal infection (Summers, 2001).

D'Herelle's next step was to extend his trials to humans. The procedures for conducting human trials in the 1920's were crude and inadequate by current standards, but d'Herelle first determined the safety of his phage preparations by self- administration. After being assured of no harmful effects, he extended the treatment to patients afflicted with bacillary dysentery (Summers, 2001). He also used phages successfully to treat four patients having bubonic plague in Egypt. This work probably attracted the most attention and was followed by large-scale human trials in India, during which phages were used especially to control cholera epidemics (Summers, 2001).

D'Herelle's commercial laboratory in Paris produced several phage based preparations directed against bacteria commonly associated with various diseases; for example, Bacte-intest-phage (phages directed against intestinal infections), Bacte – rhino- phage (phages directed against respiratory infections), Bacte-coli-phage (phages directed against coliforms), Bacte-pyo-phage (phages directed against pyogenic bacteria) etc. and they were marketed by what later became a large French company L'oreal (Sulakvelidze, 2001). Therapeutic phages were also produced for human use in the United States by, the Eli Lilly Company. These were used to treat various infections including abscesses, suppurating wounds, vaginitis, acute and chronic infections of the upper respiratory tract and mastoid infections (Sulakvelidze and Morris, 2001).

The routes of administration and the dosing regimens for the therapy were selected according to the etiological agents and the severity of the infection like: 1) orally, rectally (via enemas) to treat gastrointestinal infections; 2) topically (as tampons, rinses and creams) to treat skin, eye, ear, nasal mucosa and other infections; 3) via aerosols or intrapleural injections, to treat respiratory tract infections and 4) intravenously, to treat bacteremia (Sulakvelidze and Morris, 2001).

In Eastern Europe, and especially in the former Soviet Union, therapeutic phage research and development was actively carried out, at the Hirszfeld Institute of Immunology and Experimental Therapy (HIIET) of the Polish Academy of Sciences, Wroclaw, Poland and at the Eliava Institute of Bacteriophage, Microbiology and Virology (EIBMV) of the Georgian Academy of Sciences, Tbilisi. These institutes were instrumental in developing and producing phages for the treatment of septicemia, furunculosis, pulmonary and urinary tract infections and for the prophylaxis or treatment of postoperative and post traumatic infections (Sulakvelidze, 2001). Till date, these institutions are supplying therapeutic phages to the various hospitals, clinics and pharmaceutical companies all over the world. Several laboratories have also established a non-profit Eliava Phage Production Renaissance Foundation jointly to set up a central local and research scale production facility. Phage Therapy Centre, Tbilisi, Republic of Georgia, has recently opened a phage therapy centre at Tijuana, Mexico where patients with infected wounds that are resistant to antibiotics can travel and get treated. It is difficult to know how many patients have been treated with phages since their discovery, but millions does not seem to be an overestimate. The history of phage therapy can be divided into four periods: early enthusiasm, critical skepticism, abandonment, recent interest and appraisal (Summers, 2001).

REASONS FOR FAILURE

Although many early phage therapy trials were reported successful, there were also failures. Several factors,

including the lack of a general understanding of phage biology and imperfections in the diagnostic bacteriology available at that time, may have contributed to the failure of some early phage therapy studies (Sulakvelidze and Morris, 2001). Due to high specificity of phages and narrow host range, it was necessary to choose specific phages for a specific species / strain of bacteria but they were frequently used to treat infections by bacteria that were insensitive to phages or even against diseases that were not caused by bacteria (Bradbury, 2004). Moreover, the technology for producing large quantities of purified, stable phage preparations was not available at that time. Often the commercial phage preparations used were poorly characterized and contained no viable phages (Sulakvelidze and Morris, 2001). Also many of phage therapy trials were conducted without any controls and absence of a double blind study design (Pirisi, 2000). Interestingly, political considerations also played a role in decreasing the popularity of phage therapy in Western world by clearly tainting it as a part of 'Soviet Medicine'. Despite all these reasons, it was the overshadowing discovery of antibiotics, which seemed like 'Magic bullets' against a very broad spectrum of bacteria that made the use of phage therapy gradually fell out of favour in the United States and Western Europe (Sulakvelidze and Morris, 2001).

BACTERIOPHAGES AS THERAPEUTIC AGENTS

Bacteriophages as therapeutic agents boast of several advantages over antibiotics. Bacteriophages are very specific; usually affect only the targeted bacterial species (Sulakvelidze, 2001) as compared to antibiotics which target both pathogens and normal microflora. So, microbial balance in the patient is disturbed leading to serious secondary infections. Phages are also capable of exponential growth, replicating at the site of infection (Pirisi, 2000). Thus, are available where they are most needed. Development of resistance to antibiotics as well as its transfer among various bacterial species is major drawback of antibiotics. Although there are some exceptions, phages tend not to cross species boundaries (Carlton, 1999). Moreover, Phages are living organisms, so can undergo mutations, some of which can overcome bacterial mutations (Pirisi, 2000). Other advantage is that still not many serious side effects have been described for phage therapy (Sulakvelidze, 2001). If needed, we can also select new phage which is a relatively rapid process that can be accomplished in days or weeks than developing a new antibiotic which may take several years (Sulakvelidze, 2001).

LITERATURE ON THE USE OF PHAGE THERAPY

On thorough pubmed search, a number of articles are listed which show the potential applications of bacteriophages in the field of veterinary medicine as well as agriculture, aquaculture and food industry. Much evidence in support of the effectiveness of phages against bacterial infections are available since 1980's.William Smith et al (1982, 1983) reported the successful use of phages to treat experimental *Escherichia coli* infections in mice, calves, lambs and piglets. Soothill et al (1988, 1992 and 1994) reported the utility of phages in preventing and treating experimental disease in mice and guinea pig infected with *Pseudomonas aeruginosa* and *Acinetobacter* and they also suggested that phages might be efficacious in preventing infections of skin grafts used to treat burn patients.

Among Gram negative bacteria, extended spectrum beta lactamases (ESBL) producing bacteria pose specific threat. Similarly, among nonfermenters, imipenem resistant *Pseudomonas aeruginosa* infections are a matter of grave concern. Studies are going on which show effectiveness of phages against such bacteria when used in mouse model (Wang *et al.*, 2006). So, time has come to evaluate their therapeutic efficacy against these infections in humans also.

Nakai *et al.* (1999) explored the use of phages to treat or control fish pathogens. The use of phages to control fish diseases and other infections in aqueous environments seems particularly promising. In agriculture industry, the phages have been successfully used to clean fresh cut fruits and vegetables of *Listeria* and *Salmonella*. Unlike chemical treatment or heating, phage treatment does not harm the fruit and also targets only specific pathogen, rather than wiping out the whole microflora. Use of phages has also been explored in food processing to kill *Salmonella* and *Campylobacter* on chicken and entero-haemorrhagic *Escherichia coli* in beef. Some researchers are studying the use of phages to improve food safety by ridding food animals such as oysters and beef cattle of bacteria that are pathogenic to humans (Stone, 2002; Thacker, 2003).

FUTURE PROSPECTS FOR PHAGE THERAPY IN HUMANS

Multidrug resistant bacteria have opened up a second window for phage therapy. It can even serve as a stand-alone therapy against infections by such bacteria. Appropriate administration of phages can be used to treat lethal infections by Gram negative bacteria such as

Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella pneumoniae and *Salmonella* species and Gram positive bacteria such as *Enterococcus faecium* and *Staphylococcus aureus* (Matsuzaki, 2005).

Phages have been found to be useful in treatment of post burn infections particularly with organisms like *Pseudomonas* that are known to be notoriously resistant to variety of antimicrobials (Ahmad, 2002). Phages could be used to remove certain pathogens like *Staphylococcus aureus* including methicillin resistant *Staphylococci* (MRSA) that can colonize human skin and membranes and pose a threat to hospitalized patients with catheters or open wounds (Parffit, 2005). New models are also being developed to use phage therapy to kill intracellular bacterial pathogens like *Mycobacterium tuberculosis* (Broxmeyer, 2002).

They are also useful as a treatment for certain wounds such as diabetic ulcers, in which poor circulation hinders the use of antibiotics (Thacker, 2003). Phages could be used in hospitals as a prophylactic to decolonize immuno -compromised patients or patients waiting for surgery (Pirisi, 2000). A novel wound healing sustained release matrix based on biodegradable polyester amides and impregnated with lytic bacteriophages and an antibiotic under the name of Phagobioderm has been tried in management of infected venous stasis ulcers and poorly healing wounds (Markoishvili, 2002).

They could act as ideal candidates for co therapy with antibiotics. The co administration can help prevent the emergence of bacterial resistance to antibiotics, thereby, greatly prolonging their clinical usefulness (Carlton, 1999). Genetically engineered phages could also be prepared to deliver antimicrobial agents to bacteria as an alternative therapy for treatment of bacterial infections. Delivery of lethal agent phagemids by non-lytic phages can reduce target bacterial numbers by several folds (Westwater, 2003).

Researchers are exploring a new approach, where bacterial infection will not be treated by live phages but with specific phage enzymes that lyse bacteria. These lysins which are purified phage encoded peptidoglycan hydrolases, target specific bacteria and are reported to be effective for treatment of bacterial infections, especially by Gram positive bacteria such as *Streptococcus pyogenes, Streptococcus pneumoniae, Bacillus anthracis* and *Group B streptococci* (Matsuzaki, 2005).

It is also possible to genetically engineer phages to express new traits of potential value. Whole phage particles can be used to deliver vaccines in form of immunogenic peptides attached to modified phage coat proteins or as delivery vehicles for DNA vaccines (Clark, 2004; Jepson, 2004).

Recently, it has been suggested that potential of bacteriophages against bioterrorism could also be exploited (Inal, 2003; Walter, 2003). Confirmation and implication of the immunological role of phages could open up novel perspective for their potential use in the treatment of cardiovascular and autoimmune disease, graft rejection and cancer (Gorski, 2003).

CONCLUSIONS

Interest in phage therapy has been recently rekindled because of the emergence of multidrug resistant bacteria. Bacteriophages have several characteristics, which make them potentially attractive therapeutic agents, and time has come to carefully revisit this therapeutic option. More than a century later after a British chemist caught the first whiff of phage in water from the Ganges river, that the firm Gangagen, India (*www.gangagen.com*) is hoping to put Bangalore on the phage therapy map along with atleast ten other biotech companies including Intralytix *(www.intralytix.com)* and Exponential Biotherapies (USA), Novolytics (UK) and Phage Biotech Limited, Israel (*www.phage-biotech.com*). Phage Biotech Limited is presently conducting second round of animal pre-clinical experiments of *Pseudomonas* keratitis and endophthalmitis phage treatments with excellent preliminary results; Exponential Biotherapies focuses primarily on vancomycin resistant *enterococcus* (VRE) and have successfully completed Phase 1 clinical trials; Biochimpharm owns the large privatized production facility for phage tablets but despite all this, bureaucratic and regulatory hurdles pose a considerable challenge. Phage related studies will have to face increased scrutiny by the food and drug administration (FDA). However, FDA has not yet issued any written guidelines on how it intends to regulate phage therapy. It is not even clear, which section of the FDA will supervise phage-related products. Different sections of the FDA might regulate different aspects of phage, depending on whether it is used as a topical agent, or given intravenously or sprayed on vegetables. In addition to these regulatory and scientific uncertainties, phage enthusiasts face one more hurdle: public acceptance (Stone, 2002). It is the right time to start educating people about phages as safe, natural delivery systems instead of being just viruses. However, the positive outcome of many previous human phage

therapy trials in Eastern Europe and the former Soviet Union, and the recent promising results from animal and human studies in Great Britain and other countries suggest that phage therapy, if properly applied, can be of great value as part of an overall approach to control infectious diseases either alone or as co-therapy with antibiotics.

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BIODIVERSITY CHECKLIST OF GELECHIOID (LEPIDOPTERA) FAUNA OF HARYANA

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Abstract

Intensive and extensive surveys between months of February and November during 2000-2004 in pursuit of collecting as many examples of superfamily Gelechioidea from Haryana, a North Indian state, resulted in procurement of 80 species as against 37 recorded so far. Out of these, 41 species were new. Kalesar was observed to be the best site for collection of aforementioned fauna due its richness in well protected natural flora. Furthermore, month of August was experienced as the most ideal period both in terms of abundance of biodiversity and number of specimens of the concerned fauna.

Key words: Lepidoptera, Gelechioidea, checklist, Haryana, India.

INTRODUCTION

So far, only 37 species of superfamily Gelechioidea (Lepidoptera) have been recorded from the North Indian state of Haryana. Climatic conditions in this part of India show great amount of variability during the year, ranging from extremely dry and hot summers from April to June, moderate to heavy rainfall in July and August and winter season during December to February. In view of extremely exiguous reporting of fauna of this group by all the workers so far, repeated sampling was done during February to November of 2000-2004 in 11 localities viz., Darpur, Faridabad, Kalesar, Kalka, Morni, Naraingarh, Panchkula, Pipli, Rewari, Shahabad and Chandigarh to bring to light the unrecorded fauna. Intensive and extensive efforts of collection, using a light lure system comprising of a white sheet measuring 9'× 7' and two 160W mercury bulbs at night (Holloway *et al.,* 1984; Robinson *et al.,* 1994; Walia and Wadhawan, 2002), supplemented by net sweeping during day time yielded as many as 881 examples comprising of 542 males and 339 females of gelechioid moths.

Superfamily Gelechioidea moths are characterised by the presence of scales on the proboscis and by the absence of tympanal organs. Scoble (1995) recognised 11 families under this superfamily in comparison to 15 by Hodges (1999). However, authors have followed the classification given by Scoble (1995), the one followed by Heppner (1998) as well.

After thoroughly comparing the procured gelechioid fauna with entire concerned literature (Bengtsson, 1995; Clarke, 1955, 1963, 1965; 1969; Fletcher, 1929; Gaede, 1937; Meyrick, 1894, 1904 1906, 1908, 1909, 1910, 1910a, 1912–1916, 1913, 1916–1923, 1922, 1923–1930, 1925, 1930–1936; Pajni and Deepak Mehta, 1986; Park, 1995, 1999, 2000; Rose and Pathania, 2003, 2003a; Pathania and Rose, 2004; Robinson *et al.*, 1994; Stainton, 1858; Walker, 1864), 41 species were determined as new out of total identified 80 species belonging to families Gelechiidae (32 species), Lecithoceridae (12 species), Oecophoridae (23 species), Cosmopterigidae (8 species), Blastobasidae (3 species) and Scythrididae (2 species) (Fig. 1). In addition, 4 new combinations for already described species were also proposed. 17 new species pertaining to families Gelechiidae and Cosmopterigidae have already been published by the authors (Wadhawan and Walia, 2005; Walia and Wadhawan, 2004, 2004a, 2004b, 2005, 2005a, 2005b, 2005c, 2005d, 2006, 2006a).

Taxonomic status of the investigated species, along with number of specimens and months of collection of each is given below. Status of abundance is shown as VC : Very Common, C : Common, R : Rare, VR : Very Rare.

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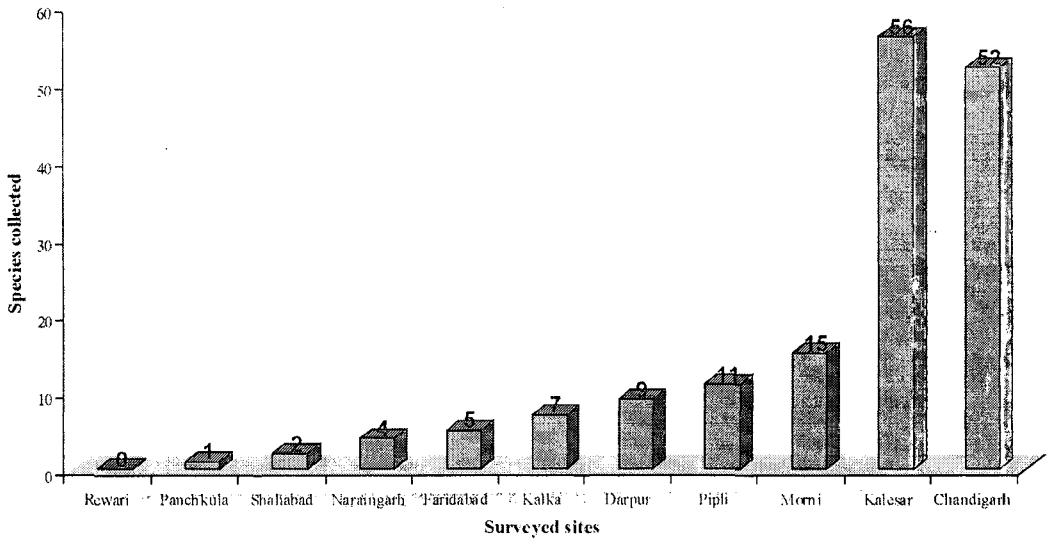

Fig. 1: Biodiversity of Gelechioid fauna in Haryana.

SYSTEMATIC ACCOUNT

ORDER : LEPIDOPTERA

SUPERFAMILY : GELECHIOIDEA

A) FAMILY : GELECHIIDAE

i) SUBFAMILY : GELECHIINAE

1. *Polyhymno alcimacha* Meyrick VC

   Polyhymno alcimacha Meyrick, 1918, *Exotic Microlepidoptera,* **2 :** 129.

   Material examined : 22♂, 17♀♀; Chandigarh; Kalesar (Forest Rest House), Kalka; June to September.

2. *Thiotricha albicephalata* Walia & Wadhawan VR

   Thiotricha albicephalata Walia & Wadhawan, 2005, *Pb. Univ. Res. Journ. (Sci.),* **55:** 81-85.

   Material examined : 1♂, 2♀♀; Kalesar (Forest Rest House), Morni (Forest Rest House); May and August.

3. *Thiotricha animosella* (Walker) VR

   Gelechia animosella Walker, 1869, *List. Lep. Het. Brit. Mus.,* **30 :** 1022.

   Material examined : 2♂♂; Chandigarh; March.

4. *Thyrsostoma shivai* Walia & Wadhawan R

   Thyrsostoma shivai Walia & Wadhawan, 2005, *Pb. Univ. Res. Journ. (Sci.),* **55:** 75-80.

   Material examined : 4♂♂; Kalesar (Forest Rest House); September.

5. *Thyrsostoma albilustra* Walia & Wadhawan VR

   Thyrsostoma albilustra, Walia & Wadhawan, 2005, *Pb. Univ. Res. Journ. (Sci.),* **55:** 75-80.

   Material examined : 2♂♂, 1♀; Kalesar (Forest Rest House); April and August.

6. *Idiophantis melanosacta* Meyrick R

   Idiophantis melanosacta Meyrick, 1907, *J. Bombay nat. Hist. Soc.,* **18 :** 149.

   Material examined : 4♂♂; Kalesar (Forest Rest House), Chandigarh; March, April, August and September.

7. *Syrmadaula signumforcipatus* Walia & Wadhawan VC

   Syrmadaula signumforcipatus Walia & Wadhawan, 2004, *Biol. Memoirs,* **30** (2) : 100-103, 3 phs., 6 figs.

Material examined : 24♂♂, 28♀♀; Chandigarh. Kalesar (Forest Rest House), Darpur (Forest Rest House), Pipli (Zoological Park); March to October.

8. *Diastaltica asymmetria* Walia & Wadhawan C

Diastaltica asymmetria Walia & Wadhawan, 2006, *Uttar Pradesh J. Zool.*, **26** (1) : 47-51.

Material examined : 5♂♂, 5♀♀; Kalesar (Forest Rest House); May and August.

9. *Brachyacma tabellata (Meyrick)* R

Paraspistis tabellata Meyrick, 1913, *J. Bombay nat. Hist. Soc.*, **22** : 770.

Material examined : 4♂♂, 1♀; Chandigarh. Kalesar (Forest Rest House); July to September.

10. *Pityocona bifurcatus* Walia & Wadhawan VC

Pityocona bifurcatus Walia & Wadhawan, 2006, *Polish Journ. of Ent.* (Communicated).

Material examined : 32♂♂, 5♀♀; Chandigarh. Kalesar (Forest Rest House), Pipli (Zoological Park), Kalka, Faridabad (Badkhal Lake); March to October.

11. *Telphusa signata* Walia & Wadhawan R

Telphusa signata Walia & Wadhawan, 2005, *J. Appl. Bioscience*, **31** (1) : 39-42.

Material examined : 3♂♂, 1♀; Kalesar (Forest Rest House), Chandigarh; April, July and August.

12. *Stegasta valvulata* Walia & Wadhawan VC

Stegasta valvulata Walia & Wadhawan, 2004, *Pb. Univ. Res. Journ. (Sci.)*, **54** : 117-126.

Material examined : 5♂♂, 10♀♀; Faridabad (Badkhal Lake), Kalesar (Forest Rest House), Chandigarh; March, June and August to October.

13. *Stegasta comissata* Meyrick VC

Stegasta comissata Meyrick, 1923, *Exotic Microlepidoptera*, **3** : 18.

Material examined : 21♂♂, 23♀♀; Kalesar (Forest Rest House), Morni (Forest Rest House), Darpur (Forest Rest House), Chandigarh; March to October.

14. *Stegasta pawani* Walia & Wadhawan C

Stegasta pawani Walia & Wadhawan, 2004, *Pb. Univ. Res. Journ. (Sci.)*, **54** : 117-126.

Material examined : 9♂♂, 1♀; Kalesar (Forest Rest House), Morni (Forest Rest House), Chandigarh; June, September and October.

15. *Anarsia reciproca* Meyrick VC

Anarsia reciproca Meyrick, 1916, *Exotic Microlepidoptera*, **2** : 300.

Material examined : 6♂♂, 15♀♀; Pipli (Zoological Park), Chandigarh; May to October.

16. *Hypatima tephroptila* (Meyrick) comb. nov. VR

Chelaria tephrophila Meyrick, 1931, *Exot. Microlepidoptera*, **4** : 70.

Material examined : 3♂♂; Kalesar (Forest Rest House), Chandigarh; May and August.

17. *Ganpati valvasymmetria* Walia & Wadhawan VC

Ganpati valvasymmetria Walia and Wadhawan, 2005, *Uttar Pradesh J. Zool.*, **25** (2) : 213-216.

Material examined : 6♂♂, 6♀♀; Kalesar (Forest Rest House), Chandigarh; August and September.

ii) SUBFAMILY : DICHOMERIDINAE

18. *Dichomeris rasilella* (Herrich-Schäffer) C

Anacampsis rasilella Herrich-Schäffer, 1855, *Schmett. Eur.*, **5** : 202.

Material examined : 7♂♂ ; Naraingarh (Forest Rest House), Chandigarh; March, September and October.

19. *Dichomeris sicasymmetria* Walia & Wadhawan VR

Dichomeris sicasymmetria Walia & Wadhawan, 2004, *Pb. Univ. Res. Journ. (Sci.)*, **54** : 83 – 100.

Material examined :1♂; Kalesar (Forest Rest House); April and August.

20. *Dichomeris kalesarensis* Walia & Wadhawan VC

Dichomeris kalesarensis Walia & Wadhawan, 2004, *Pb. Univ. Res. Journ. (Sci.)*, **54** : 83 – 100.

Material examined : 21♂♂, 21♀♀; Kalesar (Forest Rest House), Chandigarh; May, August and September.

21. *Dichomeris hansi* Walia & Wadhawan R

Dichomeris hansi Walia & Wadhawan, 2004, *Pb. Univ. Res. Journ. (Sci.)*, **54** : 83 – 100.

Material examined : 5♂♂; Morni (Forest Rest House), Kalka, Chandigarh; August and September.

22. *Dichomeris fuscodelta* Walia and Wadhawan C

Dichomeris fuscodelta Walia and Wadhawan, 2004, *Pb. Univ. Res. Journ. (Sci.)*, **54** : 83 – 100.

Material examined : 8♂♂, 4♀♀; Chandigarh. Kalesar (Forest Rest House), Kalka; June, August to October.

23. *Dichomeris bispotalis* Walia and Wadhawan VR

Dichomeris bispotalis Walia and Wadhawan, 2004, *Pb. Univ. Res. Journ. (Sci.)*, **54** : 83 – 100.

Material examined : 3♂♂; Panchkula; May.

24. *Helcystogramma hoplophora* (Meyrick) comb. nov. C

Onebala hoplophora Meyrick, 1916, *Exotic Microlepidoptera*, **1** : 577–578.

Material examined : 3♂♂, 7♀♀; Faridabad (Badkhal Lake), Kalesar (Forest Rest House), Darpur (Forest Rest House), Chandigarh; February to May and September.

25. *Helcystogramma hibisci* (Stainton) VR

Gelechia hibisci Stainton, 1858, *Trans. ent. Soc. London*, **5**(2) : 117.

Material examined : 1♂, 1♀; Chandigarh; Kalesar (Forest Rest House); April and October.

26. *Helcystogramma leucoplecta* (Meyrick) comb.nov. VR

Strobisia leucoplecta Meyrick, 1910, *J. Bombay nat. Hist. Soc.*, **20** : 729–730.

Material examined : 1♂, 1♀; Morni (Forest Rest House; March.

27. *Brachmia xerastis* (Meyrick) R

Torodora xerastis Meyrick, 1905, *J. Bombay nat. Hist. Soc.*, **16** : 599.

Material examined : 2♂♂, 2♀♀; Chandigarh; Kalesar (Forest Rest House); March and May.

28. *Brachmia arotraea* (Meyrick) VR

Cladodes arotraea Meyrick, 1894, *Trans. Ent. Soc. Lond.* **1894** : 15.

Material examined : 2♂♂, 1♀; Chandigarh; Kalesar (Forest Rest House); March and August.

iii) SUBFAMILY : SYMMOCINAE

29. *Veinspastus bicornuta* sp. nov. VC

Material examined : 8♂♂, 16♀♀; Morni (Forest Rest House), Naraingarh, Chandigarh; March to July.

30. *Indospastus fuscospotalis* sp. nov. R

Material examined : 2♂♂, 4♀♀; Kalesar (Forest Rest House), Morni (Forest Rest House), Chandigarh; March and June.

31. *Apiuncusa asignata* sp. nov. VC

Material examined : 7♂♂, 7♀♀; Chandigarh, Kalesar (Forest Rest House); March and July to September.

32. *Apiuncusa discata* sp. nov. R

Material examined : 4♂♂, 1♀; Chandigarh, Morni (Forest Rest House); April to June.

B) FAMILY : LECITHOCERIDAE

i) SUBFAMILY : LECITHOCERINAE

33. *Heteralcis spatulata* sp. nov. R

Material examined : 5♂♂; Kalesar (Forest Rest House); July to September.

34. *Eridachtha xanthocephalata* sp. nov. R

Material examined : 4♂♂, 1♀; Morni (Forest Rest House), Kalesar (Forest Rest House), Chandigarh; March, May, August and September.

35. *Homaloxestis xylotripa* Meyrick R

Homaloxestis xylotripa Meyrick, 1918, *Exotic Microlepidoptera*, **2** : 102.

Material examined : 4♂♂, 2♀♀; Kalesar (Forest Rest House), Chandigarh; March, April and September.

36. *Homaloxestis fuscoannulata* sp. nov. VR

Material examined : 2♂♂; Darpur (Forest Rest House), Kalesar (Forest Rest House); June and August.

37. *Lecithocera xanthoantennalis* sp. nov. VC

Material examined : 5♂♂; 7♀♀; Kalesar (Forest Rest House), Naraingarh, Chandigarh; March, April and June.

38. *Lecithocera acuta* sp. nov. VR

Material examined : 1♂; Chandigarh; September.

39. *Lecithocera xanthocostalis* sp. nov. C

Material examined : 6♂♂, 2♀♀; Kalesar (Forest Rest House), Pipli (Zoological Park); August to October.

ii) SUBFAMILY : TORODORINAE

40. *Cophomantella lysimopa* (Meyrick) comb. nov. C

Cophomantis lysimopa Meyrick, 1933, *Exotic Microlepidoptera,* **4** : 357.

Material examined : 3♂♂, 5♀♀; Chandigarh, Kalesar (Forest Rest House), Darpur, Morni; March and April.

41. *Chlorolychnis faridabadensis* sp. nov. R

Material examined : 4♂♂; Fardiabad (Badkhal Lake); October.

42. *Torodora quadrangulata* sp. nov. VR

Material examined : 1♂; Chandigarh; June.

43. *Torodora biovalis* sp. nov. VR

Material examined : 2♂, 1♀; Chandigarh, Naraingarh; March, April and September.

44. *Torodora proxianuliata* sp. nov. VC

Material examined : 10♂♂, 7♀♀; Kalesar (Forest Rest House); September.

C) FAMILY : OECOPHORIDAE

i) SUBFAMILY : STATHMOPODINAE

45. *Stathmopoda theoris* (Meyrick) VR

Aeoloscis theoris Meyrick, 1906, *J. Bombay. nat. Hist. Soc.,* **17** : 410.

Material examined: 12♂♂, 34♀♀; Kalesar (Forest Rest House), Chandigarh, Faridabad (Badkhal Lake), Kalka, Pipli (Zoological Park); March to October.

46. *Stathmopoda anconias* Meyrick R

Stathmopoda anconias Meyrick, 1910, *Rec. Indian Mus.,* **15** : 223.

Material examined : 2♂, 3♀♀; Kalesar (Forest Rest House), Kalka; May, August and September

47. *Stathmopoda bifascialis* sp. nov. VR

Material examined : 1♂, 2♀♀; Kalesar (Forest Rest House), Chandigarh; August and September.

ii) SUBFAMILY : AUTOSTICHINAE

48. *Apethistis insulsa* (Meyrick) VC

Brachmia insulsa Meyrick, 1913, *Journ. Bombay nat. Hist. Soc.,* **22** : 774.

Material examined : 73♂♂, 10♀♀; Chandigarh, Pipli (Zoological Park), Kalesar (Forest Rest House), Morni (Forest Rest House); March to October.

49. *Apethistis arorai* sp. nov. VR

Material examined : 1♂, 2♀♀; Chandigarh; April and August.

iii) SUBFAMILY : HYPERTROPHINAE

50. *Eupselia isacta* Meyrick VC

Eupselia isacta Meyrick, 1910, *J. Bombay nat. Hist. Soc.,* **20** : 153–154.

Material examined : 10♂♂, 1♀; Kalesar (Forest Rest House), Chandigarh; July.

iv) SUBFAMILY : XYLORYCTINAE

51. *Aeolanthes rhodochrysa* Meyrick C

Aeolanthes rhodochrysa Meyrick, 1907, *J. Bombay nat. Hist. Soc.,* **17**(4) : 739.

Material examined : 7♂♂, 3♀♀; Kalesar (Forest Rest House); August and September.

52. *Aeolanthes sagulata* Meyrick VR

Aeolanthes sagulata Meyrick, 1917, *Exotic Microlepidoptera,* **2** : 53.

Material examined : 1♀; Kalesar (Forest Rest House); September.

53. *Odites notocapna* Meyrick R

Odites notocapna Meyrick, 1925, in Caradja, *Mem. Sect. Sti. Acad. Românâ,* **3** : 382.

Material examined : 4♂♂, 2♀♀; Pipli (Zoological Park), Shahabad, Chandigarh; April, June and October.

v) SUBFAMILY : ETHMIINAE

54. *Ethmia didyma* Kun C

Ethmia didyma Kun, 2002. *Annales His.-nat. Mus. natn. hung.,* **94** : 171.

Material examined : 6♂♂, 2♀♀; Kalesar (Forest Rest House), Morni (Forest Rest House); March and October.

55. *Ethmia acontais* Meyrick C

Ethmia acontias Meyrick, 1906, *J. Bombay nat. Hist. Soc.,* **17** : 410.

Material examined : 8♂♂, 1♀; Kalesar (Forest Rest House), Morni (Forest Rest House), Chandigarh; March, April and July to October.

vi) SUBFAMILY : OECOPHORINAE

56. *Periacma plumbea* Meyrick R

Periacma plumbea Meyrick, 1919, *Exotic Microlepidoptera,* **2** : 238.

Material examined : 2♂♂, 2♀♀; Kalesar (Forest Rest House), Chandigarh; April, May and August.

57. *Periacma unequispinosa* sp. nov. VR

Material examined : 1♂, 2♀♀; Kalesar (Forest Rest House), Darpur (Forest Rest House); March and October.

58. *Periacma trispinosa* sp. nov. R

Material examined : 1♂, 3♀♀; Kalesar (Forest Rest House), Kalesar (Forest Rest House); July.

59. *Promalactis semantris* (Meyrick) VC

Epicallima semantris Meyrick, 1906, *J. Bombay nat. Hist. Soc.,* **17** : 409.

Material examined: 28♂♂, 13♀♀; Kalesar (Forest Rest House), Pipli (Zoological Park), Chandigarh; March, April, June to September.

60. *Promalactis sponsalis* Meyrick R

Promalactis sponsalis Meyrick, 1920, *Exotic Microlepidoptera,* **2** : 365.

Material examined: 3♂♂, 1♀; Morni (Forest Rest House), Chandigarh; March and September.

61. *Eonympha erythrozona* Meyrick R

Eonympha erythrozona Meyrick, 1906, *J. Bombay nat. Hist. Soc.,***17** : 407, by monotypy.

Material examined : 3♂♂, 3♀♀; Kalesar (Forest Rest House); August.

vii) SUBFAMILY : DEPRESSARIINAE

62. *Acria xanthosaris* Meyrick R

Acria xanthosaris Meyrick, 1908, *J. Bombay nat. Hist. Soc.,* **18** : 636.

Material examined : 4♂♂; Kalesar (Forest Rest House), Chandigarh; March, May, June and August.

63. *Cryptolechia dimorphica* sp. nov. R

Material examined : 4♂♂, 1♀; Kalesar (Forest Rest House); March and August.

64. *Cryptolechia tegumenta* sp. nov. VR

Material examined : 1♂, 1♀; Chandigarh; September.

65. *Tonica niviferana* (Walker) VR

Binsitta niviferana Walker, 1864, *List. Lep. Het. Brit. Mus.,* **29** : 832.

Material examined : 1♂, 2♀♀; Kalesar (Forest Rest House), Chandigarh; April and August

66. *Tonica gypsopis* Meyrick VR

Tonica gypsopis Meyrick, 1928, *Exotic Microlepidoptera,* **3** : 474.

Material examined : 2♂♂; Kalesar (Forest Rest House); August.

67. *Psorosticha saculata* sp. nov. VC

Material examined : 5♂♂, 6♀♀;Chandigarh, Kalesar (Forest Rest House); May, July and August.

D) FAMILY : COSMOPTERIGIDAE

68. *Macrobathra gentilis* Meyrick VC

Macrobathra gentilis Meyrick, 1918, *Exotic Microlepidoptera,* **2** : 212.

Material examined : 8♂♂, 11♀♀; Chandigarh, Kalesar (Forest Rest House); March to October.

69. *Macrobathra ochrefasciata* sp. nov. VR

Material examined : 3♀♀; Kalesar (Forest Rest House); March and June.

70. *Harpograptis basoxanthis* sp. nov. VR

Material examined : 2♀♀; Morni (Forest Rest House); October.

71. *Labdia molybdaula* (Meyrick) VC

Pryoderces molybdaula Meyrick, 1915, *Exotic Microlepidoptera,* **1** : 309–310.

Material examined : 7♂♂, 16♀♀; Chandigarh, Kalesar (Forest Rest House); February to April, August and September.

72. *Labdia symbolias* (Meyrick) VR

Pyroderces symbolias Meyrick, 1915, *Exotic Microlepidoptera,* **1** : 313.

Material examined : 1♂; Kalesar (Forest Rest House); March and August.

73. *Cosmopterix albilinearis* Wadhawan & Walia C

Cosmopterix albilinearis Wadhawan & Walia, 2005, *Polish Journ. of Ent.*, **74** (4): 471-477, 9 figs.

Material examined : 1♂, 6♀♀; Chandigarh, Pipli (Zoological Park), Darpur (Forest Rest House); March, June, August and October.

74. *Cosmopterix mimetis* Meyrick C

Cosmopterix mimetis Meyrick, 1909. *J. Bombay nat. Hist. Soc.*, **19** : 417.

Material examined : 6 ♂♂; Kalka, Chandigarh March; June and August to October.

75. *Stagmatophora dorsophanes* Meyrick VC

Stagmatophora dorsophanes Meyrick, 1918, *Exotic Microlepidoptera*,**2** : 412–413.

Material examined : 16♂♂, 13♀♀; Chandigarh; March to June and September.

E) FAMILY : BLASTOBASIDAE

76. *Blastobasis ochromorpha* Meyrick R

Blastbasis ochromorpha Meyrick, 1928, *Exotic Microlepidoptera*, **3** : 145.

Material examined : 5♂♂; Naraingarh, Pipli (Zoological Park), Chandigarh; May, July and August.

77. *Blastobasis fuscocephalis* sp. nov. R

Material examined: 5♀♀; Kalesar (Forest Rest House); June and September.

78. *Blastobasis spinalis* sp. nov. C

Material examined : 2♂♂, 4♀♀; Chandigarh; March, April and September.

F) FAMILY : SCYTHRIDIDAE

79. *Scythris persenophanes* Meyrick R

Scythris percenophanes Meyrick, 1930-1936, *Exotic Mcrolep.* **4** : 543.

Material examined : 5♀♀; Chandigarh, Kalesar (Forest Rest House), Darpur (Forest Rest House); March to May and September.

80. *Eretmocera impactella* (Walker) VC

Gelechia impactella Walker, 1864, *Cat. Lep. Het. Brit. Mus.*, **29** : 637.

Material examined: 36 ♂♂; Kalesar (Forest Rest House), Pipli (Zoological Park), Shahabad, Chandigarh; March to October.

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REPORTING OF THREE NEW SPECIES OF GENUS *LECITHOCERA* HERRICH-SCHÄFFER FROM NORTH INDIA ALONG WITH DIAGNOSIS OF A KNOWN SPECIES (LEPIDOPTERA : LECITHOCERIDAE)

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Abstract

Four species of genus *Lecithocera* Herrich-Schäffer, including three new, collected from different states of northern India are dealt with. In addition, both intra-individual and intra-specific variations in wing venation observed selectively in radial and cubital veins of forewings and that too only in two out of four species are highlighted.

Key words : Lepidoptera, Lecithoceridae, *Lecithocera*, 3 new species, North India.

INTRODUCTION

Genus *Lecithocera* Herrich-Schäffer, the largest and type genus of family Lecithoceridae is worldwide in distribution. Out of more than 200 species recorded so far under it (Park, 1999, 2000), majority are from the tropics and 60 from India. As per Park (1999), eight genera erected by previous workers viz., *Patouissa* Walker (1864), *Sarisophora* Meyrick (1904), *Brachyerga* Meyrick (1925), *Periphorectis* Meyrick (1925), *Xanthocera* Amsel (1963), *Xanthocerodes* Amsel (1955), *Leviptera* Janse (1954) and *Parrhasastris* Gozmány (1972) have been synonymised under this genus. Despite showing great degree of variations in wing venation, uniformity in significant male genitalic structures indicate that though largest yet this genus has a homogeneous assemblage.

Intensive and extensive collection surveys of various North Indian states during 2000-2004 resulted in collection of four species of this genus. One of the species was identified as *L. oxycona* Meyrick and the remaining three were found new addition to the existing fauna of this genus after thoroughly comparing with relevant literature (Amsel, 1955, 1963; Clarke, 1965; Gozmány, 1972; Janse, 1954; Meyrick, 1894, 1904, 1905, 1907, 1910, 1910a, 1911, 1912–16, 1916–1923, 1923–1930, 1925, 1930–1936; Nye and Fletcher, 1991; Park, 1999, 2000; Pathania and Rose, 2004; Walker, 1864) and are named as *Lecithocera acuta* sp. nov., *L. xanthoantennalis* sp. nov. and *L. xanthocostalis* sp. nov.

Present communication deals with detailed descriptions of the new species, along with the diagnosis of already known species. The text is supported by diagrams of wing venation and genitalia. In addition, observed variations in venation of forewings only in two species i.e. *oxycona* Meyrick and *xanthocostalis* sp. nov. are documented.

SYSTEMATIC ACCOUNT

Genus *Lecithocera* Herrich-Schäffer

Lecithocera Herrich-Schäffer, 1853, *Schmett. Eur.*, **5:** 11 (Key), 45.

ibidem, **6 :** *Microlepid.* pl. 12 *Syst. Bearbeitung* figs. 10, 11; 1854, *ibidem*, **5 :** 207.

Head with appressed scales. Antenna as long as or longer than forewing, more or less thickened towards base, simple or rarely ciliated in male; scape moderately elongate, without pecten. Labial palpi long, recurved, second segment thickened with appressed scales, often somewhat rough towards apex beneath, rarely with rough projecting scales below; third segment as long as or longer than second, slender, acute, or seldom flat in male, obtuse. Proboscis developed; maxillary palpi very short, filiform, appressed to proboscis. Forewing with R_1 from middle of anterior margin of discal cell; R_4 and sometimes R_3 out of R_5, or seldom R_4 absent or R_3, R_4 stalked, R_5 absent, R_5 if present to apex or termen; M_2 nearer to M_3 than M_1, M_3 originating far from CuA_1+CuA_2; CuA_1, CuA_2 stalked or seldom coincident; 1A+2A forming a basal fork. Hindwing as broad as or broader than forewing, trapezoidal, termen hardly sinuate, cilia two-third to four-fifth of wing width; Rs, M_1 stalked; M_2 rather approximated or nearly parallel, nearer M_3 than M_1; M_3, CuA_1 connate or stalked or sometimes coincident. Hind tibia clothed with hairs above.

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Type-species : *Carcina luticornella* Zeller, 1839, *Isis Oken, Leipzig* **1839 :** 197, by monotypy.

Key to the species of genus *Lecithocera* Herrich-Schäffer

1. Forewing without band or streak along costa; juxtal plate not bifurcated but comparatively more sclerotised at distal corners.....***xanthoantennalis* sp. nov.**

– Forewing with narrow or broad band along costa; juxtal plate bifurcated at distal end into two arms.....**2**

2. Costal margin of forewing marked with distinctly broad band; male genitalia with distal end of valvae gradually narrowed into bluntly pointed tip.....***acuta* sp. nov.**

– Costal margin of forewing bearing narrow streak; male genitalia with distal end of valvae broadly spatulate.....**3**

3. Costal streak on forewing light yellow; female genitalia with corpus bursae beset with innumerable minute denticles.....***oxycona* Meyrick**

– Costal streak on forewings dark yellow; female genitalia with corpus bursae without denticles.....***xanthocostalis* sp. nov.**

***Lecithocera xanthoantennalis* sp. nov.**

(Figs. 1 - 3, 7 - 12)

Male : Head iridescent brownish-fuscous, side tufts yellow, loosely raised; frons light brown. Antenna slightly longer than forewing, yellow; flagellum thickened at base, tapering distally, sparsely speckled with fuscous. Labial palpus yellow; second segment brownish-fuscous on outer surface; third segment shorter than second, fuscous below. Proboscis and maxillary palpi ochraceous (Fig. 1-3).

Thorax brownish-fuscous. Forewing (Fig. 7) oblong; costa slightly exarched; apex acutely angulate; termen oblique; tornus obtusely angulate. Ground colour fuscous on upper surface; a speck in middle of discal cell about one-third from base and a streak on discocellulars reaching upto inner margin blackish-fuscous; cilia along margin greyish-fuscous. Under surface greyish-fuscous, paler along costa. Discal cell about half of wing length. Hindwing (Fig. 8) as broad as forewing; costa exarched medially; apex acutely angulate; tornus obtusely angulate. Ground colour greyish-brown on upper and under surfaces; cilia along inner margin about three-fourth of wing width. Discal cell about half of wing length. Legs light ochraceous, speckled with fuscous; hind tibia whitish-ochraceous hair scaled above.

Abdomen with proximal and distal segments yellowish-ochraceous, remaining brown on upper surface; under surface brown. Male genitalia (Fig. 9, 10) with uncus bifurcated into two short lobes, each lobe setose at apex; gnathos broad and flat in basal half, narrowly beak-like and pointed in distal half; tegumen about two-third of U-like vinculum; saccus indistinct; juxtal plate comparatively more sclerotised at distal corners. Valvae broad at base gradually narrowed and bent distally, setose along ventral margin; costa bent, connected to tegumen by a strap-like process; blunt end of cucullus bearing tuft of setae; sacculus distinctly sclerotised. Aedeagus (Fig. 11) broader at anterior end, remaining narrow part of even width; vesica adorned with numerous minute and few large denticles; opening of ductus ejaculatorius anterior. Female genitalia (Fig. 12) with corpus bursae rounded, profusely denticulate; signum represented by moderately sclerotised plate beset with denticles; ductus bursae broad, about two and a half times longer than corpus bursae; ductus seminalis arisinging from S-like sclerotisation in ductus bursae, much broader at point of origin; genital plate adorned with short spines; anterior apophyses about half of posterior; papillae anales cone-like, bearing setae of varying lengths.

Alar expanse : Male : 12–13 mm; Female : 12–13 mm

Material examined : HOLOTYPE : ♂, 17.iv.2000; Kalesar (Forest Rest House), Haryana. PARATYPES : 1♀, 17.iv.2000; 1♀, 20.iv.2000; 1♀, 23.iii.2001; 1♀, 27.iii.2001; 1♂, 23.iii.2003; Kalesar (Forest Rest House), Haryana. 1♀, 22.iii.2001; Naraingarh, Haryana. 1♂, 1♀, 15.iii.2001; 1♀, 23.vi.2001; 1♂, 1♀, 11.iv.2003; Chandigarh. (Coll.: D. Wadhawan & V.K.Walia) Deposited in Entomology Section, Department of Zoology, Panjab University, Chandigarh.

Type locality : Kalesar (Haryana).

Etymology : Highlighting the yellow colouration of antennae, present species is accordingly named

Fig. 1

Fig. 2

Fig. 3

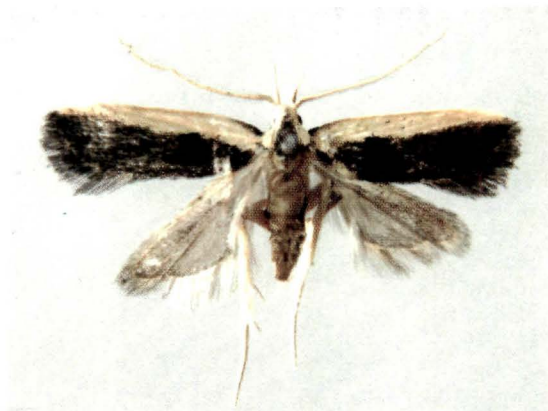


Fig. 4

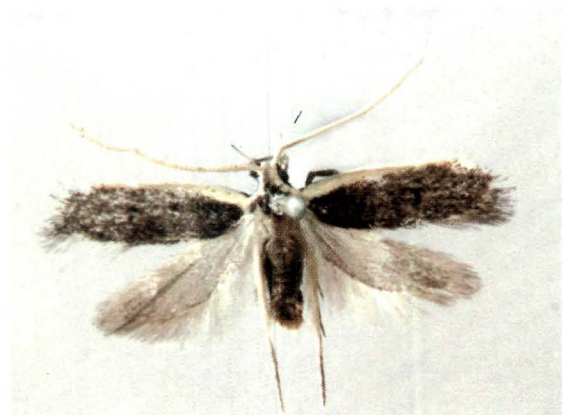


Fig. 5

Fig. 6

Figs. 1-6 : (1-3) *Lecithocera xanthoantennalis* sp. nov.- (1) dorsal view[live] , (2) lateral view[live], (3) dorsal view[stretched]; (4) *Lecithocera acuta* sp. nov.- dorsal view [stretched]; (5) *Lecithocera oxycona* Meyrick - dorsal view [stretched]; (6) *Lecithocera xanthocostalis* sp. nov. - dorsal view [stretched].

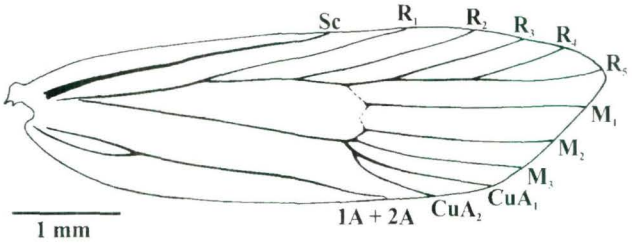


Fig. 7

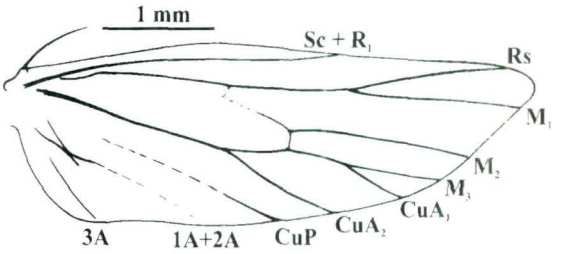


Fig. 8

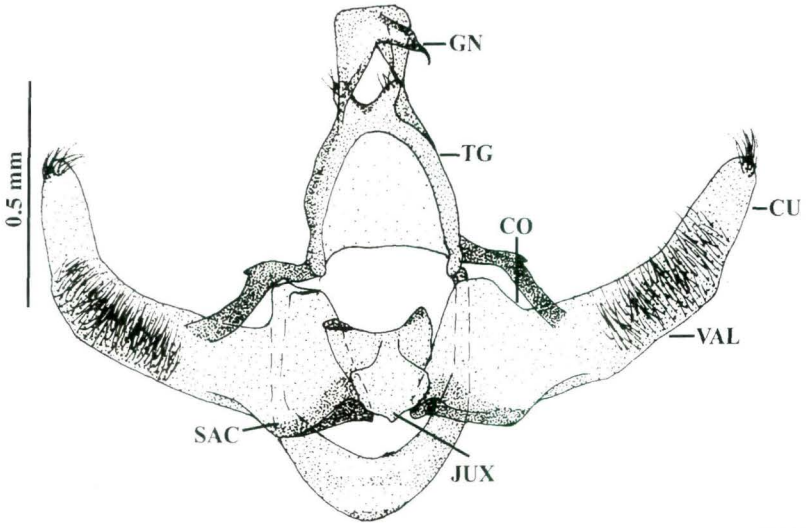


Fig. 9

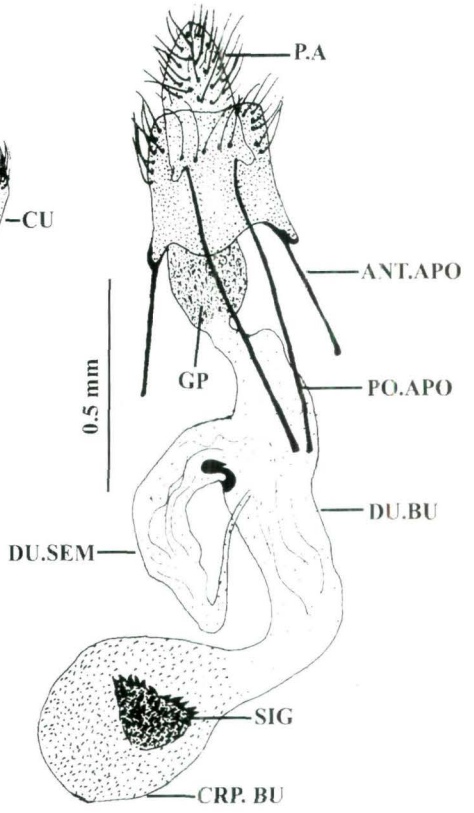


Fig. 12

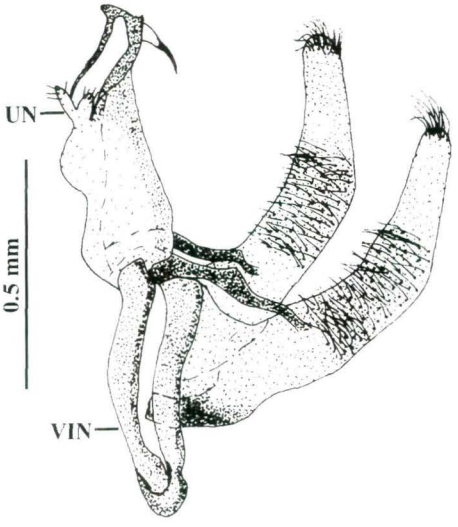


Fig. 10

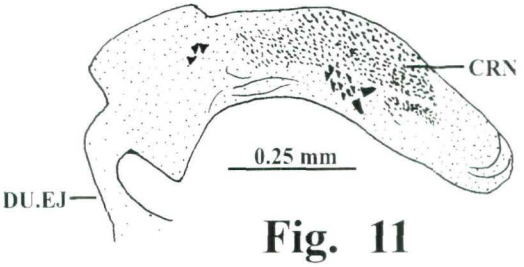


Fig. 11

Figs. 7-12 : *Lecithocera xanthoantennalis* sp. nov. - (7) forewing; (8) hindwing; (9) male genitalia (ventral view); (10) male genitalia (lateral view); (11) aedeagus; (12) female genitalia.

Lecithocera xanthoantennalis sp. nov.

Remarks : In contrast to whitish-ochraceous antennae, veins in forewings indicated by cloudy whitish-fuscous streaks and whitish-fuscous costa at base of the closely allied species *Lecithocera ichorodes* Meyrick, studied species has yellow antennae and uniformly fuscous forewings without any streak (Meyrick, 1910). Besides this, vesica in the aedeagus of the compared species is adorned with a spine at distal end which is absent in the *xanthoantennalis* sp. nov.

Live specimens of *L. xanthoantennalis* sp. nov. while resting on a substratum maintain their body in a slanting way with anterior end of body raised at an angle of about 40°. Also, both the antennae are tightly held together and projected in front of body (Fig.1,2).

Lecithocera acuta sp. nov.

(Figs. 4, 13 - 16)

Male : Head light yellow, with a longitudinal fuscous band in middle. Antenna slightly longer than forewing, light yellow; flagellum slightly broader at base, gradually narrowed towards apex. Labial palpi light yellow; second segment shaded with fuscous above, except at apex; third segment as long as second. Proboscis and maxillary palpi ochraceous. (Fig. 4)

Thorax fuscous; patagium and part of thorax below it light yellow. Forewing (Fig. 13) oblong; costa gently arched; apex acutely angulate, termen oblique; tornus obtusely angulate. Ground colour blackish-fuscous on upper surface; a light yellow broad band along costa gradually narrowed, terminating at apex; costa blackish-fuscous at base; a discal spot one-third away from base and a much larger equally away black; cilia along margin grey. Under surface grey, light yellow along costa. Discal cell nearly half of wing length, R_3 shortly stalked with R_4+R_5, R_5 to termen just below apex; CuA_1, CuA_2 stalked; CuP present only at margin. Hindwing (Fig. 14) broader than forewing; costa slightly excurved in middle; apex acutely angulate, somewhat produced; termen oblique; tornus obtusely angulate. Ground colour greyish-ochraceous on upper and under surfaces; cilia along inner margin about two-third of wing width. Discal cell about half of wing length; M_3, CuA_1 stalked. Legs yellowish-ochraceous, speckled with fuscous; hind tibia whitish-ochraceous hair scaled above.

Abdomen greyish-ochraceous on upper and under surfaces. Male genitalia (Fig. 15) with uncus short, bilobed with common base, each lobe blunt and sparsely setose; tip of falcate gnathos sharply pointed; tegumen shorter than broadly U-like vinculum; saccus wanting; juxtal plate produced into a narrow process at its latero-distal angles. Valvae broad at base, each gradually narrowed into bluntly pointed tip; costa concave, joined by strap-like process bent elbow joint-like joining tegumen; cucullus much narrower than rest of valva, profusely setose; sacculus comparatively more sclerotised along ventral edge, with a bunch of short spines near its end. Aedeagus (Fig. 16) broad proximally, narrowed and bent distally, flat at distal end, with a pair of short triangular projections on one side; vesica adorned with a group of spines of varying lengths and a large sclerotised plate adorned with numerous microscopic denticles; opening of ductus ejaculatorius anterior.

Alar expanse : Male : 14 mm ; Female : Not studied.

Material examined : HOLOTYPE : ♂, 21.ix.2001, Chandigarh.PARATYPES: 1♂, 30.viii.2002, Subathu (Forest Guest House) Himachal Pradesh. (Coll.: D. Wadhawan & V.K.Walia) Deposited in Entomology Section, Department of Zoology, Panjab University, Chandigarh.

Type locality : Chandigarh.

Etymology : Owing to acutely narrowed distal end of valvae as key feature of this species, it is named *Lecithocera acuta* sp. nov.

Remarks : Present species is closely allied to *L. caecilia* Meyrick of Ceylon as far as general body colouration, pattern, alar expanse, shape of valvae and aedeagus in male genitalia is concerned (Meyrick, 1916–1923; Clarke, 1965). However, it differs from compared species due to fuscous thorax, light yellow patagium and absence of marginal dots along apex and termen, in comparison to, ochraceous thorax, fuscous patagium and fuscous marginal dots around apex and termen in *L. caecilia.* Furthermore, ground colour of forewings of closely allied species is ochraceous in contrast to broadly light yellow costa and blackish-fuscous remaining part.

Lecithocera oxycona Meyrick

(Figs. 5, 17 - 22)

Lecithocera oxycona Meyrick, 1910, *Journ. Bombay nat. Hist. Soc.*, **20** : 444.

Male : Head with vertex light yellow, with a broad longitudinal medial band fuscous; frons fuscous.

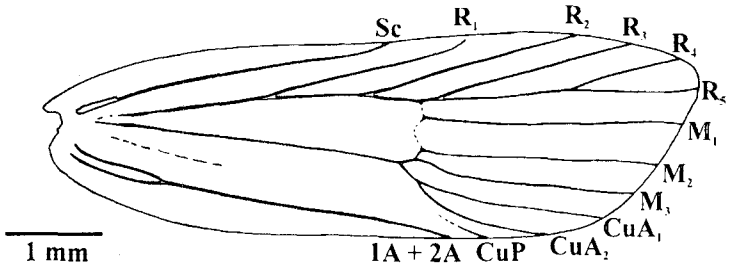


Fig. 13

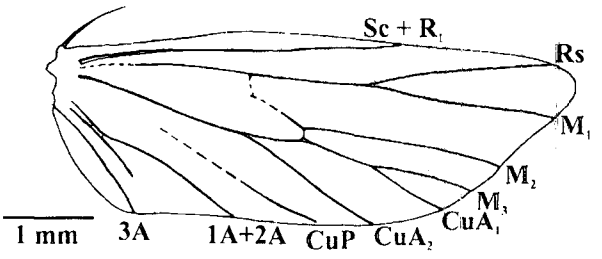


Fig. 14

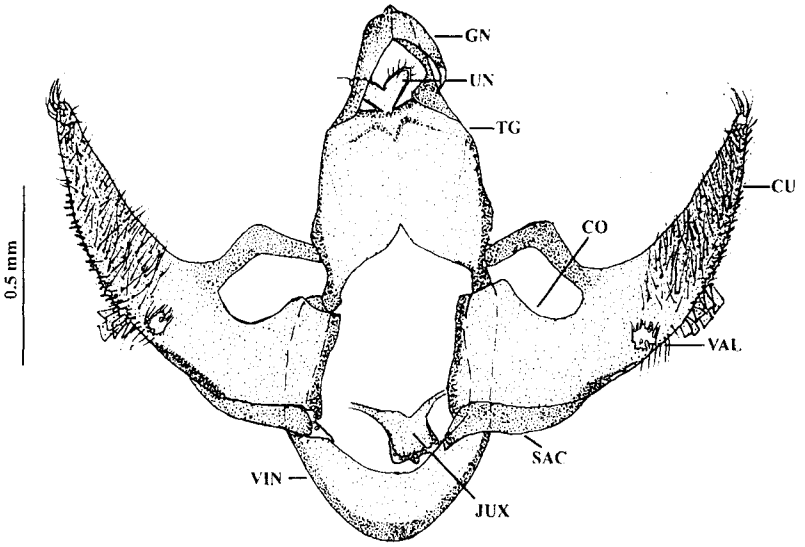


Fig. 15

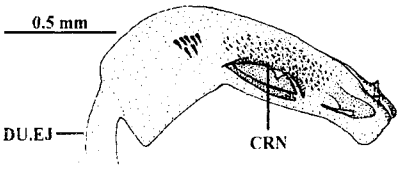


Fig. 16

Figs. 13-16 : *Lecithocera acuta* sp. nov. - (13) forewing; (14) hindwing ; (15) male genitalia (ventral view); (16) aedeagus.

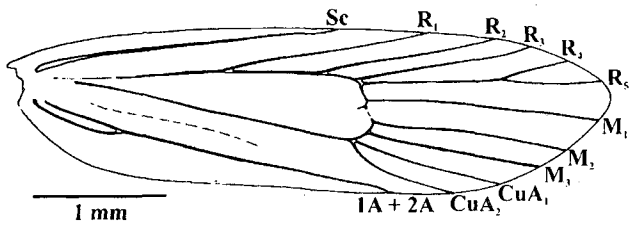


Fig. 17

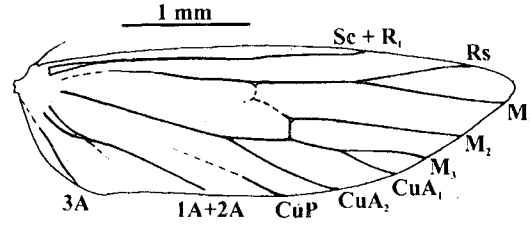
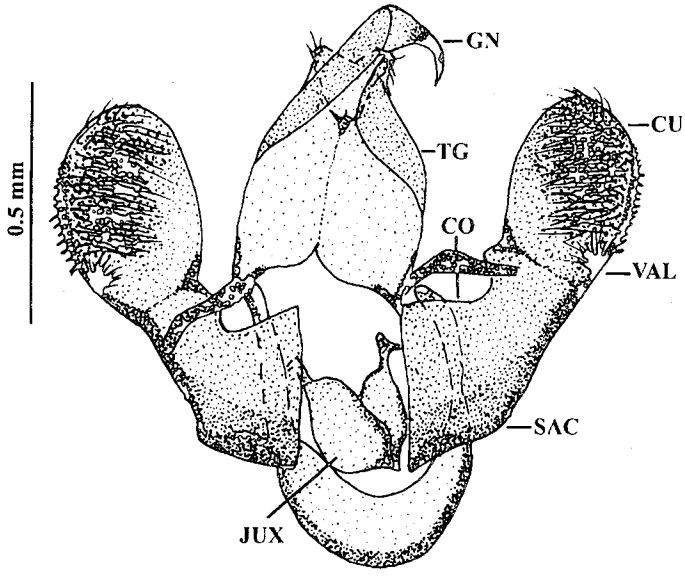


Fig. 18



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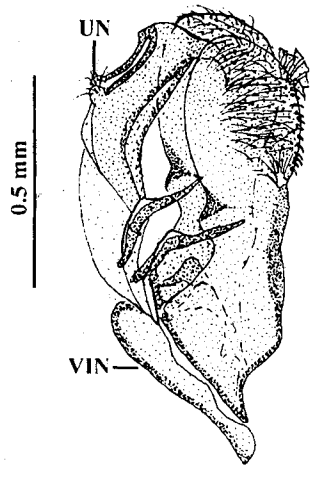
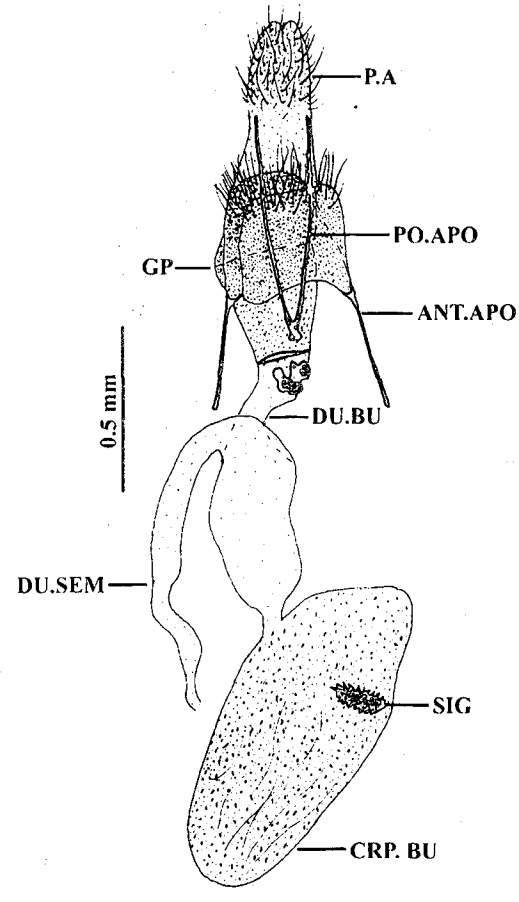


Fig. 20



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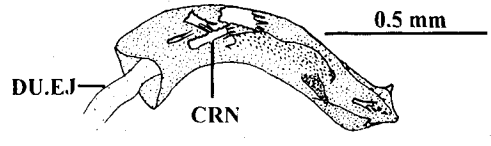

Fig. 21

Figs. 17-22 : *Lecithocera oxycona* Meyrick - (17) forewing; (18) hindwing; (19) male genitalia (ventral view); (20) male genitalia (lateral view); (21) aedeagus; (22) female genitalia.

Forewing with ground colour blackish-fuscous above; costal margin light yellow, broader at base and gradually narrowed posteriorly upto near apex; a speck in discal cell one-third away from base and another bigger between it and apex black; cilia along margin greyish-fuscous (Fig. 5); vein R_3 connate with R_4+R_5, R_5 to costa just above apex; CuA_1, CuA_2 connate (Fig. 17). Hindwing (Fig. 18) with ground colour on upper and under surfaces grey, suffused with fuscous. Legs brownish-fuscous; hind tibia and tarsi light yellow, tibia suffused with brownish-fuscous scales.

Abdomen grey on upper surface, distal end light yellow. Male genitalia (Fig. 19, 20) with distal margin bifurcation of uncus less pronounced, lobes short and setose; gnathos strongly falcate; tegumen slightly shorter than vinculum; vinculum broader in proximal half, U-like; saccus wanting; latero-distal angles of juxtal plate continued into a narrow process bent at right angle. Valvae with costa concave; sclerotised band connecting costa of each to base of tegumen slightly bent in middle; cucullus broadly spatulate, densely setose, its ventral margin bearing row of short and blunt spines in addition to knob-like process adorned with sharp spines on ventral margin; saccus indistinct. Aedeagus (Fig. 21) cylindrical, curved, apex blunt with a conical projection on each side; vesica adorned with a denticulate plate, preceeded by four unequal bunches of long spines and another of much shorter spines near posterior end; opening of ductus ejaculatorius anterior. Female genitalia (Fig. 22) with corpus bursae oval, beset with innumerable minute denticles; signum represented by a denticulate margined plate; ductus bursae comparatively broader in proximal two-third, with ductus seminalis originating from about one-third away from distal end; genital plate adorned with numerous minute spines; anterior apophyses half of posterior in length, apices of latter broad and flat; papillae anales beset with setae of varying lengths.

Alar expanse : Male : 11–13 mm; Female : 11–13 mm

Material examined : 1♂, 1♀, 09.x.2002; 1♂, 10.x.2002; 1♂, 11.x.2002; Jharoal, Rajasthan. 2♀♀, 12.x.2002; 1♀, 13.x.2002; Ranakpur (Kumbhalgarh Wildlife Sanctuary), Rajasthan. 2♂♂, 1♀, 06.x.2002; 5♂♂, 6♀♀, 07.x.2002; 1♂, 1♀, 08.x.2002; Mamer (Phulwari Ki Nal Sanctuary), Rajasthan. (Coll.: D. Wadhawan & V.K.Walia) Deposited in Entomology Section, Department of Zoology, Panjab University, Chandigarh.

Old distribution : India – North Coorg (3500 feet), Gooty, Konkan; (Meyrick, 1910; Clarke, 1965).

Remarks : This species is being reported for the first time from three localities of Rajasthan. Exact matching of colour pattern and structure of dissected male genitalia of the collected examples with documented description and figures authenticated its identification. (Meyrick, 1910; Clarke, 1965) Despite all these similarities, specimens collected from localities with entirely different physiographic conditions than known so far in having uniformly light yellow labial palpi in contrast to dark fuscous anterior edge of third segment of labial palpi. (Meyrick, 1910)

According to generic characters, CuA_1 and CuA_2 in the forewing are either stalked or coincident. Most of the studied examples of the present species showed stalked condition of these veins. In contrast, a few others revealed variations in their association both of intra-individual (i) and intra-specific (ii) type which are as given below:

(i) Veins CuA_1 and CuA_2 connate in right but separate in left wing (1female each from Ranakpur and Mamer).

(ii) –Wings of both sides with CuA_1 and CuA_2 separate (1 male from Mamer)

–Wings of both sides with CuA_1 and CuA_2 connate (1 female from Mamer).

Lecithocera xanthocostalis sp. nov.

(Figs. 6, 23 - 28)

Male : Head with vertex fuscous, side tufts dark yellow, somewhat raised; frons fuscous. Antenna slightly longer than forewing, dark yellow; scape long; flagellum with basal two-fifth fusiform, distal part of even width, yellow. Labial palpi with second segment brownish-fuscous; third segment slightly longer than second, dark yellow, apex speckled with fuscous. Proboscis and maxillary palpi fuscous. (Fig.6)

Thorax fuscous, patagium dark yellow at base. Forewing (Fig. 23) oblong, narrow; costa gently exarched; apex acutely angulate; termen oblique; tornus obtusely angulate. Ground colour fuscous on upper surface; a dark yellow streak along costa broader at base and terminating two-third away; a diffused spot in discal cell one-third away from base and another equally away bit darker than ground colour; cilia along margin greyish-brown. Under surface

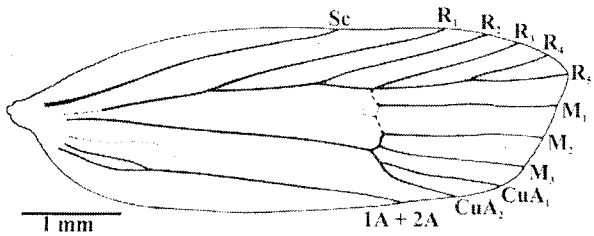

Fig. 23

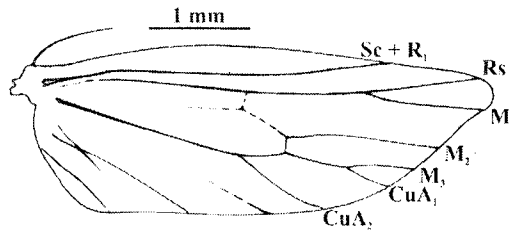

Fig. 24

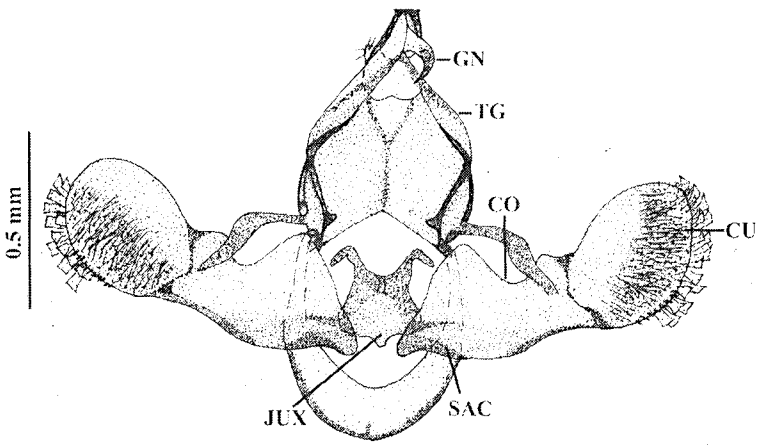

Fig. 25

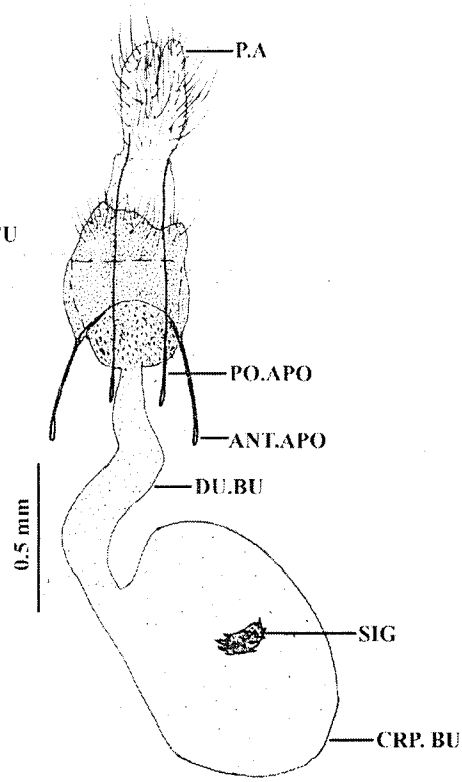

Fig. 28

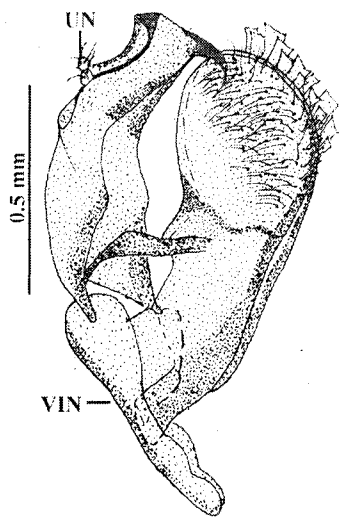

Fig. 26

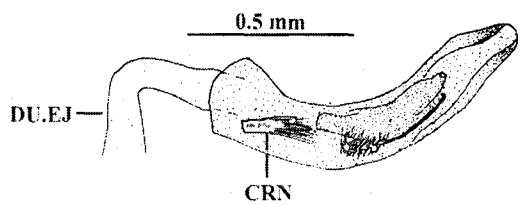

Fig. 27

Figs. 23-28 : *Lecithocera xanthocostalis* sp. nov. - (23) forewing; (24) hindwing ; (25) male genitalia (ventral view); (26) male genitalia (lateral view); (27) aedeagus; (28) female genitalia.

greyish-fuscous, grey along inner margin in basal two-third. Discal cell two-third of wing length; R_3 minutely stalked with long stalk of R_4, R_5 latter to apex; CuA_1, CuA_2 on a short stalk. Hindwing (Fig. 24) slightly narrower than forewing; costa excurved medially; apex acute somewhat produced; termen oblique; tornus obtusely angulate. Ground colour light brown on upper and under surfaces, latter speckled with fuscous; basal half of costa light yellow on former surface; cilia along inner margin about half of wing width. Discal cell about half of wing length; M_3, CuA_1 long stalked. Legs fuscous; hind tibia brown speckled with fuscous, brownish-ochraceous hair scaled above.

Abdomen greyish-brown, irrorated with fuscous on upper surface; under surface brownish-fuscous. Male genitalia (Fig. 25,26) with uncus bifurcated distally into two short and blunt setose lobes; gnathos strongly falcate, pointed at tip; tegumen nearly equal to broad and U-like vinculum; saccus absent; juxtal plate with two latero-distal arms, each bent at right angle; valvae with costa concave, joined to tegumen by strap-like process slightly bent in middle; cucullus broadly spatulate, densely setose, distinctly along ventro-distal margin; sacculus comparatively more sclerotised. Aedeagus (Fig. 27) cylindrical, curved, blunt at distal end; vesica adorned with a bunch of long spines near anterior end and another bunch of short spines in middle associated with large plate; opening of ductus ejaculatorius anterior. Female genitalia (Fig. 28) with corpus bursae oval; signum denticulate all over; ductus bursae about five-sixth of corpus bursae in length, more sclerotised distally; sclerotised genital plate beset with numerous spines having broader base; anterior apophyses about three-fifth of posterior, apices of both broad; papillae anales oval, beset with setae of varying lengths.

Alar expanse : Male : 14–15 mm; Female : 14 mm

Material examined : HOLOTYPE : ♂, 04.viii.2002; Kalesar (Forest Rest House), Haryana. PARATYPES : 1♀, 07.viii.2002; 1♂, 17.ix.2002; 1♀, 20.ix.2002; 1♂, 28.ix.2002; 1♀, 29.ix.2002; Kalesar (Forest Rest House), Haryana. 1♂, 09.x.2001; Pipli (Zoological Park), Haryana. 1♂, 06.x.2002; Mamer (Forest Rest House), Rajasthan. (Coll.: D. Wadhawan & V.K.Walia) Deposited in Entomology Section, Department of Zoology, Panjab University, Chandigarh.

Type locality : Kalesar (Haryana).

Etymology : Name of the new species i.e. *Lecithocera xanthocostalis* pertains to the yellow colouration of costa.

Remarks : A cursory glance at the collected specimens of this species suggested its closeness to *Lecithocera oxycona* Meyrick due to almost alike body colouration and pattern. Nevertheless critical observations brought to the fore differences in alar expanse, colouration of costal margin, antennae and point of termination of vein R_5 in forewing. The present species measured 14–15 mm, dark yellow costal margin, broadly fusiform antennae at base and vein R_5 to apex in forewing, as against, alar expanse 11–13 mm, light yellow costa, moderately fusiform antennae and R_5 to costa just above apex in forewing of *L. oxycona*. Besides this, the two species clearly differed in the shape of uncus, juxta, cornuti in aedeagus and presence of a knobbed structure bearing spines on ventral margin of valvae of *Lecithocera oxycona* only (Clarke, 1965).

As per generic characters, in the forewing, vein R_3 is either stalked with R_4+R_5 or seldom R_4 is absent or R_3,R_4 are stalked but R_5 is absent. The collected examples of this particular species revealed presence of veins R_3, R_4 and R_5. Observed variations in their association both of intra-individual (i) and intra-specific (ii) nature are as given below:

(i) Right forewing with R_3 connate with R_4+R_5 and in left wing R_3 stalked with R_4+R_5 in a male collected from Kalesar (Forest Rest House).

(ii) Both wings with R_3 connate with R_4+R_5

DISCUSSION

During the course of present investigations it has been observed that all the four studied species, namely *xanthoantennalis* sp. nov., *acuta* sp. nov., *oxycona* Meyrick and *xanthocostalis* sp. nov. showed all morphological features characteristic of genus *Lecithocera* Herrich-Schäffer, except variations in the condition of particular veins in forewing. Existence of such variations of different nature in the members of this genus is a well known fact. Despite these, notable similarities in structure of various component parts of male genitalia were noticed in all four species, like distally bifurcated uncus, falcate gnathos, broadly U-like vinculum, absence of saccus, presence of juxtal plate, costa of each valva deeply concave joined to tegumen by angled strap-like structure, sclerotised base of sacculus, vesica in aedeagus adorned with cornuti and opening of ductus ejaclatorius anterior. Likewise, consistency in presence

of denticulate surfaced signum in three species whose female genitalia could be observed further affirmed not only the congeneric status but also homogenous group of the studied species. The same inference can also be drawn considering their alike resting posture as presented for one of the investigated species i.e. *Lecithocera xanthoantennalis* sp. nov. in this communication (Fig. 1&2).

ACKNOWLEDGEMENTS

The authors are sincerely thankful to the Ministry of Environment and Forests for sanctioning an All India Coordinated project on Taxonomy for research on Microlepidoptera (AICOPTAX) No. J-22018/58/99-CSC (BC). Authors are indebted to the Forest Department in the concerned states for rendering co-operation during collection of research material. We are also grateful to Prof. S. Chaudhry, Chairman, Department of Zoology, Panjab University, Chandigarh for providing necessary facilities and secretarial assistance.

ABBREVIATIONS

1 A + 2 A = vein representing fused first and second anal vein; 3A = third anal vein; ANT. APO = anterior apophysis; CRP. BU = corpus bursae; CuA_1 = first anterior cubital vein; CuA_2 = second anterior cubital vein; Cu.P = posterior cubital vein; DU. BU = ductus bursae; DU. EJ = ductus ejaculatorius; DU. SEM = ductus seminalis; GN = gnathos; JUX = juxta; M_1 = first median vein; M_2 = second median vein; M_3 = third median vein; P. A = papilla analis; PO. APO = posterior apophysis; R_1 = first radial vein; R_2 = second radial vein; R_3 = third radial vein; R_4 = fourth radial vein; R_5 = fifth radial vein; Rs = radial sector vein; SA = saccus; SAC = sacculus; Sc + R_1 = fused subcosta and first radial veins; Sc = subcosta; TG = tegumen; UN = uncus; VIN = vinculum; VAL = valva.

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CUMULATIVE EFFECTS OF GARLIC OIL AND VITAMIN E AGAINST STAVUDINE INDUCED MUTAGENICITY IN MICE

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Abstract

Stavudine (d4T), a member of class antiretroviral is most widely used for the treatment of HIV patients. It also produces mutagenic and clastogenic effects by producing reactive oxygen species. During the present investigations the cumulative effects of garlic oil (low and high doses) and vitamin E have been evaluated for 24h, 48h and 72h against the mutagenicity induced by stavudine in bone marrow and multiple organs of mice by applying the chromosomal analysis and micronucleus test respectively. All kinds of structural and numerical aberrations were observed after the treatment of stavudine. It also produced micro-nucleated polychromatic cells (MnPCCs) in different organs (liver, kidney, lungs, spleen and testes). The pretreatment of both the antioxidants (Garlic oil and Vitamin E) separately or in combination reduced the chromosomal aberrations and formation of poly MnPCCs by quenching the reactive oxygen molecules. Maximum reduction in the frequencies of chromosomal aberrations and MnPCCs induced by stavudine was recorded in mice pretreated with high dose of garlic oil (0.5ml) and vitamin E rather than with low doses of garlic oil (0.25ml) and vitamin E, when administered together or separately.

Key words: stavudine, garlic oil, vitamin E, mutagenicity, chromosomal analysis, micronucleus assay.

INTRODUCTION

Stavudine, a member of the class antiretroviral is a thymidine analogue with potent anti-human immunodeficiency virus activity comparable to zidovudine (AZT) (Lin *et al,* 1988; Mansuri *et al,* 1989; Saint Marc *et al,* 1999 and Carpenter *et al,* 2000). It is sequentially phosphorylated by cellular kinases to stavudine 5'- triphosphate (the active form) which competitively inhibits thymidine 5'- triphosphate incorporation by human immunodeficiency virus reverse transcriptase and causes chain termination (Lea and Faulds, 1996). It also prevents the cell from producing new viruses and thus decreases the amount of virus in the body.

Stavudine is effectively used in combination with other medicines for delaying the development of AIDS and increasing CD4 cell counts. It blocks the virus reverse transcription, causes mitochandrial toxicity by affecting its DNA replication and also causes tissue specific toxicities (McComsey and Lonergon, 2004). Stavudine produces reactive oxygen species (ROS) and causes oxidative DNA damages (Sawyer and Colucci, 2000). It also acts as clastogenic, mutagenic and carcinogenic agent (Odinecs *et al.,* 1996).

Supplementation with antioxidants prevents the oxidative modification of DNA in lymphocytes of HIV infected patients (Jaruga *et al.,* 2002; George *et al.,* 2003 and Astley *et al.,* 2004). Allyl sulphur compounds present in the garlic oil reduce oxidative stress induced DNA damage by chemicals, suppress bioacivity of carcinogens, reduce cell proliferation and apoptosis (Pan *et al.,* 1997; Banerjee *et al.,* 2003 and Kaur *et al.,* 2004). AGE (aged garlic extract) and ajoene, an extract of garlic, enhance the CD4+/CD8+ ratio and block the destruction of CD4T cells (Morioka, 1993 and Walder *et al.,* 1997).

Vitamin E (∝-tocopherol) prevents the propagation of free radicals in the biomembranes and thus prevents the damage (Niki, 1996). It enhances the CD4+/CD8+ ratio, reduce the viral load in HIV infected patients (Spada *et al.,* 2002) and reduces the number of modified DNA bases in the HIV-infected patients (Jaruga *et al.,* 2002).

The cumulative antioxidant protection of garlic oil and vitamin E have not yet been tested against stavudine. Therefore, the present work was undertaken to evaluate the cumulative effect of garlic oil and vitamin E against the mutagenicity of stavudine in bone marrow and multiple organs of mice by employing chromosomal analysis and the micronucleus test (also a chromosomal test).

MATERIAL AND METHODS

For the present investigations, male albino mice of laca strain (*Mus musculus*) weighing about 25 g each, were

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procured from the Central Animal House of Punjab University, Chandigarh. Stavudine was procured from the National Institute of Pharmaceutical Education and Research (NIPER), SAS Nagar, Mohali. The garlic oil and vitamin E were procured from Ranbaxy Laboratories Ltd. Bombay and Intas Pharmaceutical Ltd. Ahmedabad respectively.

The mice were divided into eleven groups, each having five mice. A single dose of stavudine (5mg/25g body weight dissolved in distilled water) was given orally to one group. To the second group low dose of garlic oil, i.e. 0.25 ml of garlic oil diluted with olive oil 1:1v/v and to the third group 0.5ml of garlic oil (high dose) were given for six days. To the fourth group a dose of vitamin E (10mg/25g) was administered daily for six consecutive days. The other two groups were administered a cumulative dose of low dose of garlic oil (0.25 ml) + vitamin E and a high dose of garlic oil (0.50ml) + vitamin E for six consecutive days and then was followed by a single dose of stavudine (5mg). N-methyl-N'nitro- N-nitrosoguanidine (MNNG) (0.3mg/25g body weight) was used as positive control for one group while to another group the distilled water (0.5ml/25g body weight) was given for the negative control. The mice were dissected to take out bone marrow and other organs (liver, kidney, lung, spleen and testes) after 24, 48 and 72 h of the last treatment.

For the chromosomal analysis from the bone marrow, the air-drying technique of Das (1966) was followed. Prior to the sacrifice each animal was injected 4mg/kg body weight of colchicine intraperitonally. The various organs were taken and transferred to a chilled homogenizing buffer and processed for getting a single cell suspension by following the method of Sasaki *et al.* (1997). For analysing the chromosomal breakages in the cells the micronucleus test was employed (Schmid, 1976).

RESULTS

All kinds of numerical and structural aberrations were observed in the groups treated with stavudine and MNNG. These aberrations include centromeric gaps, centric fusions, tendency to form rings, stickiness, chromatid breaks, hypoploidy, hyperploidy, ring formation and the chromosomal distortions. The percentage of chromosomal distortions, centromeric gaps and the tendency to form rings was higher than the other kinds of aberrations. The percentage of the chromosomal distortions was maximum after the treatment of stavudine for 24 h and thereafter it reduced after 48 h and 72 h respectively. Pretreatment of the garlic oil or vitamin E reduced the percentage of aberrations as compared to the group treated with stavudine. The cells mostly showed the centric fusions and the chromosomal elongations but a few cells with hyperploidy and hypoploidy were also observed. A further reduction in the chromosomal aberrations, were observed when vitamin E and the garlic oil were administered together. The highest reduction of chromosomal aberrations was observed when a high dose of the garlic oil and vitamin E were administered before the treatment of stavudine (Table 1 and Figures 1 & 2).

The perusal of table 2 and Figures 3–5 indicate that the treatment with stavudine and MNNG produced mono, bi, tri and polynucleated cells in the different organs. In the stavudine treated group liver and kidney showed maximum number of polynucleated cells. The maximum number of MnPCCs was found in the liver and least in spleen up to group VII. Gradation wise it was liver > kidney > testes > lung > spleen. With the pretreatment of garlic oil (low dose and high dose) and vitamin E the number of MnPCCs reduced considerably.

In the group VIII, the administration of vitamin E caused least effect in testes and more in the liver. Gradation of frequency of MnPCCs was liver > lung > kidney > spleen > testes. The high dose of the garlic oil showed more amelioration than low dose. The mutagenicity of the medicine was reduced considerably in the group where cumulative pretreatment of high dose of the garlic oil and vitamin E was administered before stavudine treatment. A reduction of 40 to 50% in the polynucleated cells was observed in these groups (group X, XI). In these two groups mainly mono and bi nucleated cells were observed and a few polynucleated cells were also observed.

DISCUSSION

A significant increase in the amount of modified DNA bases causing mutagenicity, clastogenicity etc have been observed in the HIV infected patients. *In vitro* assays stavudine produced an increased frequency of the chromosomal aberrations while in the *in vivo* micronucleus assay stavudine was clastogenic to bone marrow cells of mice (Schilling *et al.*, 1995). The formation of micronuclei has been associated with the production of hydroxyl radicals. The stavudine produced mono, bi, tri and polynucleated cells in different organs during the present investigations.

Table 1 : Effects of various treatments on frequency distribution of %age aberrations in bone marrow cells of mice

S.No.	Dose (per 25 gm body weight of mice)	Time duration (hours)*	Total aberrant cells scored**	Aberrations per cell (% ± S.E.)
I.	Distilled water	24	5	1.0 ± 0.07
	(0.5ml)	48	6	1.2 ± 0.08
		72	7	1.4 ± 0.06
II.	MNNG	24	315	63.0 ± 1.10
	(0.3mg)	48	345	69.0 ± 1.18
		72	384	77.0 ± 1.30
III.	Low dose of garlic oil	24	30	6.0 ± 0.23
	(0.25ml)	48	37	7.4 ± 0.25
		72	41	8.2 ± 0.27
IV.	High dose of garlic oil	24	52	10.4 ± 0.28
	(0.5ml)	48	64	12.8 ± 0.35
		72	68	13.6 ± 0.29
V.	Stavudine	24	275	55.0 ± 0.74
	(5mg)	48	247	49.4 ± 0.70
		72	200	40.0 ± 0.67
VI.	Low dose of garlic oil +	24	225	45.0 ± 0.78
	Stavudine (0.25ml + 5mg)	48	205	41.0 ± 0.68
		72	185	37.0 ± 0.65
VII.	High dose of garlic oil +	24	165	33.0 ± 0.63
	Stavudine (0.5ml + 5mg)	48	154	31.0 ± 0.61
		72	130	26.0 ± 0.50
VIII.	Vitamin E	24	17	3.4 ± 0.20
	(10 mg)	48	26	5.2 ± 0.23
		72	30	6.0 ± 0.24
IX.	Vitamin E + Stavudine	24	237	47.2 ± 0.68
	(10mg + 5mg)	48	223	44.6 ± 0.77
		72	196	39.2 ± 0.68
X.	Vitamin E + low dose of	24	152	30.4 ± 0.74
	garlic oil + Stavudine	48	147	28.2 ± 0.63
	(10mg + 0.25ml + 5mg)	72	127	25.4 ± 0.59
XI.	Vitamin E + high dose of	24	139	28.0 ± 0.64
	garlic oil + Stavudine	48	131	26.2 ± 0.60
	(10mg + 0.5ml + 5mg)	72	115	23.0 ± 0.57

* Total number of animals used for each experiment were 5.
** Total number of cells scored for each experiment were 500.

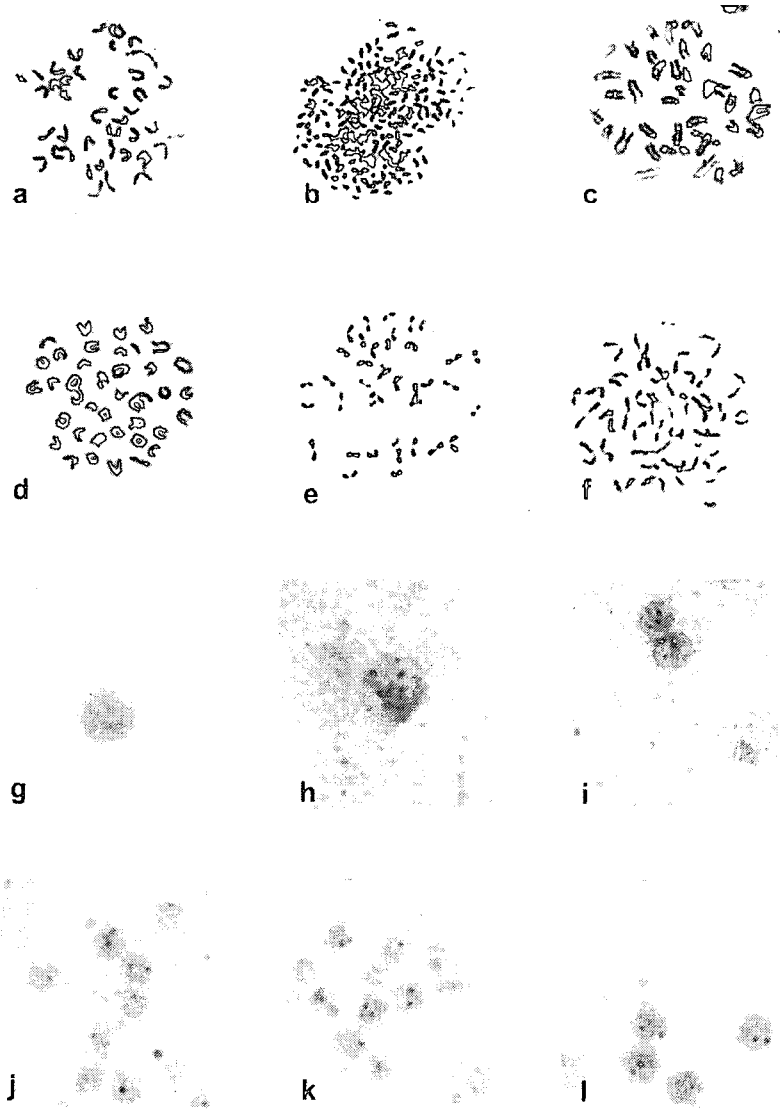

Fig.1: Effect of various treatments on bone marrow cells and different organs of mice.

a-f: Effect of various treatment on bone marrow cells on mice – a: Normal chromosomes; b: Effect of stavudine; c: Effect of garlic oil pretreatment and stavudine; d: Effect of vit.E pretreatment and stavudine; e: Effect of low dose of garlic oil + vit.E pretreatment and stavudine; f: Effect of high dose of garlic oil + vit.E pretreatment and stavudine.

g-l: Production of micronuclei after the effect of various treatments on different organs of mice – g: Normal cell; h: Effect on stavudine on liver cell; i: Effect of stavudine on liver cell pretreated with garlic oil; j: Effect of stavudine on kidney cell pretreated with garlic oil; k: Effect of stavudine on lung cell pretreated with vit.E; l: Effect of stavudine on testis cell pretreated with vit.E.

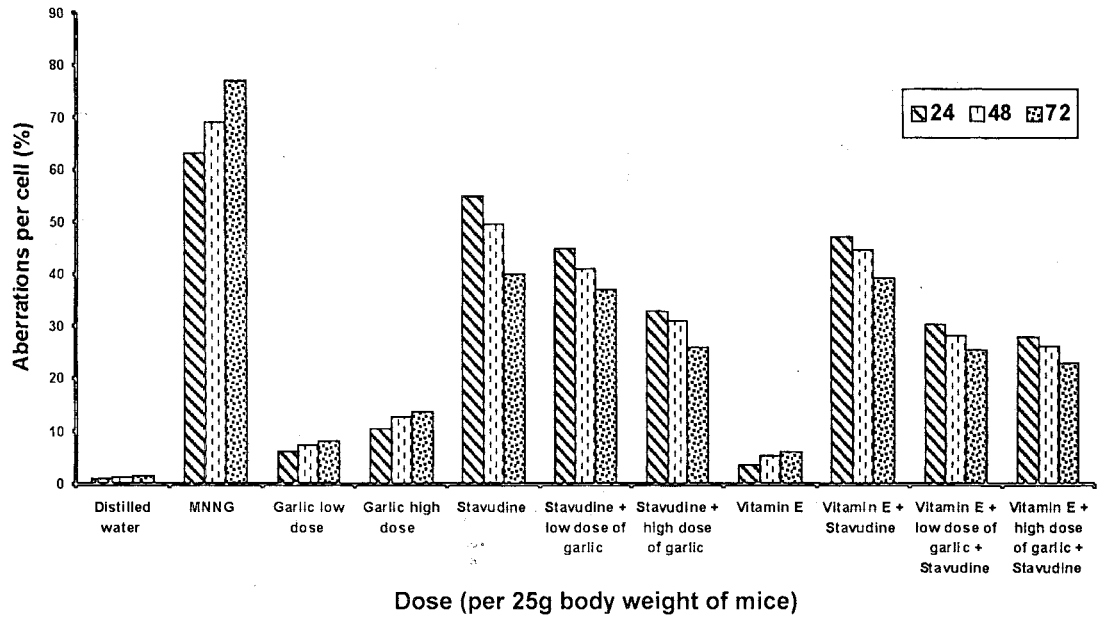

Fig. 2: Effects of various treatments on frequency distribution of percentage of aberrations in bone marrow cells of mice.

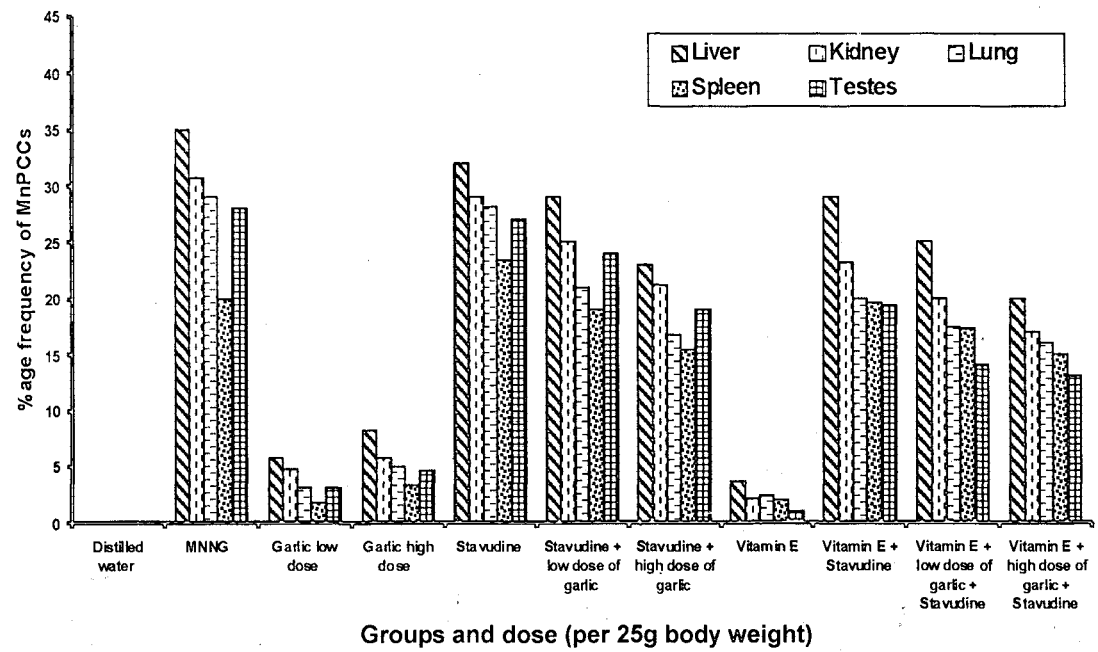

Fig. 3: Effects of various treatments on frequency distribution of MnPCCs in different organs of mice for 24 hours

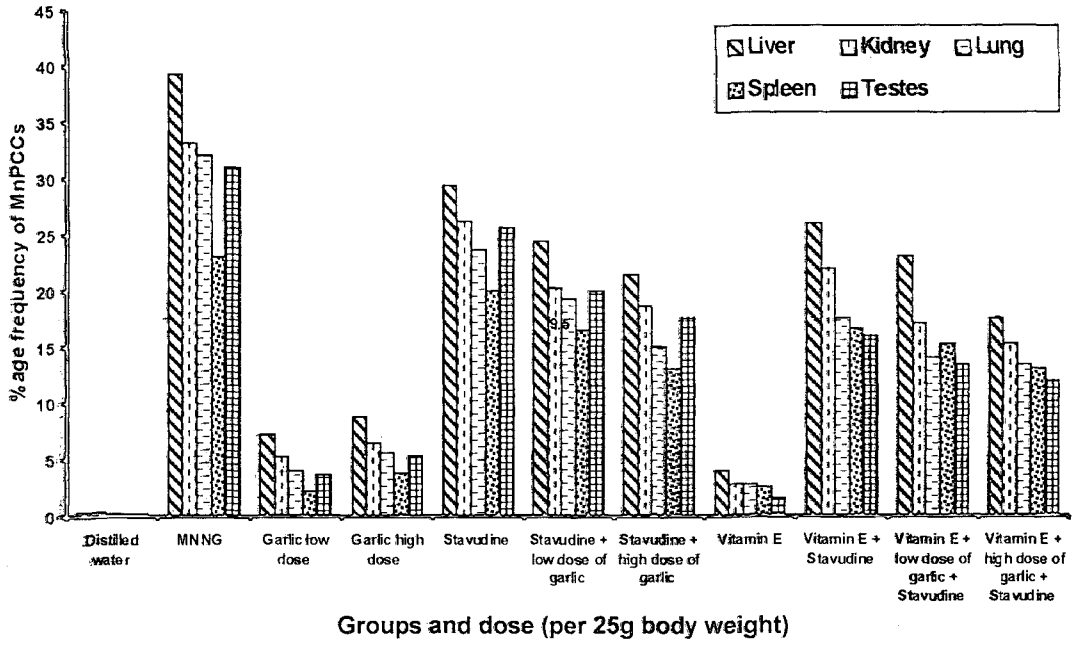

Fig. 4: Effects of various treatments on frequency distribution of MnPCCs in different organs of mice for 48 hours

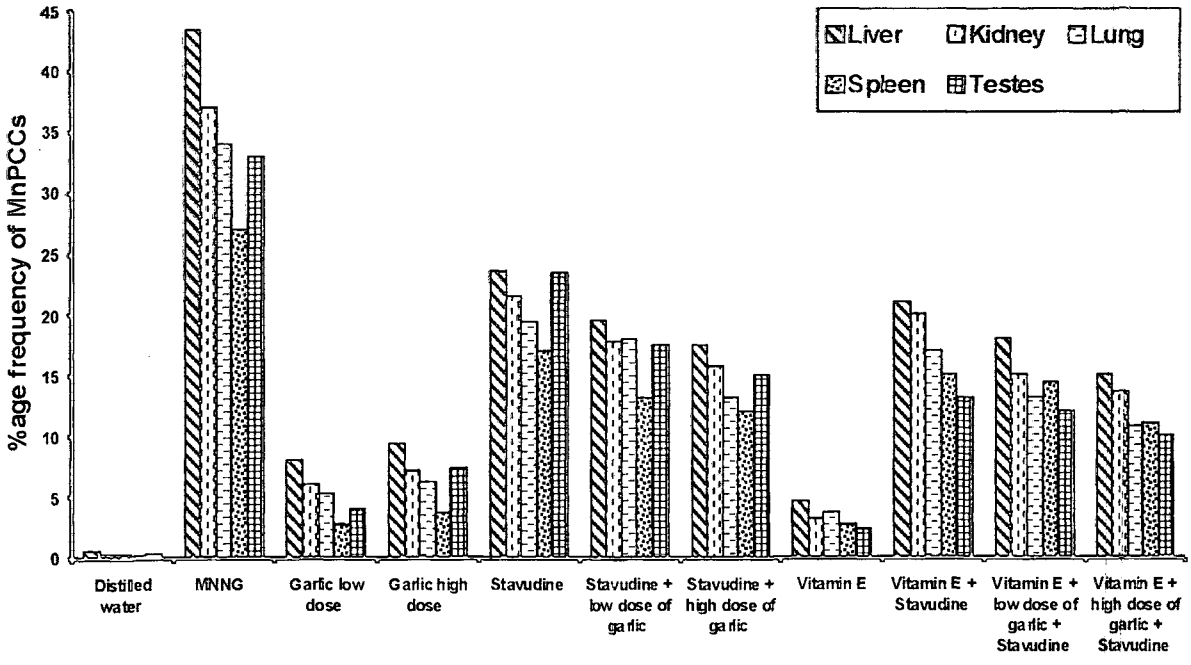

Fig. 5: Effects of various treatments on frequency distribution of MnPCCs in different organs of mice for 72 hours

Table 2 : Effects of various treatments on frequency distribution of micronucleated cells in different organs of mice

S.No.	*Groups and dose (per 25gm body weight)	Time duration (hours)	**Frequency of micronucleated polychromatic cells (MnPCCs) in (% ± S.E.)				
			Liver	Kidney	Lung	Spleen	Testes
I.	Distilled water (0.5 ml)	24	0.2 ± 0.04	0.2 ± 0.03	0.2 ± 0.04	0.2 ± 0.03	0.2 ± 0.03
		48	0.4 ± 0.06	0.4 ± 0.05	0.2 ± 0.04	0.2 ± 0.05	0.2 ± 0.03
		72	0.6 ± 0.08	0.4 ± 0.07	0.4 ± 0.06	0.2 ± 0.06	0.4 ± 0.07
II.	MNNG (0.3mg)	24	35.0 ± 0.59	30.8 ± 0.54	29.0 ± 0.53	20.0 ± 0.44	28.0 ± 0.52
		48	39.2 ± 0.62	33.2 ± 0.57	32.0 ± 0.56	23.0 ± 0.47	31.0 ± 0.55
		72	43.4 ± 0.65	37.0 ± 0.60	34.0 ± 0.58	27.0 ± 0.51	33.0 ± 0.57
III.	Low dose of garlic oil (0.25ml)	24	5.8 ± 0.24	4.8 ± 0.21	3.2 ± 0.17	1.8 ± 0.13	3.2 ± 0.18
		48	7.2 ± 0.26	5.2 ± 0.22	4.0 ± 0.20	2.2 ± 0.15	3.6 ± 0.19
		72	8.0 ± 0.28	6.0 ± 0.24	5.2 ± 0.22	2.8 ± 0.16	4.0 ± 0.20
IV.	High dose of garlic oil (0.5ml)	24	8.2 ± 0.24	5.8 ± 0.23	5.0 ± 0.21	3.4 ± 0.18	4.6 ± 0.21
		48	8.8 ± 0.31	6.4 ± 0.25	5.6 ± 0.23	3.8 ± 0.19	5.4 ± 0.22
		72	9.4 ± 0.30	7.2 ± 0.26	6.2 ± 0.24	3.8 ± 0.19	7.4 ± 0.27
V.	Stavudine (5mg)	24	32.0 ± 0.56	29.0 ± 0.53	28.2 ± 0.57	23.4 ± 0.48	27.0 ± 0.51
		48	29.4 ± 0.54	26.2 ± 0.51	23.6 ± 0.47	20.0 ± 0.45	25.6 ± 0.50
		72	23.6 ± 0.47	21.6 ± 0.45	19.4 ± 0.43	17.0 ± 0.41	23.4 ± 0.47
VI.	Low dose of garlic oil + Stavudine (0.25ml + 5mg)	24	29.0 ± 0.53	25.0 ± 0.40	21.0 ± 0.45	19.0 ± 0.43	24.0 ± 0.48
		48	24.4 ± 0.48	20.2 ± 0.44	19.2 ± 0.43	16.4 ± 0.40	20.0 ± 0.45
		72	19.6 ±	17.8 ±	18.0 ±	13.2 ±	17.6 ±

			0.43	0.42	0.41	0.38	0.42
VII.	High dose of garlic oil + Stavudine (0.5ml + 5mg)	24	23.0 ± 0.47	21.2 ± 0.46	16.8 ± 0.38	15.4 ± 0.39	19.0 ± 0.43
		48	21.4 ± 0.45	18.6 ± 0.43	15.0 ± 0.39	13.0 ± 0.35	17.6 ± 0.42
		72	17.6 ± 0.42	15.8 ± 0.40	13.2 ± 0.36	12.0 ± 0.34	15.0 ± 0.39
VIII.	Vitamin E (10mg)	24	3.6 ± 0.18	2.2 ± 0.13	2.4 ± 0.15	2.0 ± 0.14	1.0 ± 0.09
		48	4.0 ± 0.19	2.8 ± 0.16	2.8 ± 0.16	2.6 ± 0.16	1.6 ± 0.12
		72	4.6 ± 0.21	3.2 ± 0.17	3.8 ± 0.19	2.8 ± 0.18	2.4 ± 0.15
IX.	Vitamin E + Stavudine (10mg + 5mg)	24	29.0 ± 0.53	23.2 ± 0.47	20.0 ± 0.45	19.6 ± 0.43	19.4 ± 0.44
		48	26.0 ± 0.49	22.0 ± 0.46	17.6 ± 0.42	16.6 ± 0.40	16.0 ± 0.40
		72	21.0 ± 0.45	20.0 ± 0.40	17.0 ± 0.41	15.0 ± 0.39	13.2 ± 0.38
X.	Vitamin E + low dose of garlic oil + Stavudine (10mg + 0.25ml + 5mg)	24	25.0 ± 0.49	20.0 ± 0.44	17.4 ± 0.41	17.2 ± 0.42	14.0 ± 0.37
		48	23.0 ± 0.47	17.0 ± 0.41	14.0 ± 0.40	15.2 ± 0.41	13.4 ± 0.38
		72	18.0 ± 0.43	15.0 ± 0.39	13.2 ± 0.37	14.4 ± 0.38	12.0 ± 0.31
XI.	Vitamin E + high dose of garlic oil + Stavudine (10mg + 0.5ml + 5mg)	24	20.0 ± 0.45	17.0 ± 0.41	16.0 ± 0.39	15.0 ± 0.14	13.0 ± 0.36
		48	17.6 ± 0.42	15.2 ± 0.40	13.4 ± 0.37	13.0 ± 0.36	12.0 ± 0.35
		72	15.0 ± 0.39	13.6 ± 0.37	10.8 ± 0.30	11.0 ± 0.31	10.0 ± 0.29

*Total number of animals used for each experiment was 5.

** The total number of cells scored for each experiment were 500.

Reduction of genotoxicity of stavudine by the interactive effect of the garlic oil and vitamin E has been reported by using the comet assay test (Kaur and Singh, 2005). Garlic along with its dially sulphide compounds have already been reported as ameliorating agents against the genotoxicity of doxorubicin (Kaur *et al.*, 2004) and zidovudine induced oxidative stress (Kaur and Malvika, 2004). It also acts as a protective agent in kidney of hypertensive rats (Sharifi *et al.*, 2003) and reduced inflammatory effects in humans (Keiss *et al.*, 2003).

Antiretroviral therapy in association with ∝-tocopherol is more effective in reducing viral load and also in increasing the CD4+/CD8+ ratio (Spada *et al.*, 2002). It is found to reduce the level of modified DNA bases in HIV infected patients (Jaruga *et al.*, 2002) and suppress CD95L mRNA expression and protects T cells from CD95 mediated apoptosis (Li–Weber *et al.*, 2002). ∝-tocopherol restores the concentrations of some micronutrients (Vitamin A and E, Zn and Cu) in liver, intestine, serum and thymus (Wang *et al.*, 1994) and prevents nonylphenol induced oxidative stress in the testes of rats (Chitra and Mathur, 2004).

It may thus be concluded that both the antioxidants (garlic oil and vitamin E) act as ameliorating agents against the mutagenicity induced by stavudine and the protective effects were more pronounced in the high dose of garlic oil treated group. The cumulative effects of both the antioxidants prior to or in combination with stavudine proved to be more ameliorating against the mutagenicity caused by stavudine.

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PERFORMANCE ANALYSIS OF ROUTING PROTOCOLS OF WIRELESS AD-HOC NETWORKS FOR A CONFERENCE TYPE ENVIRONMENT

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Abstract

Mobile Ad-hoc Network (MANET) is the network that is different from traditional wireless network for its dynamically changing topology, no base station support, and no fixed routers. Routing is a major problem in this type of network. In this paper we have analyzed the performance of various reactive and proactive routing protocols for MANET. We consider an environment of a medium size conference in which there are at most 50 nodes traveling from one point to another. Proactive routing protocols are table driven protocols, which continuously evaluate the routes to various mobile nodes. These types of protocols are efficient and show less latency in route discovery. The main problem in these types of protocols is that lot of information goes wasted, as that is not required at any time. The other type of protocols is reactive protocols, which are On Demand Type Protocols. Route discovery is carried out using some global search method in these protocols. Comparative evaluation of Dynamic Source Routing (DSR), Temporal Ordered Routing Algorithm (TORA), Direct Sequenced Distance Vector (DSDV) and Ad-hoc On Demand Distance Vector (AODV) protocols has been performed and it is found that DSDV protocol is best-suited protocol as it shows a lower delay, high throughput and low routing overhead.

Key words : MANET, Routing, DSDV, DSR, AODV, TORA.

INTRODUCTION

A mobile ad hoc network is a collection of wireless, mobile nodes, dynamically forming a temporary network without the use of any existing infrastructure or centralized administration. It has Multi-hop communication capability. In this type of network each node works as an independent router. Each host uses wireless RF transceivers as network interface. Example applications of MANET are conferences or meetings, emergency search-and-rescue operations, war etc., where users need to deploy network immediately, without base stations or fixed network infrastructure.

MANET is self-organizing network. Every time a mobile host moves, it needs to re-discover which mobile hosts are reachable. It does this by sending a "ping" message in all directions and listens for corresponding "pong" messages. The strength of the "ping" message weakens as distance increases giving the mobile host a limited range within which "ping" messages can be heard. This range is called the scan range of the mobile host.

MANET is fully decentralized. No central server exists in a MANET environment. Therefore every mobile host is equally important within the network. MANET has very dynamic topology. Therefore within MANET systems, one will find that communication end-points frequently move independently of one another.

Various routing protocols for MANET can be classified into two categories: Proactive protocols and Reactive protocols. Proactive protocols are table driven protocols, which continuously evaluate routes to various mobile hosts. So there is no latency in route discovery. A drawback of these protocols is that a lot of routing information may never be used. On the other hand, reactive protocols make on-demand searches for a path, which can be less costly when host mobility is high. In these protocols route discovery is done through some global search method. The global search method depends upon the location management scheme. A bottleneck in this scheme is high latency in route discovery. So these types of protocols are not suited for real time communication. We will now discuss various protocols used in MANET for routing.

DESTINATION SEQUENCED DISTANCE VECTOR (DSDV) PROTOCOL (Charles and Bhagwat, 1994)

In this protocol each route in the network is tagged with a sequence number originated by destination. Hosts perform periodic & triggered updates, issuing a new sequence number. Triggered updates are necessary for immediate topological changes. These updates will immediately update all the routing information on all nodes. A constraint on how frequent these updates are

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broadcasted must be taken into consideration. This is because in a frequently changing network these updates will make the network congested. Sequence number indicates the freshness of a route. Routes with more recent sequence numbers are preferred for packet forwarding. If two or more routes have same sequence number, then one with smallest metric is used.

This protocol gives a special importance to the management of network when topology changes. The following are the main features of this protocol to handle any type of topological changes.

- Broken links are assigned a metric of ∞.
- Any route through a hop with a broken link is also assigned a metric of ∞.
- ∞-routes are assigned new sequence numbers by any host & immediately broadcasted via triggered update.
- If a node has an equal/later sequence number with a finite metric for a ∞-route, then a route update is triggered.

Damping fluctuation may occur in case of frequent changes in the network topology. In that case, those routes are preferred having later sequence numbers, or smaller metric for same sequence numbers. This criterion may cause problems and leads to table fluctuations if worse metrics are received first, causing a ripple of triggered updates. The solution to this problem is to use average settling time as a parameter before advertising routes. This is equivalent to using two tables, one for forwarding packets and another for advertising routes.

DYNAMIC SOURCE ROUTING – DSR

Dynamic Source Routing (DSR) (Jhonson *et. al.*, 2004) belongs to the class of reactive protocols and allows nodes to dynamically discover a route across multiple network hops to any destination. Source routing means that each packet in its header carries the complete ordered list of nodes through which the packet must pass. There are certain variations to this protocol that have also been proposed (Hui Yao *et. al.*, 2005). Idea proposed in this paper is based on 2-level hierarchical scheme: the 1-cell cluster and 2- server cluster. Route discovery is transferred to 2-server cluster to prevent from network flooding. But here we have used the basic DSR protocol. It uses no periodic routing messages (e.g. no router advertisements), thereby reducing network bandwidth overhead, conserving battery power and avoiding large routing updates throughout the ad-hoc network. Instead DSR relies on support from the MAC layer (the MAC layer should inform the routing protocol about link failures). The two basic modes of operation in DSR are route discovery and route maintenance.

A. Properties

DSR uses the key advantage of source routing. Intermediate nodes do not need to maintain up-to-date routing information in order to route the packets they forward. There is also no need for periodic routing advertisement messages, which will lead to reduced network bandwidth overhead, particularly during periods when little or no significant host movement is taking place. Battery power is also conserved on the mobile hosts, both by not sending the advertisements and by not needing to receive them; a host could go down to sleep instead.

This protocol has the advantage of learning routes by scanning for information in packets that received a route from A to C through B means that node learns the route to C, but also that it will learn the route to B. The source route will also mean that B learns the route to A and C and that C learns the route to A and B. This form of active learning is very good and reduces overhead in the network.

However, each packet carries a slight overhead containing the source route of the packet. This overhead grows when the packet has to go through more hops to reach the destination. So the packets sent will be slightly bigger, because of the overhead.

Running the interfaces in promiscuous mode is a serious security issue. Since the address filtering of the interface is turned off and all packets are scanned for information. A potential intruder could listen to all packets and scan them for useful information such as passwords and credit card numbers. Applications have to provide the security by encrypting their data packets before transmission. The routing protocols are prime targets for impersonation attacks and must therefore also be encrypted. One way to achieve this is to use IP-sec (Stephen and Atkinson, 1988).

DSR also has support for unidirectional links by the use of piggybacking the source route a new request. This can increase the performance in scenarios where we have a lot of unidirectional links. We must however have a MAC protocol that also supports this.

B. Route Discovery

Route discovery is the mechanism whereby a node X

wishing to send a packet to Y, obtains the source route to Y. Node X requests a route by broadcasting a Route Request (RREQ) packet. Every node receiving this RREQ searches through its route cache for a route to the requested destination. DSR stores all known routes in its route cache. If no route is found, it forwards the RREQ further and adds its own address to the recorded hop sequence. This request propagates through the network until either the destination or a node with a route to the destination is reached. When this happen a Route Reply (RREP) is uni-casted back to the originator. This RREP packet contains the sequence of network hops through which it may reach the target.

In Route Discovery, a node first sends a RREQ with the maximum propagation limit (hop limit) set to zero, prohibiting its neighbors from re-broadcasting it. At the cost of a single broadcast packet, this mechanism allows a node to query the route caches of all its neighbors.
Nodes can also operate their network interface in promiscuous mode, disabling the interface address filtering and causing the network protocol to receive all packets that the interface overhears. These packets are scanned for useful source routes or route error messages and then discarded.

The route back to the originator can be retrieved in several ways. The simplest way is to reverse the hop record in the packet. However this assumes symmetrical links. To deal with this, DSR checks the route cache of the replying node. If a route is found, it is used instead. Another way is to piggyback the reply on a RREQ targeted at the originator. This means that DSR can compute correct routes in the presence of asymmetric (unidirectional) links. Once a route is found, it is stored in the cache with a time stamp and the route maintenance phase begins.

C Route Maintenance

Route maintenance is the mechanism by which a packet sender S detects if the network topology has changed so that it can no longer use its route to the destination D. This might happen because a host listed in a source route, move out of wireless transmission range or is turned off making the route unusable. A failed link is detected by either actively monitoring acknowledgements or passively by running in promiscuous mode, overhearing that a neighboring node forwards a packet.

When route maintenance detects a problem with a route in use, a route error packet is sent back to the source node. When this error packet is received, the hop in error is removed from this host route cache, and all routes that contain this hop are truncated at this point.

AD-HOC ON DEMAND DISTANCE VECTOR (AODV) PROTOCOL. (Charles *et al.*, 2003).

The Ad-hoc On Demand Distance Vector (AODV) protocol, a reactive protocol for routing, uses sequence numbers for building loop free routes. Key difference from a similar type of proactive protocol DSR is that the source route is no longer required. There are two methods of discovery of paths in the protocol. One is reverse path setup and the other is forward path setup. Other features of AODV are its Table management, path maintenance, and local connectivity management.

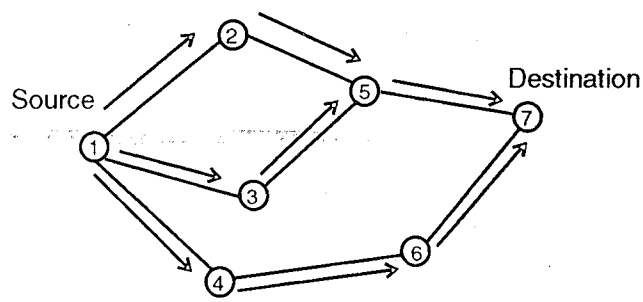

Figure 1(a): Propagation of Route Request (RREQ) Packet

To find a path to the destination, the source broadcasts a route request packet. The neighbors in turn broadcast the packet to their neighbors till it reaches an intermediate node that has recent route information about the destination or till it reaches the destination (Figure-1(a)). A node discards a route request packet that it has already seen. The route request packet uses sequence numbers to ensure that the routes are loop free and to make sure that if the intermediate nodes reply to route requests, they reply with the latest information only (Figure-1(b)).

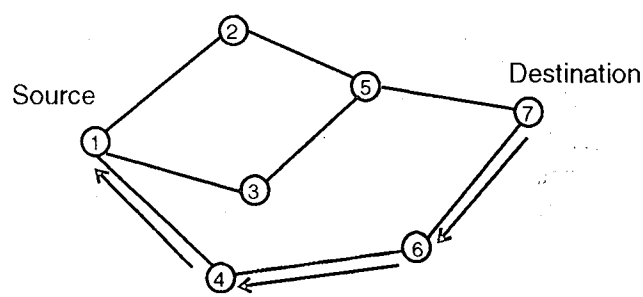

Figure 1(b): Path taken by the Route Reply (RREP) Packet

A. Reverse Path Setup

The actual algorithm uses two counters: Sequence number & Broadcast id. The protocol is as follows:

1. Broadcast route reque st (RREQ) < source_addr, source_sequence-#, broadcast_id, dest_addr, dest_sequence_#, hop_cnt >
2. RREQ uniquely identified by <source_addr, broadcast_id>
3. Route reply (RREP) if neighbor is the target, or knows a higher dest_sequence_#
4. Otherwise setup a pointer to the neighbor from whom RREQ was received
5. Maintain reverse path entries based on timeouts

B. Forward Path Setup

1. RREQ arrives at a node that has current route to the destination (larger/same sequence number).
2. unicast request reply (RREP) <source_addr, dest_addr, dest_sequence_#, hop_cnt,lifetime> to neighbor
3. RREP travels back to the source along reverse path
4. Each upstream node updates dest_sequence_#, sets up a forward pointer to the neighbor who transmit the RREP.

Figure 2 & Figure 3 represents setting up of path.

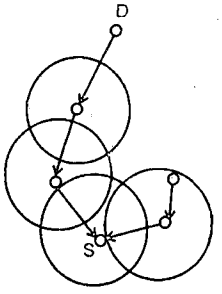

FIGURE 2: REVERSE PATH FORMATION

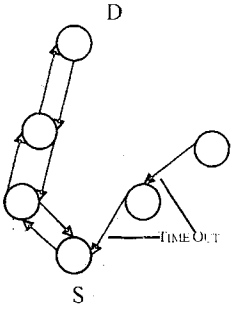

FIGURE 3: FORWARD PATH FORMATION

C. Protocol Maintenance

C.1 Router Table Management

Route request expiration timer purges reverse paths that do not lie on active route. Active neighbor relays a packet within active_route_timeout. Route cache timer purges inactive routes. New route is preferred if it has higher destination sequence number or lower metric.

C.2 Path Maintenance

Upon link breakage, affected node propagates an unsolicited RREP <dest_sequence_#+1, ∞> to all upstream nodes. Source may restart route discovery process.

C.3 Local Connectivity Management

Broadcasts used to update local connectivity information. Inactive nodes in an active path required to send "hello" messages.

TEMPORALLY ORDERED ROUTING ALGORITHM (TORA) (Park and Corson, 1997).

The Temporally Ordered Routing Algorithm (TORA) is a highly adaptive, efficient and scalable distributed routing algorithm based on the concept of link reversal. TORA is proposed for highly dynamic mobile, multi-hop wireless networks. It is a source-initiated on-demand routing protocol. It finds multiple routes from a source node to a destination node. The main feature of TORA is that the control messages are localized to a very small set of nodes near the occurrence of a topological change. To achieve this, the nodes maintain routing information about adjacent nodes. The protocol has three basic functions: Route creation, Route maintenance, and Route erasure. Each node has a quintuple associated with it -

- Logical time of a link failure.
- The unique ID of the node that defined the new reference level.
- A reflection indicator bit.
- A propagation ordering parameter.
- The unique ID of the node.

The first three elements collectively represent the reference level. A new reference level is defined each time a node loses its last downstream link due to a link failure. The last two values define a delta with respect to the reference level. Route Creation is done using query (QRY) and update (UPD) packets. The route creation algorithm starts with the height (propagation ordering parameter in the quintuple) of destination set to 0 and all other node's height set to NULL (i.e. undefined).

The source broadcasts a QRY packet with the destination node's id in it. A node with a non-NULL height responds with a UPD packet that has its height in it. A node receiving a UPD packet sets its height to one more than that of the node that generated the UPD.

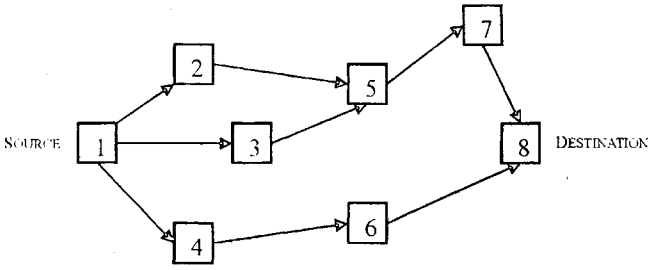

Figure 4: Propagation of QRY packet

A node with higher height is considered upstream and with lower height downstream. In this way a directed acyclic graph is constructed from source to the destination. Figure-4 illustrates a route creation process in TORA and node 5 does not propagate QRY from node 3 as it has already seen and propagated QRY message from node-2.

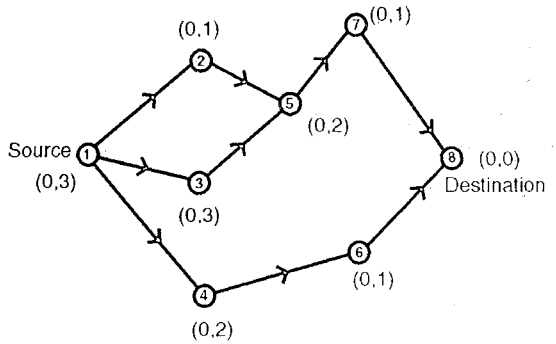

Figure 5: Height of each node updated as a result of UPD packet

In Figure-5, the source (i.e. node 1) may have received a UPD each from node 2 or node 3 but since node 4 gives it lesser height, it retains that height. When a node moves, the directed acyclic graph (DAG) route is broken, and route maintenance is needed to reestablish a DAG for the same destination. When the last downstream link of a node fails, it generates a new reference level. This results in the propagation of that reference level by neighboring nodes as shown in Figure-5. Links are reversed to reflect the change in adapting to the new reference level.

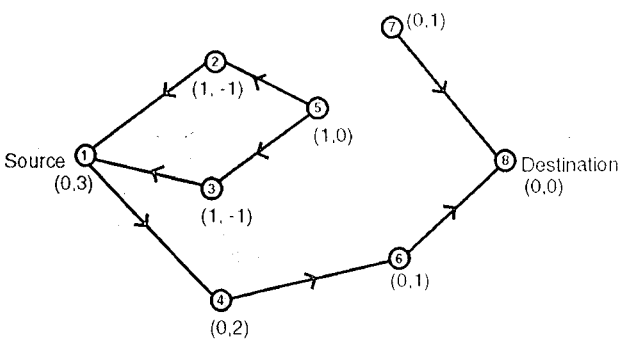

Figure 6: Re-establishing route on failure of link 5-7

This has the same effect as reversing the direction of one or more links when a node has no downstream links. In the route erasure phase, TORA floods a broadcast clear packet (CLR) throughout the network to erase invalid routes.

In TORA there is a potential for oscillations to occur, especially when multiple sets of coordinating nodes are concurrently detecting partitions, erasing routes, and building new routes based on each other. Because TORA uses inter-nodal coordination, its instability problem is similar to the "count-to-infinity" problem in distance-vector routing protocols, except that such oscillations are temporary and route convergence will ultimately occur.

SIMULATION SETUP

A discrete event simulator for wired and wireless networks called Network Simulator (ns-2) is used for comparative evaluation of these protocols. The simulator is based on UNIX and UNIX like platforms. The nodes inside the network can move within a rectangular boundary. Random Waypoint Mobility Model (Josh *et al.,* 1998) has been implemented in ns-2 for generating the node movement. The main performance measures that are substantially affected by the routing algorithm, the average end-to-end Throughput, the average end-to-end delay and the routing overhead. The performance of MANET routing protocol depends on the network conditions, which includes mobility, congestion and transmission condition etc. In (Liang and Kunz, 2006) authors discuss the impact of mobility on the performance of the routing protocols, using three different mobility models. It further looks into different parameters which change with the mobility from the point of view of individual nodes, showing that nodes experience different levels of mobility at different points of time. These parameters are important from the point of view of the user/application, so these define the external behavior of the protocol. In (Taha and Xian, 2005) authors evaluated the performance through a long-tailed distribution called the compound log-normal distribution. It also investigates overall system reliability of ad hoc networks with multi path routing.

The parameters that we have chosen to evaluate these routing protocols are mobility, network load and size of network environment. We have chosen a Conference type environment whose area is about 1000m x 1000m in dimension. To simulate a Conference type environment of around 1 hour in 200 sec we have chosen a network load of 5 packets per second and a total of 100 constant bit rate flows in the total simulation time. Bandwidth of the medium was kept at 2 Mbps, transmitter range of 250 m and pause time of 2s with a total of 50 nodes in the environment.

RESULTS

A. End-to-End Delays

Delays are the amount of time taken by the routing protocol to deliver a packet to the destination. DSDV protocol is showing a minimum delay, whereas DSR is showing a highest delay of packets. AODV and TORA show results that are comparable to DSDV.

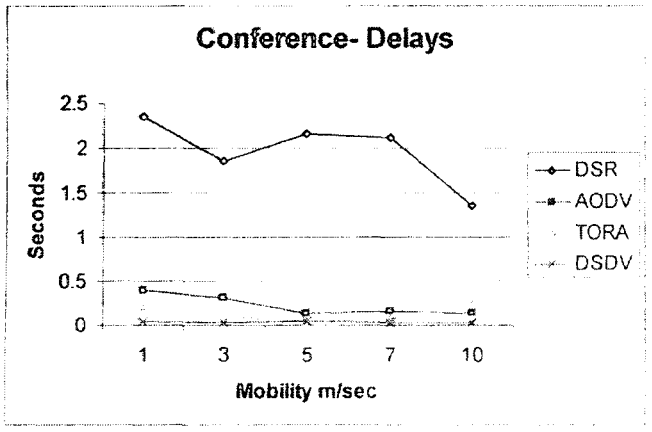

Figure 7: End-to-End Delay

The main observation from graph in Figure-7 is that DSDV performance is not affected by the mobility. Delays almost remained same during all mobility simulations. Another reason for this small delay is that only those packets belonging to valid routes at the sending instant are transmitted, a lot of packets are lost until new route table entries have been propagated through the network by the route update messages in DSDV.

B. Routing Overhead

AODV and DSDV are showing a good result as far as the routing overhead is concerned. The graph in Figure-8 is logarithmic graph that depicts the less routing traffic in AODV and DSDV. Overhead in TORA is the highest, which

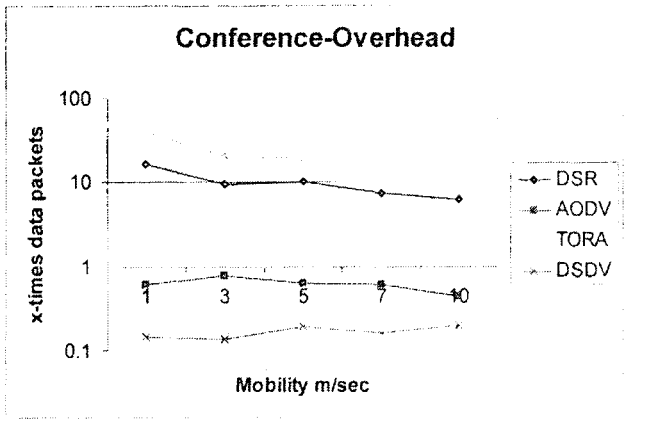

Figure 8: Routing Overhead

Various QRY and UPD packets would increase the overall overhead in TORA. Overhead here has been calculated as the ratio of the total number of packets sent by router to the total number of packet sent by the agents. Due to mobility, DSR is also showing higher routing overhead. Mobility means change in topology which means more updates being sent.

C End-to-End Throughput

End-to-End throughput is the measure of delivery of packets from source to the destination. According to graph in Figure-9, DSR is showing a good throughput. TORA is the least effective protocol as far as throughput is concerned. AODV and DSDV show a decline in throughput as the mobility increases. After mobility 3, these protocols shows the same results. DSDV is showing little better results than the AODV protocol, may be due to a constant update interval so that it could adapt to changing topology, whereas in AODV it is communicated only when the new route has to be established.

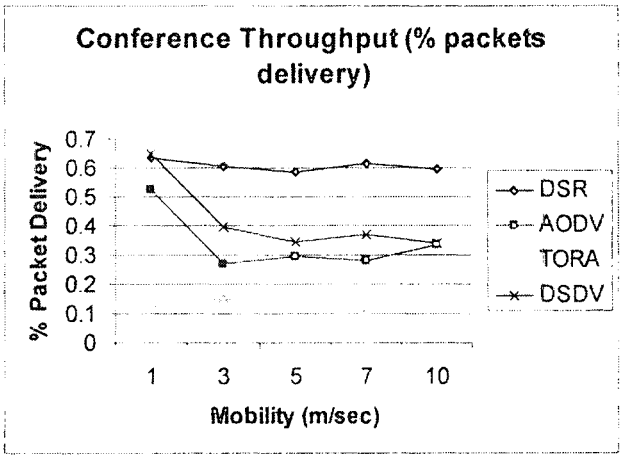

Figure 9: Throughput

CONCLUSIONS

To conclude, Mobile Ad-hoc Networks are the collection of mobile routers. In these networks status of communication links between routers at any given time is the function of their positions, transmission power levels, antenna pattern, and co-channel interference levels. For communication between various nodes inside a MANET, a shortest path needs to be established. Adaptive shortest path, and Link state routing algorithms are inefficient algorithms. For these networks, two approaches of Table-driven and on-demand routing techniques were discussed in the paper. The routing techniques are very much at development stages but some of them need to be enhanced for efficient communication between nodes. Today's Mobile revolution depicts a great future for these mobile ad-hoc networks, and before the demand came we need to be prepared for the best solution possible. Among the protocol studied in this paper, DSDV is coming

out to be the best-suited protocol for this environment, as it shows a lower delay, high throughput and low routing overhead. AODV is the second best in this order. Other two protocols DSR and TORA do not show effective results. Though DSR shows high throughput, but delays and the routing overhead are not proving its superiority over the other protocols.

FUTURE WORK

In evaluating these protocols, more work needs to be undertaken by creating more environments. Other environments may be created by varying packet sending rate, changing the size of network environment, or number of flows during the whole simulation, considering 3-dimensional environment in which node can also move along Z-axis.

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PERFORMANCE EVALUATION OF LAN FOR PARALLEL COMPUTING

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Abstract

The use of interconnected PC's, as single logical computational resources has become a widespread approach to a cost-effective high-performance computing. Though dedicated high end supercomputers still have their place in the market yet combined unused CPU cycles of desktop PCs available in the campus network can form comparable virtual supercomputers. Consequently, Parallel Processing in a network of PCs are attracted a boost of attention and becoming one of the most promising areas of large scale scientific computing. The parallel computing in a network of PC is greatly influence by the network performance metrics latency and bandwidth. In this paper, we studied the effect of peer to peer based network architecture and conducted experiments to measure latency and bandwidth of Level-1 Ethernet based interconnected PCs so that network parallel computing should become latency and bandwidth tolerant. The design is relatively easy to use, inexpensive to apply and extremely accurate. The highly accurate results provided by TCP/IP ping-pong can be coupled with any macro-benchmark such as parallel matrix multiplication benchmark to evaluate the performance of Local Area Network of local nodes or non-local nodes for parallel processing.

Key words: bandwidth measurement, latency, network performance, parallel computing.

INTRODUCTION

The demand of high performance computers is always a hunt for scientist and researcher in universities. Parallel computing in local area network is the area, which is becoming prominent to achieve the high performance supercomputing for complex problems. The computational power of COTS (commodity off the shelf) PCs has doubled about every 18 months. Of the top ten super computers, seven are COTS clusters: and the price of the third place COTS cluster is only 1.3 % of the cost of the supercomputer. So COTS has provided a cost-effective solution for high performance computing.

Unfortunately, most communication libraries PVM (http://www.netlib.org/pvm3/) and MPI (Farrel and Ong. 2004); http://www-unix.mcs.anl.gov/mpi/ are not yet optimized to harness the power of cluster computing. The majority of the application developed using these libraries are based on shared memory architecture and parallelized for multithreaded SMP systems, while only a few applications have been created to exploit distributed memory using message passing architecture.

There are several network interconnects that provide low latency (less than 10 microseconds) and high bandwidth (several GB per seconds). Ethernet network technology is now virtually ubiquitous; it has evolved from 10 base – T in 1993, Fast Ethernet in 1995, 1 Gigabit in 1998 to 10 Gigabit in 2002. The 10-gigabit Ethernet standard provides a significant increase in bandwidth while maintaining compatibility with the installed based of Fast Ethernet standard interfaces to protect existing investments in Ethernet technology and without much extra overhead required to change network protocols.

Ethernet not only dominates the LAN market, but is also taking hold in the MAN market. It has extended into WAN arena as both its distance and capacity has increased. Ethernet provides user level access to network interface cards for performing communication and support access to remote process memory address spaces. More sophisticated models such as LogP (Culler *et al.*, 1993) and BSP (http://www.bsp-worldwide.org/) are also used for network performance of interconnects such as Myrinet (Boden *et al.*, 1995; http://www.myri.com/) and Quadric (http://www.quadrics.com/quadrics/QuadricsHome.nsf/DisplayPages/Homepage).However these models focus on general parallel computing and don't address main features presents in these emerging commercials interconnects. Most clusters still continue to use IP as preferred higher level protocol for interconnection network communication. Beowulf project, world most popular Cluster, is based on Ethernet and Fast Ethernet.

NETWORK DESIGN AND PERFORMANCE METRICS

The proposed design consists of three components.

1. Master PC
2. Server
3. Cluster of nodes

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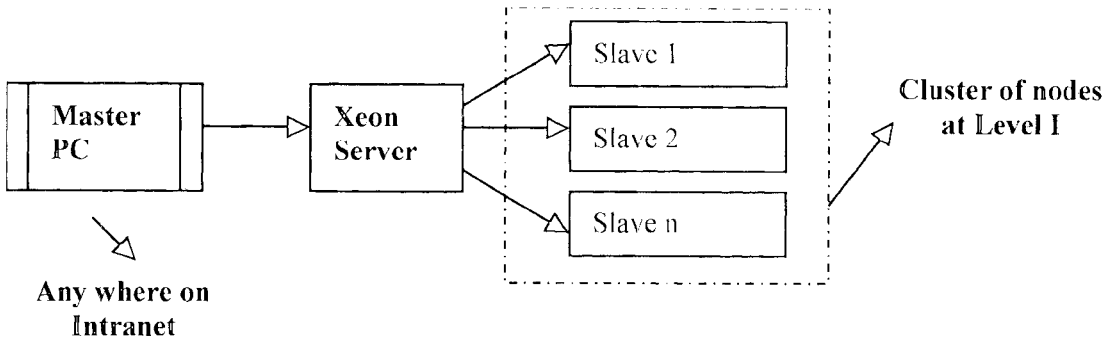

Figure 1 : Design of DeskOne

The design is shown in the figure 1 where in, Master PC is responsible for defining the job and submitting the job to Server. It can be anywhere in the Intranet and connected to server in the peer-to-peer network fashion at the time of submission of the job and getting back the results from the server.

Server breaks the job and allocates tasks to slaves and sends back the final result to Master PC to display the results. Server can be centrally located so-that average distance of the all the nodes to server may be equally poised. The main advantage of using server is to partition the job according to the computing power of the available nodes so that load balance may be maintained and server should not come under the situation of starvation.

Cluster of slave nodes at level I i.e. under single switch actually execute the code of the task and submit the results back to server. All the nodes are kept under level I so that sending and receiving messages (code) may become bandwidth and latency tolerant.

Metrics helps in comparing and characterizing the network. In network evaluation, Latency and Bandwidth are the two parameters, which affect the performance of both data intensive and compute intensive applications. In this paper, certain parametric values of TCP/IP are suggested which improves the latency and bandwidth. "Ping" is a standard tool to check one way communication and two-way way communication in a network of PCs. A Ping utility sends specifically marked packets from the local computer to a remote computer /device. Besides determining whether the remote computer is currently 'alive', ping also provides indicators of the general speed or reliability of the network connection. Ping utility will be used to see the network traffic, no. of hops, and zero-TTL conditions.

The network parameters latency and bandwidth are related to TCP window size. The TCP window size is the amount of buffering allowed by the receiver before an acknowledgement is required.

The formula that governs the optimum window size (MacDonald and Barkley, 2004) is

TCP Windows Size = Bandwidth (in Bytes) X Latency (RTT)

Effective bandwidth always varies and can be affected by high latency. Too much latency in too short a period of time can create a bottleneck and prevents data from "filling the pipe", thus decreasing effective bandwidth. Network tool like 'traceroute' measure latency by determine the time it takes to travel from source to destination and back the so-called round-trip time. Round-trip time is not the only way to specify latency but it is the most common.

IMPLEMENTATION

The environment is implemented using DeskGrid API and DLL files. DeskGrid is a full implementation of communication over TCP sockets Microsoft Win32 Platforms. This feature opens up the possibility of utilizing resources commonly excluded from network parallel computing systems such as Macintosh and Windows based PCs. For the experiment, DeskGrid communication library is loaded on the server and the contributor is loaded on the each local and non-local PCs in the local area network (LAN) in the background with high priority. It forms a grid of computers. The submitters are located any where in the LAN and submit the jobs to server. Tasks are managed by background daemon which is resident

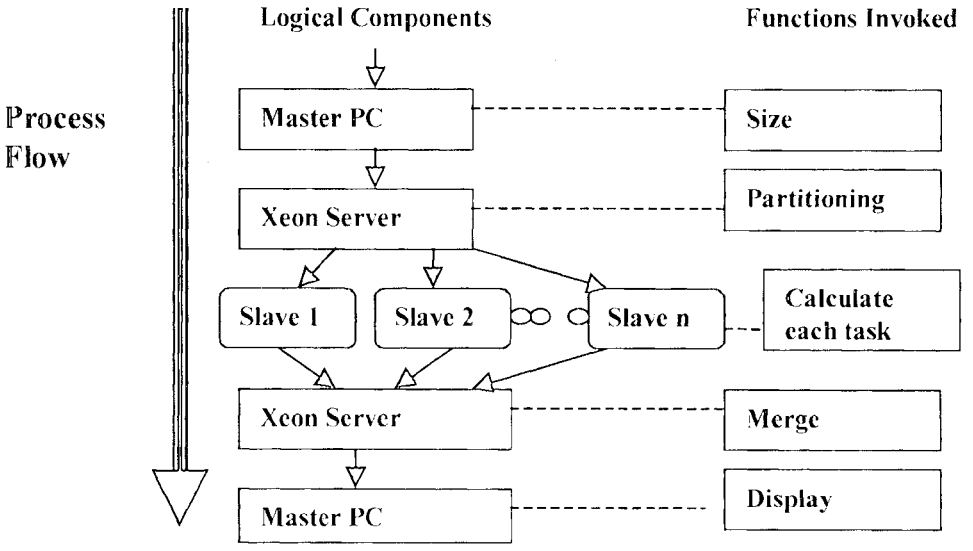

Figure 2 : Communication Protocol

on each node of the grid enabled cluster. The daemon communicates with each other using TCP protocol. The message size is kept to the packet size of 4k keeping in view the BDP (Bandwidth Delay Product) value calculated based on the Windows TCP Buffer size of 64K. The buffer size is set statically on each node. For implementing the parallel applications in LAN, a set of four best suitable computers , whose performance are identical, from the grid is taken as a PC cluster for executing the sub-tasks (jobs).

Moreover, we assume that at any given instant only one parallel program is in execution on the cluster, and that the main memory of each desktop system is large enough to accommodate the working set of the parallel process it executes. Finally, we assume that the communication network carries only traffic generated by the desktop PC in the cluster (both by the parallel program and from jobs executed by other desktop PC). The communication between tasks is shown in Fig. 2.

RESULTS AND ANALYSIS

Desktop PC loaded with windows XP, is having the 16 KB TCP window size as the default value. We evaluated our network performance with 64KB TCP windows size. The network load during the experiment was kept at zero level so that results with more accuracy and stability can be collected. The other TCP/IP (DWORD values) parameter, we added or modified are Global MAX TCP Windows Size (in bytes) and TCP1323Opts to be 3.

Table I contains the data collected when TCP size is kept

Table- I
Network Performance Metrics
(64 KB)

No. of hops	Bandwidth (Mbps)	Latency (ms)
1 (P2P)	62	1.1
2	50	1.8
3	16	4.0
4 (Distant)	13	4.8

Table- II
Network Performance Metrics
(32 KB)

No. of hops	Bandwidth (Mbps)	Latency (ms)
1 (P2P)	11.2	1.8
2	7.0	2.3
3	6.2	2.5
4 (Distant)	5.9	2.7

Table- III
Network Performance Metrics (16KB)

No. of hops	Bandwidth (Mbps)	Latency (ms)
1 (P2P)	10.6	1.5
2	7.1	2.2
3	5.9	2.7
4 (Distant)	5.5	2.9

at 64kb and nodes are varied from single hop to multiple hops. As the nodes in the grid are taken from multiple hops, the bandwidth decrease because of the network congestion and poor acknowledgement of the sent data. Data shows that with Windows Socket Size of 64 KB and clearly indicates Level-I Fast Ethernet Interconnections Network are suitable for coarse grain applications. As the nodes are moved across the switches, the bandwidth and latency both disfavor the design of the network.

Table II and Table III contains the data when the buffer size is kept at the default values. The analysis of these two tables reflects that nodes from multiple hops with 64kb size are better than level-I Ethernet interconnection with smaller TCP window size i.e 16kb and 32kb.

CONCLUSIONS

The aim of the study was see the effect of TCP socket size for the local nodes and non-local nodes in a grid enabled PC Cluster for parallel compuing. This paper shows that the obvious answer is to use Level I Ethernet Network with socket size 64KB along with other parameter TcpWindowSize value and additional parameter of GlobalWindowSize. as studied in the paper. DeskOne has performed better in peer to peer topology and is well suitable for both data intensive applications and compute intensive applications. When the clients move across the switches then DeskOne is not suitable for data intensive applications as the bandwidth chokes. Hopefully, in future the work in this area will lead to the development of even better tools and methods for creating high speed, low latency interconnection networks.

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METRIC APPROXIMATION PROPERTY IN THE TENSOR PRODUCT OF BANACH ALGEBRAS

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Abstract

Let X and Y be two Banach spaces and $X\otimes_\alpha Y$ be the completion of the algebraic tensor product $X\otimes Y$ w.r.t. the cross norm α. In this paper, we prove that if X and Y have the metric approximation property, then $X\otimes_\alpha Y$ has also the same provided the cross norm α is uniform. The converse of this result is also established for the projective tensor norm. Moreover, we investigate the conditions for the existence of the metric approximation property in the dual space of the projective tensor product of X and Y.

Key words: metric approximation property, tensor product, trace mapping, operator Ideal.

§1. INTRODUCTION

In 2004, Lima and Oja established necessary and sufficient conditions involving trace mappings and Hahn-Banach extension operators for a Banach space to have the metric approximation property (MAP). Lima and Lima (2004) proved that a Banach space X has the MAP iff for any Banach space Y, F(Y,X), the space of all finite rank operators from Y to X, is an ideal in L(Y,X), the space of all bounded linear operators from Y to X. In this paper, we extend these concepts to study the MAP in the tensor product of two Banach spaces. Let X and Y be two Banach spaces and α be a cross norm on the algebraic tensor product $X\otimes Y$. Let $X\otimes_\alpha Y$ be the completion of $X\otimes Y$ w.r.t. α. In the §2 of our paper, we prove that the existence of MAP in X and Y ensures the existence of the same in $X\otimes_\alpha Y$ for the uniform cross norm a. The converse of this also holds for the projective tensor norm. Moreover, criteria for the existence of MAP in the dual space of the projective tensor product $X\otimes_\gamma Y$ is also given in terms of operator ideals.

First we highlight some notations and terminologies which are initially needed for our purpose.

For the Banach spaces X and Y, we denote by L(X, Y) the Banach space of bounded linear operators from X to Y, and by F(X, Y) its subspace of finite rank operators.

1.01 Projective tensor product: Given normed spaces X, Y, the projective tensor norm $\|.\|_\gamma$ on $X\otimes Y$ is defined as:

$$\|u\|_\gamma = \inf\{\sum_i \|x_i\|\,\|y_i\| : u=\sum_i x_i\otimes y_i\}$$

where the infimum is taken over all (finite) representations of u. The completion of $(X\otimes Y, \|.\|_\gamma)$ is called the projective tensor product of X and Y, and is denoted by $X\otimes_\gamma Y$.

1.02 Metric approximation property: A Banach space X is said to have the metric approximation property (MAP) if for every compact set K in X and every $\varepsilon>0$, there is an operator $T\in F(X,Y)$ with $\|T\|\le 1$ such that $\|Tx-x\|\le\varepsilon$ for all $x\in K$.

1.03 Trace mapping: The trace mapping V from the projective tensor product $X^*\otimes_\gamma X$ to $F(X,X)^*$, the dual space of F(X,X) is defined by (Vu)(T) = trace(Tu), $u\in X^*\otimes_\gamma X$, $T\in F(X,X)$, i.e., if $u=\sum_{n=1}^{\infty} x_n^*\otimes x_n$, then $(Vu)(T)=\sum_{n=1}^{\infty} x_n^*(Tx_n)$.

In a linear normed space X, we denote the closed unit ball by B_X. Also, X is regarded as a subspace of X**. Thus the identity operator I_X on X is also considered as the embedding, identifying I_X with the canonical embedding $j_X: X\to X^{**}$.

F(X,X) is always equipped with the norm topology from L(X,X). Thus the trace mapping V: $X^*\otimes_\gamma X \to F(X,X)^*$ has norm one.

1.04 Hahn-Banach extension operator: Let F be linear subspace of a Banach space E. A linear operator

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$\varphi: F^* \to E^*$ is called a Hahn-Banach extension operator if $(\varphi f^*)(f) = f^*(f)$ and $\|\varphi f^*\| = \|f^*\|$ $\forall f \in F$ and $f^* \in F^*$.

The set of all Hahn-Banach extension operators $\varphi: F^* \to E^*$ is denoted by HB(F,E). We say that F is an ideal of E iff HB(F,E) is non-empty.

1.05 Metric approximation properties with conjugate operators: The dual space X* of a Banach space X is said to have the MAP with conjugate operators if I_X belongs to the closure of the subset $\{T^*: T \in F(X,X)\}$ of $L(X^*, X^*)$ with respect to the topology of uniform convergence on compact subsets of X*.

1.06 Definition: Let X and Y be two Banach spaces and let α be a crossnorm on $X \otimes Y$. We call $X \otimes_\alpha Y$ a tight tensor product of X and Y if the following two conditions hold:

(i). There is K>0 so that for all $S \in \overline{F(X)}$, $T \in \overline{F(Y)}$, the operator on $X \otimes Y$ given by:
$(S \otimes T)(x \otimes y) = Sx \otimes Ty$ $(x \in X, y \in Y)$ has α-operator norm not exceeding $K\|S\|.\|T\|$.

(ii). span $\{S \otimes T: S \in \overline{F(X)}, T \in \overline{F(Y)}\}$ is dense in $\overline{F(X \otimes_\alpha Y)}$.

§2. MAIN RESULTS

The following Theorem is very useful in proving our main results.

2.01 Theorem: A Banach space X has the MAP if and only if $I_X \in V^*(B_{F(X,X)^{**}})$ for the trace mapping $V: X^* \otimes_\gamma X \to F(X,X)^*$.

Proof: By definition, X has the MAP if and only if I_X belongs to the closure of $B_{F(X,X)}$ in the locally convex topology on L(X,X) of uniform convergence on compact subsets of X. By the identification of the dual space of the locally convex space L(X,X) due to Grothendieck, this is well-known to be equivalent to the fact that I_X belongs to the closure of $B_{F(X,X)}$ in the weak topology $\sigma(L(X,X), X^* \otimes_\gamma X)$, i.e.,

$$I_X \in \overline{B}_{F(X,X)}^{\sigma(L(X,X), X^* \otimes_\gamma X)} = L(X,X) \cap \overline{B}_{F(X,X)}^{\sigma(L(X,X^{**}), X^* \otimes_\gamma X)} \quad \ldots\ldots(1)$$

We consider the dual systems $\langle X^* \otimes_\gamma X, L(X,X^{**}) \rangle$ and $\langle F(X,X)^*, F(X,X)^{**} \rangle$. By an easy and straightforward verification, looking at $B_{F(X,X)}$ as a subset of $L(X,X^{**})$, we have that $B^\circ_{F(X,X)} = V^{-1}(B_{F(X,X)^{**}})$. Therefore, by elementary facts from the duality theory and by the bipolar theorem,

$$V^{-1}(B_{F(X,X)^{**}}) = V^{-1}(B_{F(X,X)^{**}}) = V^*(B^\circ_{F(X,X)^{**}})^0 \text{ and}$$

$$(V^*(B^\circ_{F(X,X)^{**}}))^{00} = B^{\circ\circ}_{F(X,X)} = \overline{B}^{\sigma(L(X,X^{**}), X^* \otimes_\gamma X)}_{F(X,X)}$$

This implies that (1) is equivalent to the condition $I_X \in V^*(B_{F(X,X)^{**}})$. □

2.02 Corollary: Let X be a Banach space. If the trace mapping $V: X^* \otimes_\gamma X \to F(X,X)^*$ *is isometric, then X has the MAP.*

Now, we can prove the first part of our main result as:

2.03 Theorem: Let X_1 and X_2 be two Banach spaces having MAP and α be a uniform cross norm on $X_1 \otimes X_2$. Then $X_1 \otimes_\alpha X_2$ has also the MAP.

Proof: Using Corollary 2.02, we have to show that the trace mapping

$V : (X_1 \otimes_\alpha X_2)^* \otimes_\gamma (X_1 \otimes_\alpha X_2) \to F(X_1 \otimes_\alpha X_2, X_1 \otimes_\alpha X_2)^*$ **is isometric.**

Let $u = \sum_{n=1}^{\infty} y_n^* \otimes z_n \in (X_1 \otimes_\alpha X_2)^* \otimes_\gamma (X_1 \otimes_\alpha X_2)$ **where** $y_n^* \in (X_1 \otimes_\alpha X_2)^*$

and $z_n = \sum_{k=1}^{\infty} x_{1nk} \otimes x_{2nk} \in X_1 \otimes_\alpha X_2$. **Clearly,** $\|Vu\| \le \|V\|.\|u\|_\gamma = \|u\|_\gamma$. **[since** $\|V\| = 1$. **]**

To show the converse:

Since $((X_1 \otimes_\alpha X_2)^* \otimes_\gamma (X_1 \otimes_\alpha X_2))^* = L((X_1 \otimes_\alpha X_2), (X_1 \otimes_\alpha X_2)^{**})$, $\exists T \in L((X_1 \otimes_\alpha X_2), (X_1 \otimes_\alpha X_2)^{**})$ **with**

$\|T\| = 1$ **such that** $\|u\|_\gamma = \text{trace } (Tu) = \sum_{k=1}^{\infty} T(z_n)(y_n^*)$. **Now,** $u = \sum_{n=1}^{\infty} y_n^* \otimes z_n$ **and**

$z_n = \sum_{k=1}^{\infty} x_{1nk} \otimes x_{2nk}$, **we may assume that** $x_{1nk} \to 0$ **and** $x_{2nk} \to 0$ **as** $k \to \infty$ **and** $\sum_{n=1}^{\infty} \|y_n^*\| = 1$.

Let $\varepsilon>0$. Since $\{0, x_{1n_1}, x_{1n_2},\ldots\}$ is a compact set in X_1 and X_1 has MAP, so, $\exists\ S_1 \in B_{F(X_1, X_1)}$ such that $\|S_1 x_{1nk} - x_{1nk}\| \le \varepsilon\ \forall\ n, k \in N$.

Similarly, since $\{0, x_{2n_1}, x_{2n_2},\ldots\}$ is a compact set in X_2 and X_2 has MAP, so, $\exists\ S_2 \in B_{F(X_2, X_2)}$ such that $\|S_2 x_{2nk} - x_{2nk}\| \le \varepsilon\ \forall\ n, k \in N$.

We define: $S_1 \otimes S_2 : X_1 \otimes_\alpha X_2 \to X_1 \otimes_\alpha X_2$ by

$$(S_1 \otimes S_2)\left(\sum_{n=1}^{\infty} x_{1n} \otimes x_{2n}\right) = \sum_{n=1}^{\infty} S_1(x_{1n}) \otimes S_2(x_{2n}).$$

$$\|(S_1 \otimes S_2)(z_n) - z_n\| = \left\|(S_1 \otimes S_2)\left(\sum_{k=1}^{\infty} x_{1nk} \otimes x_{2nk}\right) - \left(\sum_{k=1}^{\infty} x_{1nk} \otimes x_{2nk}\right)\right\|$$

$$= \left\| \sum_{k=1}^{\infty} S_1(x_{1nk}) \otimes S_2(x_{2nk}) - \left(\sum_{k=1}^{\infty} x_{1nk} \otimes x_{2nk}\right)\right\|$$

$$= \left\| \sum_{k=1}^{\infty} S_1(x_{1nk}) \otimes S_2(x_{2nk}) - \sum_{k=1}^{\infty} x_{1nk} \otimes S_2(x_{2nk}) + \sum_{k=1}^{\infty} x_{1nk} \otimes S_2(x_{2nk}) - \sum_{k=1}^{\infty} x_{1nk} \otimes x_{2nk}\right\|$$

$$= \left\| \sum_{k=1}^{\infty} (S_1(x_{1nk}) - x_{1nk}) \otimes S_2(x_{2nk}) + \sum_{k=1}^{\infty} x_{1nk} \otimes (S_2(x_{2nk}) - x_{2nk})\right\|$$

$$\le \sum_{k=1}^{\infty} \|S_1(x_{1nk}) - x_{1nk}\| \cdot \|S_2(x_{2nk})\| + \sum_{k=1}^{\infty} \|x_{1nk}\| \cdot \|S_2(x_{2nk}) - x_{2nk}\| \le \varepsilon\ \forall\ n \in N.$$

Now, $T: X_1 \otimes_\alpha X_2 \to (X_1 \otimes_\alpha X_2)^{**}$. So, $T \circ (S_1 \otimes S_2) : X_1 \otimes_\alpha X_2 \to (X_1 \otimes_\alpha X_2)^{**}$

We have, $\|T(z_n) - (T \circ (S_1 \otimes S_2))(z_n)\| = \|T(z_n) - (T(S_1 \otimes S_2))(z_n)\|$

$$\le \|T\| \|(S_1 \otimes S_2)(z_n) - z_n\| \le \varepsilon\ \forall\ n \in N.$$

Also, $T \circ (S_1 \otimes S_2) \in B^{F(X_1 \otimes_\alpha X_2, (X_1 \otimes_\alpha X_2)^{**})}$, $\forall u \in F(X_1 \otimes_\alpha X_2, X_1 \otimes_\alpha X_2)^*$.

$$\left\|\|u\|_\gamma - (Vu)(T \circ (S_1 \otimes S_2))\right\| = \left|\sum_{n=1}^{\infty} (Tz_n)(y_n^*) - \text{trace}(T \circ (S_1 \otimes S_2))(u)\right|$$

$$= \left|\sum_{n=1}^{\infty} (Tz_n)(y_n^*) - \sum_{n=1}^{\infty} ((T \circ (S_1 \otimes S_2))(z_n))(y_n^*)\right| = \left|\sum_{n=1}^{\infty} (Tz_n - (T \circ (S_1 \otimes S_2))(z_n))(y_n^*)\right|$$

$$\le \sum_{n=1}^{\infty} \|y_n^*\| \cdot \|Tz_n - (T \circ (S_1 \otimes S_2))(z_n)\| \le \varepsilon \Rightarrow \|u\|_\gamma - |(Vu)(T \circ (S_1 \otimes S_2))| \le \varepsilon$$

$$\Rightarrow \|u\|_\gamma \le |(Vu)(T \circ (S_1 \otimes S_2))| + \varepsilon \le \|Vu\| + \varepsilon.$$ **Hence,** $\|u\|_\gamma = \|Vu\|$.

So, V is an isometric embedding which implies that $X_1 \otimes_\alpha X_2$ has MAP. □

Next, we state the following enlightening Theorem.

2.04 Theorem: A Banach space X has the MAP if and only if F(Y,X) is an ideal in L(Y,X) for any Banach space Y.

As a converse to Theorem 2.03, we prove:

2.05 Theorem: Let X_1 and X_2 be two Banach spaces such that $X_2 \otimes_\gamma X_1$ has the MAP. Then each of X_1 and X_2 has also MAP.

Proof: Since $X_2\otimes_\gamma X_1$ has MAP, so, for any Banach space Y, $F(Y, X_2\otimes_\gamma X_1)$ is an ideal in $L(Y, X_2\otimes_\gamma X_1$ So, $\exists$ a Hahn-Banach extension operator $\varphi:F(Y, X_2\otimes_\gamma X_1)^*\to L(Y,X_2\otimes_\gamma X_1)^*$ such that $(\varphi f^*)(f)=f^*(f)$ and $\|\varphi f^*\| = \|f^*\|$ $\forall f\in F(Y, X_2\otimes_\gamma X_1)$, $f^*\in F(Y,X_2\otimes_\gamma X_1)^*$.

Claim: *Each $S\in F(Y, X_2\otimes_\gamma X_1)$ gives rise to an operator $\hat{S}\in F(Y, X_1)$ such that $\|\hat{S}\|\le\|S\|$.*

Proof: Let $x_2\in X_2$ such that $x_2 \# 0$. Let $e_1=\frac{x_2}{\|x_2\|}$. Then, $\|e_1\|=1$.

By Hahn-Banach theorem, $\exists f\in X_2^*$ such that $\|f\|=1$ and $f(x_2)=\|x_2\|$. So, $f(e_1)=1$.

We define: $T: X_2\otimes_\gamma X_1\to X_1$ by $T(\sum_{n=1}^{\infty} x_{2n}\otimes x_{1n})=\sum_{n=1}^{\infty} f(x_{2n})x_{1n}$.

$$\left\|T(\sum_{n=1}^{\infty} x_{2n}\otimes x_{1n})\right\| = \left\|\sum_{n=1}^{\infty} f(x_{2n})x_{1n}\right\| \le \sum_{n=1}^{\infty}\|f\|.\|x_{2n}\|.\|x_{1n}\| \le \sum_{n=1}^{\infty}\|x_{2n}\|.\|x_{1n}\|.$$

Thus, $\|Tz\|\le\|z\|$ $\forall z\in X_2\otimes_\gamma X_1$. So, $\|T\|\le 1$. also, $T(e_1\otimes x) = x$ $\forall x\in X_1$.

Now, $S\in F(Y, X_2\otimes_\gamma X_1)$. Let $\hat{S}=ToS$. Clearly, $\hat{S}\in F(Y, X_1)$.

For $y\in Y$, $\|\hat{S}(y)\| = \|(T\circ S)(y)\| = \|T(S(y))\| \le \|T\|.\|S\|.\|y\| \le \|S\|.\|y\|$. Thus, $\|\hat{S}\|\le\|S\|$.

Now, for $f_1^*\in F(Y, X_1)^*$, we define $\hat{f_1}^*(S)= f_1^*(\hat{S})$ where $S\in F(Y, X_2\otimes_\gamma X_1)$. Then, $\hat{f_1}^*\in F(Y, X_2\otimes_\gamma X_1)^*$.

Claim: *Corresponding to each $L\in L(Y, X_1)$ $\exists$ an operator $L_1\in L(Y, X_2\otimes_\gamma X_1)$ such that $\|L_1\| = \|L\|$ and if L is of finite rank, then L_1 is also of finite rank.*

Proof: $L\in L(Y, X_1)$. For $y\in Y$, $L(y)\in X_1$.

We define: $L_1 : Y\to X_2\otimes_\gamma X_1$ by $L_1(y)= e_1\otimes L(y)$. Then, L_1 is well-defined and linear.

$\|L_1(y)\| = \|e_1\otimes L(y)\|_\gamma = \|e_1\|.\|L_1(y)\| = \|L(y)\| \Rightarrow \|L_1\| = \|L\|$.

So, L_1 is bounded. Thus, $L_1\in L(Y, X_2\otimes_\gamma X_1)$.

Clearly, if L is of finite rank, then L_1 is also of finite rank.

We define: $\psi: F(Y, X_1)^*\to L(Y, X_1)^*$ by $(\psi f_1^*)(L)= (\varphi\hat{f_1}^*)(L_1)$

where $L\in L(Y, X_1)$, $f_1^*\in F(Y, X_1)^*$. Then, ψ is linear.

For $L\in F(Y, X_1)$, $(\psi f_1^*)(L)= (\varphi\hat{f_1}^*)(L_1)= \hat{f_1}^*(L_1)= f_1^*(\hat{L_1})$.

Now, for $y\in Y$, let $L(y) =x_1$. So, $L_1(y)= e_1\otimes x_1$.

$\hat{L_1}(y)=(ToL_1)(y)=T(L_1(y))=T(e_1\otimes x_1)= x_1= L(y)$ $\forall y\in Y \Rightarrow \hat{L_1}=L$.

So, $(\psi f_1^*)(L)= f_1^*(L)$ $\forall L\in F(Y, X_1)$, $f_1^*\in F(Y, X_1)^*$.

Now, $\|\psi f_1^*\| = \sup\{\|\psi f_1^*(L)\| : \|L\|\le 1\}$

$$=\sup\{\|\varphi\hat{f_1}^*(L_1)\| : \|L_1\|\le 1\} = \|\varphi\hat{f_1}^*\| = \|\hat{f_1}^*\| = \sup\{\|\hat{f_1}^*(S)\| : \|S\|\le 1\} = \sup\{\|f_1^*(\hat{S})\| : \|\hat{S}\|\le 1\} = \|f_1^*\|.$$

Hence, ψ is a Hahn-Banach extension operator.

So, for any Banach space Y, $F(Y, X_1)$ is an ideal in $L(Y, X_1)$, Hence, by Theorem 2.04, X_1 has the MAP. Similarly, we can prove that X_2 also has the MAP. □

Now, we concentrate on the study of MAP in the dual space of the tensor product of two Banach spaces. We observe that if X_1 and X_2 are two Banach spaces such that X_1^* and X_2^* have the MAP with conjugate operators, then $(X_1\otimes\gamma X_2)^*$ may not have the MAP with conjugate operators. For example, consider the

space l_2. Since $\{e_i\}$ is a (Schauder) basis for l_2, so, it has BAP, and so, has AP. $l_2^*=l_2$. Now, l_2 is a separable dual space with AP. By an important result of Grothendieck, separable dual spaces with AP has MAP. Hence l_2 has the MAP. Again $(l_2\otimes_\gamma l_2)^*= L(l_2, l_2^*) =L(l_2)$. But for a Hilbert space H, L(H) does not have the AP[6]. Hence, $(l_2\otimes_\gamma l_2)^*$ does not have the AP and so, MAP. Now, naturally the question arises under which conditions the dual space of the projective tensor product of two Banach spaces can have the MAP? We give a solution to this problem by the following:

2.06 Theorem: Let X_1 and X_2 be two Banach spaces such that X_1^* and X_2^* have the MAP with conjugate operators. If $\overline{F(X_1)}\otimes\overline{F(X_2)}$ is dense in $\overline{F(X_1\otimes_\gamma X_2)}$ and the dual norm of γ is uniform on $X_1^*\otimes X_2^*$, then $(X_1\otimes_\gamma X_2)^*$ has the MAP.

To prove this, first we state the following Theorem:

2.07 Theorem: The dual space X* of a Banach space X has the MAP if and only if X* has the MAP with conjugate operators.

[For the proof, we refer to Lima and Oja (2004).]

We recall that a tensor z of the algebraic tensor product $X_1\otimes X_2$ can be identified with a bounded linear transformation T_z: $X_1^*\to X_2$ of finite rank. If z is represented by $z=\sum_{i=1}^{n} x_{1i}\otimes x_{2i}$ **for** $x_{1i}\in X_1$, $x_{2i}\in X_2$, **then**

for $x_1^*\in X_1^*$, $T_z(x_1^*)=\sum_{i=1}^{n}\langle x_{1i}, x_1^*\rangle x_{2i}=\sum_{i=1}^{n} x_1^*(x_{1i})x_{2i}$

Following Grothendieck, we refer to z as a kernel of T_z. The transformation T_z is always compact and continuous w.r.t. the weak*-topology of X_1^* and the weak-topology of X_2.

Now, if $u\in X_1^*\otimes X_1$ has a representation $u=\sum_{i=1}^{n} x_{1i}^*\otimes x_{1i}$ the trace of u is defined by, trace(u) = $\sum_{i=1}^{n}\langle x_{1i}, x_{1i}^*\rangle=\sum_{i=1}^{n} x_{1i}^*(x_{1i})$. Clearly, trace(u) does not depend on the particular representation of u.

Let $S\in L(X_1,X_2^*)$. We define the two tensors $S\times z\in X_1\otimes X_1^*$ and $z\times S\in X_2^*\otimes X_2$ by the relations

$$S\times z=\sum_{i=1}^{n} x_{1i}\otimes S^* x_{2i} \text{ and } z\times S=\sum_{i=1}^{n} Sx_{1i}\otimes x_{2i},$$

$$\text{where } z=\sum_{i=1}^{n} x_{1i}\otimes x_{2i}\in X_1\otimes X_2.$$

We define, $\langle z,S\rangle$ **= trace(S**$\times$**z).**

Now, an embedding is defined from $X_1^*\otimes X_2^*$ into $L(X_1, X_2^*)$ as follows:

Each $t\in X_1^*\otimes X_2^*$ is a kernel of a bounded linear transformation S_t: $X_1^{**}\to X_2^*$. But S_t is continuous w.r.t. the weak*-topology of X_1^{**} and the weak-topology of X_2^*. Consequently, it is completely determined by its action on $X_1\subset X_1^{**}$. We identify this transformation with its restriction to X_1, for which we use the same symbol S_t, whenever there is no possibility of confusion. We refer to this embedding as the natural mapping from $X_1^*\otimes X_2^*$ to $L(X_1, X_2^*)$.

Thus, we have that the natural image of $X_1^*\otimes X_2^*$ is contained in $L(X_1, X_2^*)=(X_1\otimes_\gamma X_2)^*$. For

$\sum_{i=1}^{n} x_{1i}\otimes x_{2i}\in X_1\otimes_\gamma X_2$ **we have,**

$$\langle z, S_{x_1^*\otimes x_2^*}\rangle = \text{trace}(S_{x_1^*\otimes x_2^*}\times z)=\text{trace}\left(\sum_{i=1}^{n} S_{x_1^*\otimes x_2^*}.x_{1i}\otimes x_{2i}\right)=\sum_{i=1}^{n}\langle x_{2i},\langle x_1^*, x_{1i}\rangle x_2^*\rangle$$

$$=\sum_{i=1}^{n}\langle\langle x_{1i}, x_1^*\rangle x_{2i}, x_2^*\rangle=\text{trace}\left(\sum_{i=1}^{n} x_2^*\otimes\langle x_{1i}, x_1^*\rangle x_{2i}\right)$$

$$=\text{trace}\left(x_2^*\otimes\sum_{i=1}^{n}\langle x_{1i}, x_1^*\rangle x_{2i}\right)=\text{trace}(x_2^*\otimes T_z(x_1^*))=\langle T_z(x_1^*), x_2^*\rangle.$$

The norm of the dual space $(X_1 \otimes_\gamma X_2)^*$ is denoted by γ^*, and it is called the dual norm of γ. Clearly, under the embedding of $X_1^* \otimes X_2^*$ into $(X_1 \otimes_\gamma X_2)^*$, the dual norm γ^* induces a norm on $X_1^* \otimes X_2^*$, which we continue to denote by γ^*.

Now, we show that the dual norm of γ is also a cross norm:

Let $x_1^* \otimes x_2^* \in X_1^* \otimes X_2^*$ **and** $z = \sum_{i=1}^{n} x_{1i} \otimes x_{2i} \in X_1 \otimes_\gamma X_2$.

$$\|(x_1^* \otimes x_2^*)(z)\| = \|x_2^*(T_z x_1^*)\| = \left\|x_2^*\left(\sum_{i=1}^{n} x_1^*(x_{1i})x_{2i}\right)\right\| \le \|x_1^*\|\|x_2^*\| \cdot \sum_{i=1}^{n} \|x_{1i}\|\|x_{2i}\|$$

$$\Rightarrow \|x_1^* \otimes x_2^*\|_{\gamma^*} \le \|x_1^*\|\|x_2^*\|.$$

$$\|x_1^* \otimes x_2^*\|_{\gamma^*} \ge \sup\{\|(x_1^* \otimes x_2^*)(x_1 \otimes x_2)\| : \|x_1 \otimes x_2\|_\gamma = 1\}$$

$$= \sup\{\|x_2^*(T_{x_1 \otimes x_2} x_1^*)\| : \|x_1\| = \|x_2\| = 1\} = \sup\{\|x_2^*(x_1^*(x_1)x_2)\| : \|x_1\| = \|x_2\| = 1\}$$

$$= \sup\{|x_1^*(x_1)|\|x_2^*(x_2)\| : \|x_1\| = \|x_2\| = 1\} = \|x_1^*\|\|x_2^*\|$$

Thus, $\|x_1^* \otimes x_2^*\|_{\gamma^*} = \|x_1^*\|\|x_2^*\|$. **Hence,** γ^* **is also a cross norm.**

A cross norm is called reasonable if the dual norm is also a cross norm. Thus, we get that the projective tensor norm γ is reasonable.

With the aid of the following Proposition by Gronback, Johnson and Willis a relation between the tightness of the tensor product and the dual norm can be obtained.

2.08 Proposition: $X \otimes_\alpha Y$ is a tight tensor product of X and Y if and only if:

(i) $(X \otimes_\alpha Y)^*$ is a right Banach $\overline{F(X)}$ - and $\overline{F(Y)}$ - submodule of $L(Y, X^*)$.

(ii) $X^* \otimes Y^*$ is norm dense in $(X \otimes_\alpha Y)^*$.

2.09 Corollary: Suppose that α is a reasonable cross norm on $X \otimes Y$ and that the module property 2.08(i) holds. Then, $X \otimes_\alpha Y$ a tight tensor product if and only if $(X \otimes_\alpha Y)^* = X^* \otimes_{\alpha^*} Y^*$, where α^* denotes the dual norm.

Proof of Theorem 2.06 : Let $S \in \overline{F(X_1)}$, $T \in \overline{F(X_2)}$. We define:

$$S \otimes T: X_1 \otimes X_2 \to X_1 \otimes X_2 \text{ by } (S \otimes T)(x_1 \otimes x_2) = Sx_1 \otimes Tx_2 \quad (x_1 \in X_1, x_2 \in X_2).$$

Let $u = \sum_{i=1}^{n} x_{1i} \otimes x_{2i} \in X_1 \otimes X_2$. **Then,** $\|(S \otimes T)(u)\|_\gamma = \left\|(S \otimes T)\left(\sum_{i=1}^{n} x_{1i} \otimes x_{2i}\right)\right\|_\gamma$

$$= \left\|\sum_{i=1}^{n} S(x_{1i}) \otimes T(x_{2i})\right\|_\gamma \le \sum_{i=1}^{n} \|S(x_{1i})\|\|T(x_{2i})\| \le \|S\|\|T\| \sum_{i=1}^{n} \|x_{1i}\|\|x_{2i}\|.$$

Thus, $\|S \otimes T\| \le \|S\|\|T\|$ .....(*)

Also, span{ $S \otimes T$: $S \in \overline{F(X_1)}$, $T \in \overline{F(X_2)}$ **}** $= \overline{F(X_1)} \otimes \overline{F(X_2)}$ **is dense in** $\overline{F(X_1 \otimes_\gamma X_2)}$.

SO, $X_1 \otimes \gamma X_2$ is a tight tensor product of X_1 and X_2. So, the condition(i) of Proposition2.08 holds. Again, γ is a reasonable cross norm. Hence, by Corollary 2.09, $(X_1 \otimes \gamma X_2)^* = X_1^* \otimes \gamma^* X_2^*$.

Since X_1^* and X_2^* have the MAP with conjugate operators, so, by Theorem 2.07, X_1^* and X_2^* have the MAP. Again, by the given condition, γ^* is a uniform tensor norm. So, applying Theorem2.03, we get that $X_1^* \otimes \gamma^* X_2^*$ has also the MAP, i.e., $(X_1 \otimes \gamma X_2)^*$ has the MAP.

Remark: The above Theorem can be generalized to the case of uniform reasonable cross norms on $X_1 \otimes X_2$, whose dual norms are also uniform. A tensor norm α on $X_1 \otimes X_2$ is called uniform if, for every $t \in X_1 \otimes X_2$, $S \in L(X_1)$ and $T \in L(X_2)$, we have $\|(S \otimes T)(t)\|_\alpha \leq \|S\| . \|T\| . \|t\|_\alpha$ Clearly, for such α, the condition (*) is easily satisfied and it follows that $(X_1 \otimes \alpha X_2)^*$ has the MAP. But it is not known whether the stated condition is sufficient for any other tensor norm. The following problem arises in this context:

what are the sufficient conditions for $(X_1 \otimes \alpha X_2)^*$ to have the MAP for any tensor norm α, if X_1^* and X_2^* have the MAP ?

Again regarding the converse, another interesting problem can be raised as:

does the existence of MAP in $(X_1 \otimes \alpha X_2)^*$ ensures the existence of the same in X_1^* and X_2^* also?

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FIBER PRODUCTS AND HENSELIZATIONS OF LOCAL RINGS

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Abstract

In this paper we will prove that the henselization of fiber product of two local rings is the fiber product of their respective henselizations.

Key words: flat and faithfully flat modules, fiber product, etale′ extension and henselization.

All the rings considered in this *paper* are commutative with identity. We will be dealing with local rings by which we mean a noetherian ring with a unique maximal ideal consisting of all non-units of the ring. The main objective of this note is to establish the permutability of henselization and fiber product formation. More explicitly, we show that if **(R, M)** and **(S, N)** are two local rings with the same residue class field **k**, then the fiber product over **k** of their henselization and the henselization of their fiber product are isomorphic. The henselization ${}^{h}\mathbf{R}$ of a ring **R** at an ideal of **R** can be realized in several ways. In this paper we realize the henselization by way of passing to the inductive limit over etale′ extensions.

For the convenience of the reader and for setting up the notations, we give all the necessary definitions and known results. While proving the main result we show that

(i) The fiber product of faithfully flat extensions is faithfully flat and (ii) the fiber product of two etale′ extensions has a vanishing module of differentials.

The Fiber product of **(R, M)** and **(S, N)** over $\mathbf{k} \cong \mathbf{R}/\mathbf{M} \cong \mathbf{S}/\mathbf{N}$ is defined as the ring consisting of all pairs (x, y) in $\mathbf{R} \times \mathbf{S}$ such that $x^* \equiv y^*$ in **k**. Here x* (y*) denote the image of x in **R/M** (y in **S/N**)

Given a ring **R** and an **R** –module M and a sequence $\mathfrak{R}$ of **R** – modules

$$\ldots \to N \to N_1 \to N_2 \to \ldots$$

We denote by $\mathfrak{R} \otimes \mathbf{M}$ the sequence

$$\ldots \to N \otimes M \to N_1 \otimes M \to N_2 \otimes M \to \ldots$$

Obtained by tensoring the sequence $\mathfrak{R}$ with **M** over **R**. We say that **M** is **R** –flat (or flat over **R**) if $\mathfrak{R} \otimes \mathbf{M}$ is exact whenever $\mathfrak{R}$ is exact. We say **M** is faithfully flat over R if $\mathfrak{R} \otimes \mathbf{M}$ is exact if and only if $\mathfrak{R}$ is exact. Secondly, we use the local criterion of flatness. This criterion is given in the following result (Matsumura, H. 1980, pp. 146-147) Let **R** be a ring **A** be an ideal of **R** and M an **R** module. Set $\mathbf{R}_0 = \mathbf{R}/\mathbf{A}$ and $M_0 = M/AM$ Suppose (i) **A** is nilpotent or (ii) **R** is noetherian and M is such that $\mathbf{B} \otimes \mathbf{M}$ is separated in the **A** – adic topology for every ideal **B** of **R**. Then the following are equivalent

(1) M is **R** – flat.
(2) $\mathrm{Tor}_1^{R}(\mathbf{N}, \mathbf{M}) = 0$ for all $\mathbf{R}_0$ -module **N**.
(3) $\mathbf{M}_0$ is $\mathbf{R}_0$ - flat and $\mathbf{A} \otimes_R \mathbf{M}$ is isomorphic to **A** M.
(4) $\mathbf{M}_0$ is $\mathbf{R}_0$ - flat and $\mathrm{Tor}_1^{R}(\mathbf{R}_0, \mathbf{M}) = 0$
(5) $\mathbf{M}_0$ is $\mathbf{R}_0$ - flat and the canonical maps.

$$\gamma_n : \mathbf{A}^n/\mathbf{A}^{n+1} \otimes_{R0} \mathbf{M}_0 \to \mathbf{A}^n\mathbf{M}/\mathbf{A}^{n+1}\mathbf{M}$$

For each n, are isomorphisms.

(6) $\mathbf{M}_n = \mathbf{M}/\mathbf{A}^{n+1}\mathbf{M}$ is $\mathbf{R}_n$ - flat for each $n \geq 0$ where $\mathbf{R}_n = \mathbf{R}/\mathbf{A}^{n+1}$

Lemma. Let **(R, M), (S, N)** be as before and **(S′, N′)** be flat (faithfully flat) extension of **(S , N).** If n is in **N′** and r is in **R** then $(0, n) \otimes (r, 0) = 0$

Proof : As $n \in \mathbf{N}' = \mathbf{N}.\mathbf{S}'$, $n = \sum_i n_i s_i$ where $n_i \in \mathbf{N}$ and $s_i \in \mathbf{S}'$, We now find $r_i \in \mathbf{R}$ such that r_i and s_i have the same image in $\mathbf{k} = \mathbf{R}/\mathbf{M} = \mathbf{S}'/\mathbf{N}'$.

Therefore, $(0, n) = \sum_i (0, n_i)(r_i, s_i)$

Hence $(0, n) \otimes (r, 0) = \sum_i (0, n_i)(r_i, s_i) \otimes (r, 0)$

$= \sum_i (0, n_i)(r, 0) \otimes (r_i, s_i)$

$= \sum_i (0, 0) \otimes (r_i, s_i)$

$= 0.$

We first prove the following.

Proposition 1 . Let **(R′ , M′)** and **(S′ , N′)** be flat (faithfully flat) extensions of **(R , M)** and **(S , N)** respectively and having the same residue class field **k** , then $\mathbf{R}' \times_{\mathbf{k}} \mathbf{S}'$ is a flat (faithfully flat) extension of $\mathbf{R} \times_{\mathbf{k}} \mathbf{S}$

Proof : For proving this proposition we observe that it is sufficient to show that $\mathbf{R} \times_{\mathbf{k}} \mathbf{S}'$ is flat (faithfully flat) over

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$R \times_k S$. This is so because of the fact that the composition of two Flat extensions $R \times_k S'$ and $R' \times_k S'$ over $R \times_k S$ and $R \times_k S'$ respectively is again flat .Such is the case for faithfully flat extensions.

Using (3) of the local criterion it suffices to show that

$(M \times_k N') \otimes_{R \otimes S} (R \times_k S') \rightarrow (R \times_k S')$ is injective.

Suppose $\sum_i (m_i, n_i) \otimes (r_i, s_i) = (\sum_i m_i r_i, \sum_i n_i s_i)$ is mapped on to zero where $m_i \in M$, $r_i \in R$, $n_i \in N'$ and $s_i \in S'$. Since S' is flat over S, $\sum_i n_i \otimes s_i = \sum_i n_i s_i = 0$ in $N' \otimes S'$. Therefore ,there exists elements s_{ij} in S and $y_j \in N$ such that $\sum_j s_{ij} y_j = n_i$ and $\sum_i s_{ij} s_i = 0$ for all i and j respectively. Now choose r_{ij} in R such that $r_{ij}^* = s_{ij}^*$ in k, then

$\sum_i (m_i, n_i) \otimes (r_i, s_i)$
$= \sum_i \{(m_i, 0) + (0, n_i)\} \otimes (r_i, s_i)$
$= \sum_i \{\sum_j (r_{ij}, s_{ij})(0, y_j) + (0, n_i)(0, y_j)\} \otimes (r_i, s_i)$
$= \sum_{ij} ((0, y_j) \otimes (r_{ij} r_i, s_{ij} s_i)) + \sum_j (0, y_j) \otimes (0, \sum_i n_i s_i)$
$= \sum_j (0, y_j) \otimes (\sum_i r_{ij} r_i, \sum_i s_{ij} s_i) + \sum_j (0, y_j) \otimes (0, 0)$
$= \sum_j (0, y_j) \otimes (\sum_i r_{ij} r_i, 0) + (0, 0)$
$= 0$ (by lemma)

We now introduce the notion of **module of differentials, etale' extensions** and state a result that gives a criterion for an extension to be etale' extension.

Definition 1. Let R be a ring and S an over ring of R, and $S^e = S \otimes_R S^o$ be the enveloping algebra of S . This S^e can be made into an R – algebra via the map λ from R to S^e given by $\lambda(a) = a \otimes 1$. Let μ be the mapping from S^e to S given by $\mu(b \otimes b') = b \, . \, b'$ and $\Im$ be the kernel of μ . Then $\Omega = \Im / \Im^2$ is an R – module under the action of λ. This Ω is called the module of differentials of S over R and is denoted by $\Omega_{S/R}$.

Definition 2 Let R be a commutative ring with identity and A be an R – algebra. We say that A is etale' over R if

(a) A is an R – algebra of finite presentation;

(b) For each R – algebra B and for each non –zero ideal J of B with $J^2 = 0$, the canonical mapping Hom. (A, B) to Hom. $(A, B/J)$ is a bijection.

The following result gives criteria for an extension to be etale'. (See chapter 5 of Raynand, M. 1970 for details)

The notations being the same as above.

1. A is etale' over R if and only if

(a) $\Omega_{A/R} = 0$

(b) A is a flat R - module.

2 .Jacobian criteria.

Suppose A is a localization of the ring $R[X_1, X_2, \ldots\ldots X_n]/(f_1, f_2 \ldots\ldots, f_n)$ and φ is the natural map from $R[X_1, X_2, \ldots\ldots X_n]$ to A/M , where M is the unique maximal ideal of A . Then A is etale' over R if

$$\varphi\left(\det \left| \partial(f_1, f_2 \ldots\ldots, f_n) \middle| \partial(X_1, X_2, \ldots\ldots X_n) \right|\right) \neq 0.$$

We introduce some notations: We shall use X for the indeterminate in the polynomial ring $S[X]$ over S. If f is a polynomial in $S[X]$, then we denote the residue class ring $S[X]/(f(X))$ by $S[x]$ where x is the image of X modulo the ideal generated by $f(X)$. By x^* we denote the class of X modulo the maximal ideal.

Proposition 2 Suppose (R', M') and (S', N') are extensions of (R, M) and (S, N) with trivial residue class extensions respectively .Then the module of differentials of $R' \times_k S'$ over $R \times_k S$ is trivial .Here k denotes the common residue class ring $R/M = S/N = R'/M' = S'/N'$.

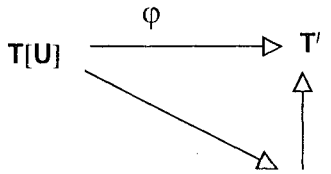
Proof : As in the case of proposition 1 , It suffices to prove the proposition in case $R = R'$ and S' is etale' extension of S .Further , since every etale' extension can be realized as a succession of standard etale' extensions, we may take S' to be standard etale' extension.

Thus $S' = (S[X]/f(X))_{g(X)}$ where $f(X)$ is a polynomial over S and the formal derivative $f'(X)$ of $f(X)$ is invertible in the localization of $S[X]/(f(X))$ at the multiplicatively closed set generated by $g(X)$.

Suppose $x^* = \alpha^*$ in k where α is in S .Observe that the maximal ideal N' of S' is generated by N and $(x - \alpha)$ .Further $\partial f / \partial x$ evaluated at α has a non – zero image in k. Now choose α' in R such that α and α' have the same image in k .If $f(X) = s_0 + s_1 X + \ldots\ldots + s_m X^m$, where $s_i \in S$,then we can find elements $r_0, r_1 \ldots r_m$ in R such that $s_i^* = r_i^*$ for each i .

Now we set $F(U) = (r_0, s_0) + (r_1, s_1)U + \ldots\ldots + (r_m, s_m)U^m$. Then $F(U)$ is a polynomial over $R \times S' = T'$ (say). We also set $T = R \times_k S$. If we now define φ from $T[U]$ to T'

by setting φ **(U)** = $(\alpha', \mathbf{x})$, then by suitably choosing the $\mathbf{r}_i$, i = 0, 1, ...m ,We may assume that φ **(F)** = 0. Therefore φ factors in the following diagram.



T [U] / (F)=T(u]

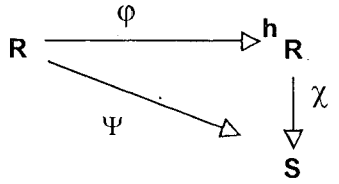
Thus we find that **T** ′ is a localization of a quotient of **T[U]**

Next we shall show that $\Omega_{T'/T} = \mathbf{0}$. For this purpose we shall use the *Jacobian criterion*. Now a straight forward calculation shows that ∂ **(F) /** ∂ **(U) =** ∂ **f /** ∂ **x** $\neq$ **0**, since the image of ∂ **f /** ∂ **x** $\neq$ **0** as **S** ′ is etale′ over **S** .Hence $\Omega_{T'/T} = \mathbf{0}$.

Definition 3 Let **(R , M)** be a local ring . Then **(R , M)** is called a *Henselian local ring* if Hensel lemma holds . that is , if the image of a monic polynomial f (x) over **R**, factors into strongly disjoint polynomials over **R / M** , then that splitting can be lifted to a decomposition over **R** itself.

A couple (^{h}**R** , φ) where ^{h}R is a local Henselian ring and φ is a local morphism of **R** in ^{h}**R** is called a Henselization of **(R , M)** , if for any Henselian local **(S , N)** and a local morphism ψ of **(R , M)** in **(S , N)**, there exists a unique local morphism χ of ^{h}**R** to **S** such that $\psi = \chi \circ \varphi$.

In other words the following diagram (wherein all morphisms are local) is commutative



The above notion of associating a henselian couple with a given local ring was first investigated by Nagata in 1960 and studied in great generality by Lafon in 1963. In 1970 Raynaud gave an approach different from that of Nagata and Lafon. We follow Raynaud's approach. The henselization we use is realized as the injective limit of local etale′ extensions with trivial residue class extensions.

Theorem. If **(R , M)** and **(S , N)** are two local rings with the same residue class field, then the henselization of their fiber product taken over **k** is isomorphic to the fiber product of their respective henselizations.

Proof. If ^{h}**R** and ^{h}**S** denote the henselizations of **R** and **S** at **M** and **N** repectively ,then the fiber product of ^{h}**R** and ^{h}**S** over **k** can be verified easily to be henselian at the unique maximul ideal **M** x_k **N**. Consequenly, from the definition of henselization of **R** x_k **S**, it is clear that ^{h}**(R** x_k **S)** is contained in ^{h}**R** x_k ^{h}**S**. For proving our theorem we need only to show that ^{h}**(R** x_k **S)** is actually equal to ^{h}**R** x_k ^{h}**S**.

For this purpose we observe that the henselization is obtained as the inductive limit of local etale′ algebras with the same residue class field . From proposition 1 and 2 we see that etale′ extension is preserved under fiber product formation. Consequently ^{h}**(R** x_k **S)**= lim. **T'**,where **T'** is a local etale′ extension of **T** = **R** x_k **S**. Therefore,

Lim . **T** ′ = lim . (**R** ′ x_k **S** ′) = lim . **R** ′ x_k lim .**S** ′ = ^{h}**R** x_k ^{h}**S** ,

Where **R** ′ and **S** ′ are local etale′ extensions of **R** and **S** over **k** .

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SYNTHESIS OF NOVEL POLYCYCLICS VIA CATALYTIC METATHESIS

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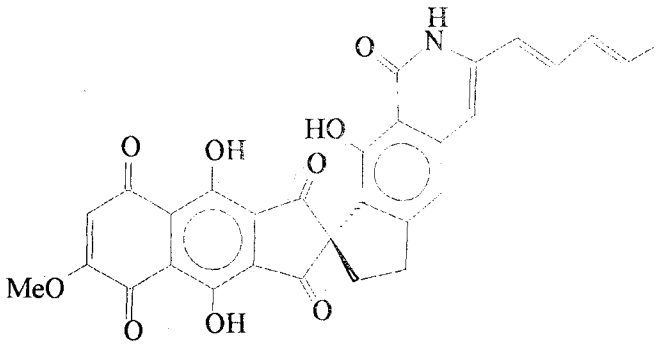
Abstract

A variety of olefinic metathesis protocols have been used to prepare polycyclic compounds and amino acid derivatives. Here we have described our efforts towards the synthesis of spirocyclic compounds.

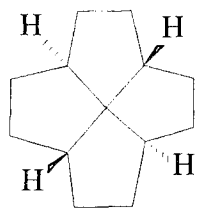
Key words: spirocyclics, metathesis, Grubbs catalyst, heterocycles.

Spirocyclic compounds have attracted the attention of organic chemists due to their unique structural and reactivity pattern. Many natural products such as fredericamycin **1** (Kita *et al.*, 2001 and references cited therein) (Figure 1), spirovetivanes, acroanes, chamigrenes, angularly fused cyclopentanoids (e.g., isocomene **7**, crinipellin-A **9**, laurenene **10**), ACAT inhibitor **11**, aphidicolin **12**, spirofornabuxine **13** and retigeranic acid **14** (Figure 2) have shown to posses spiro-linkage.



fredericamycin

1



[5.5.5.5]fenestrane

2

Figure 1

In addition to various theoretical aspects, spiro-polyenes are also of industrial interest. For example, hetero-spirenes act as photochromic systems, which can undergo reversible photochemical ring-opening and find their utility in silver-free imaging systems and as memories in data display devices. (Cox, 1975) Some of the carbocyclic spiro compounds have also been used as building blocks in thermotropic liquid crystals, which in turn are used in optical displays and screens (Feuerbacher *et al.*, 1999).

Most of the approaches to prepare spiro compounds involve: alkylation methods, transition-metal based processes, rearrangement based approaches, cleavage of bridged ring systems, ring-closure of geminally disubstituted compounds, cycloaddition tactics, and radical cyclization procedures (Figure 4) as key steps (Krapcho, 1978; Martin, 1980 and Sannigrahi, 1999). In general, these methods encounter problems associated with functional group incompatibility at one or more stages and also restricted to a single substitution pattern. Only in few instances, the newly generated ring system was left with useful functionality for further synthetic manipulation. Therefore, there is a need to generate new methods to prepare the spiro-linkage under milder reaction conditions with additional functionalities for further synthetic transformations.

Although there are numerous reports available for the synthesis of five-membered spiro cyclic systems, strategically limited numbers of methods are at hand. Therefore, we sought to investigate a conceptually new approach based on ring-closing metathesis (RCM) as a key step for spiro-annulation. The first strategy (Path A) involve the bis-allylation of cyclic β-dicarbonyl compound followed by RCM reaction (Scheme 1). In the second strategy (Path B), acyclic active methylene

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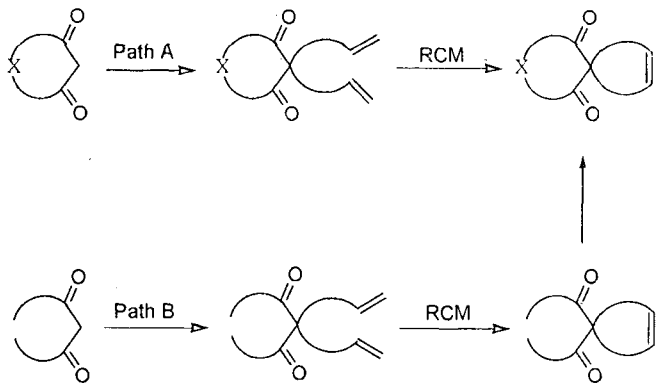


Scheme 1

X=C, heteroatom

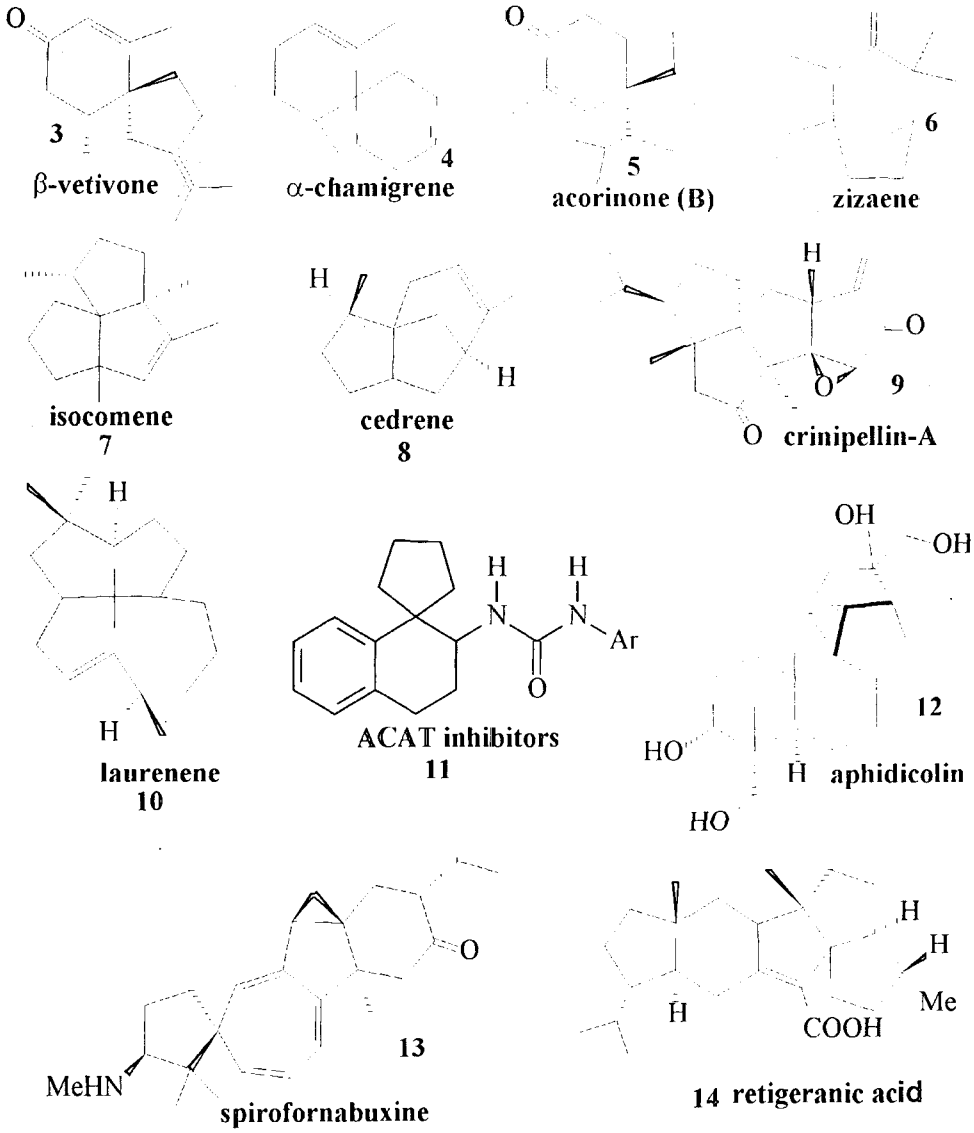


Figure 2: List of natural products containing spiro-unit.

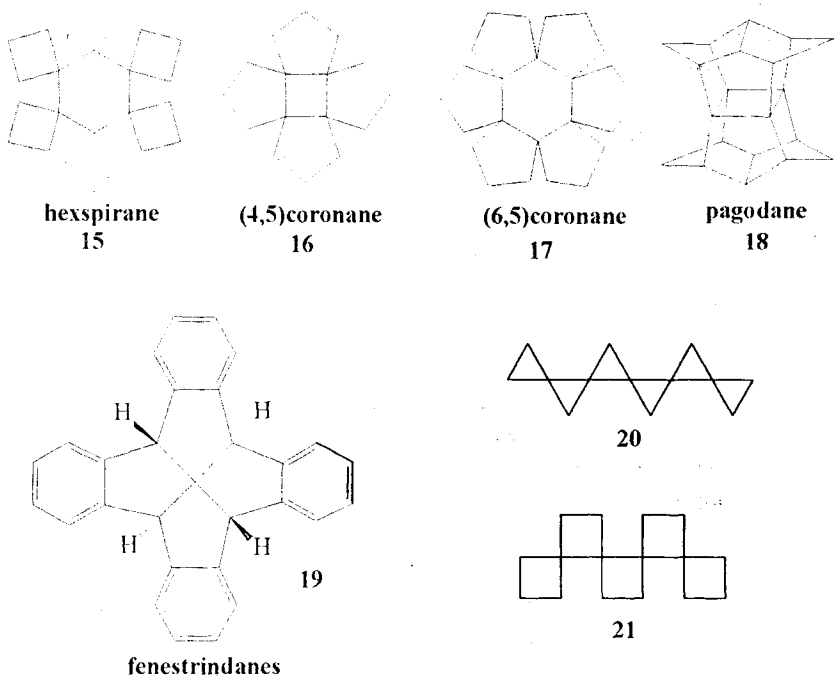


Figure 3. List of non-natural products containing spiro-unit

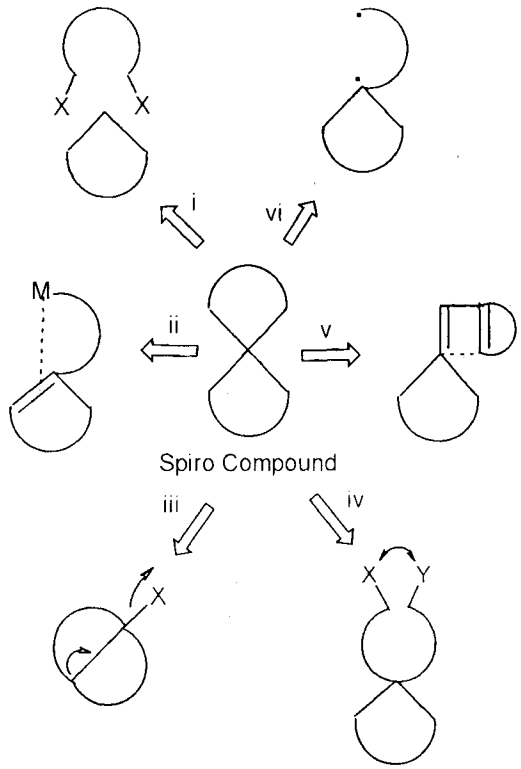


Figure 4: Various approaches to spirocyclics.

i)Alkylation methods (ii) Transition-metal based processes (iii) Rearrangement-based approaches (iv) Ring-closure of geminally disubstituted compounds (v) Cycloaddition tactics (vi) Radical cyclization method.

derivative can be diallylated and then the RCM reaction was planned. Finally, the resultant derivative may be coupled with heterocyclic precursor such as urea to generate various hetero cyclic targets. Before going into the details of our work, we would like to discuss briefly about the olefin metethesis reaction. RCM reaction is fairly common, can be induced by a variety of homogeneous catalysts and has many varied chemical applications. Recently, this methodology was applied for the preparation of several carbocyclic and heterocyclic systems (Grubbs and Chang, 1998; Armstrong, 1998; Kotha and Sreenivasachary, 2001). The two Grubbs catalysts that are generally useful for RCM reaction are shown in **Figure 5**. Ruthenium complexes **I** and **II** are less sensitive to moisture, relatively less expensive and can be handled without any special equipment. They have remarkable functional group tolerance and is stable towards air and water. Both the catalysts were used in the present study. Herein, we report the application of RCM reaction for the synthesis of spirocyclic compounds, naphthoxepin skeleton.

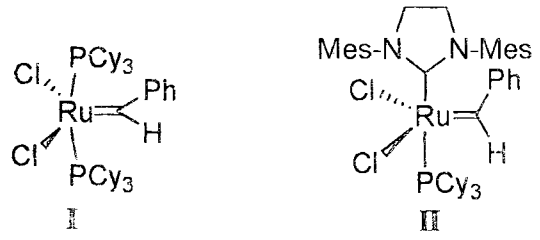
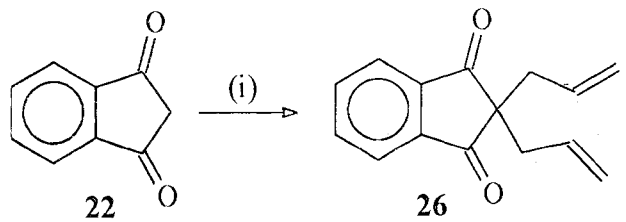


Figure 5. 1st and 2nd generation Grubbs catalyst.

Towards the realization of our approach, initially, we chose β-dicarbonyl compounds as our starting materials. To this end, the indanedione **22** was treated with allylacetate in presence of $Pd(PPh_3)_4$/DBU to deliver diallyated product **26**. The corresponding RCM product **32** was achieved by treatment of the derivative **26** with catalytic quantities of Grubbs catalyst **I**. Some occasions



Scheme 2. (i) Allylacetate, $Pd(PPh_3)_4$, DBU.

(e.g. for substrates **23-25**) the usage DBU was not required during allylation step. In a related study 9,9-diallylfluorene **27** was obtained by reaction of fluorene with ally bromide in presence of potassium metal in dioxane. Varous other β-dicarbonyl derivatives and activemethylene compounds prepared in this study are included in **Table 1** (Kotha *et al.*, 1999; Kotha and Manivannan, 2003). Some of the RCM products are worthy of a special comment. Substrates **31** and **32** constitute CD and BCD ring of fredericamycin **1** structure. Also, many other RCM products mentioned in Table 1 are potential precursors for various unknown fenestsrane frames.

In order to expand this methodology, we had also studied several other β-dicarbonyl derivatives containing heterocyclic systems. We have chosen biologically relevant molecules such as, barbituric acid, thio barbituric acid, meldrums acid, tetranoic and thiotetranoic acid derivatives as our next targets. Some of the derivatives prepared by this methodology are shown in **Tables 2** and **3**. Since several barbituric acid derivatives have useful application in pharmaceutical, material and supramolecular chemistry, we hope that this methodology will find interesting applications in these areas (Kotha *et al.*, 2005).

The generality of the present methodology was further examined to design the basic skeleton of important therapeutic agents (eg. **11**) (Kotha and Mandal, 2004). The story starts with the readily available β-naphthol **35**. Thus, O-allylation of β-naphthol **35** followed by microwave assisted Clasien rearrangement gave allylated compound **36**. Repetition of the same sequence of reactions gave the diallylated product. It is interesting to note that the loss of resonance energy of one ring when the ether is converted to the dieone approaches the extra stabilization energy of the keto over the enol form. Ruthenium-catalyzed RCM of compounds **38** and **39** with 1st generation Grubbs catalyst gave the corresponding napthoxepin and spirocyclic skeletons respectively. Two products **41** and **42** were obtained when the compound **39** was refluxed in the presence of the 1st generation Grubbs catalyst. These two compounds were found to be isomers involving isomerization of the double bond during the metathetic process. The structure of these products was firmly established on the basis of their spectral data (^1H and ^{13}C NMR) and also by a sequence of chemical reactions. We also found that the compound **41** is converted into **42** in presence of Grubbs catalyst.

Table 1: List of various spirocyclics prepared by RCM.

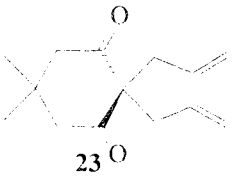
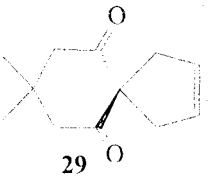
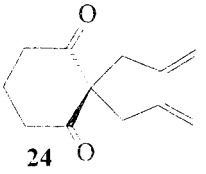
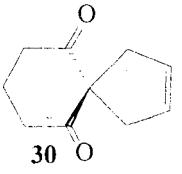
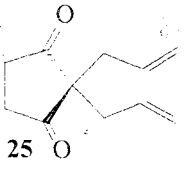
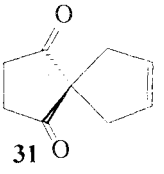
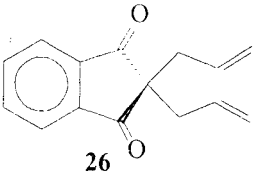
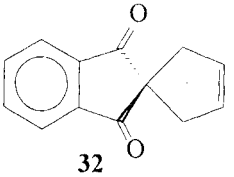
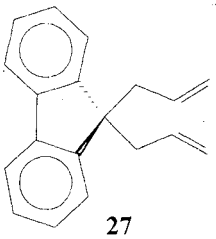
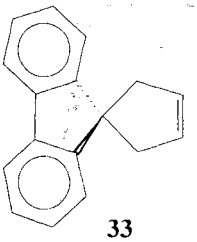
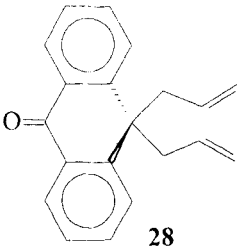
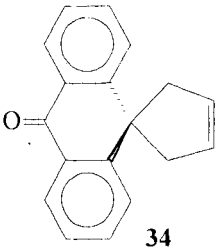
S.No	S. M	RCM product	M.P °C	Yield %
1	 **23**	 **29**	95 -96	95
2	 **24**	 **30**	64 - 65	93
3	 **25**	 **31**	89 -90	77
4	 **26**	 **32**	162 -163	80
5	 **27**	 **33**	87 -88	87
6	 **28**	 **34**	104 -105	92

Table 2: Diallylated and RCM products of barbituric acid.

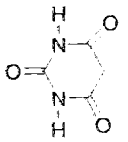
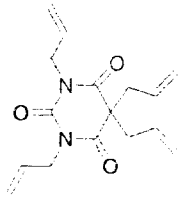
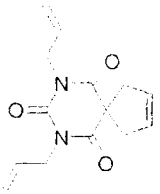
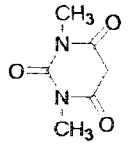
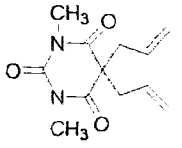
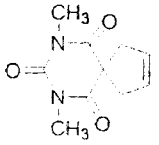
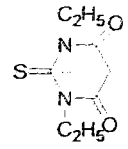
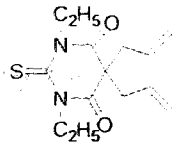
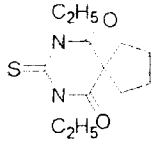
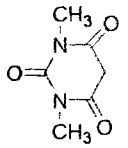
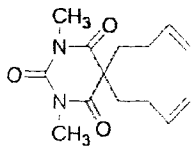
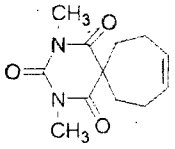
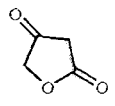
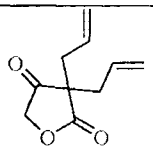
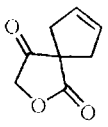
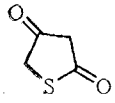
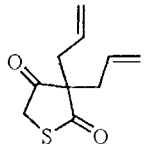
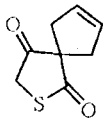
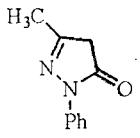
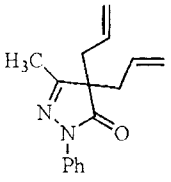
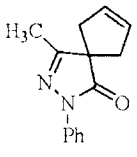
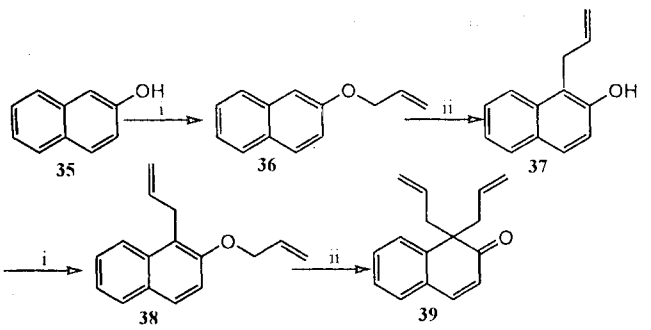
Entry	Substrate	Diallyl Products	Yield (%)	RCM Product	Yield (%)
1			52		95
2			81		88
3			85		92
4			35		81

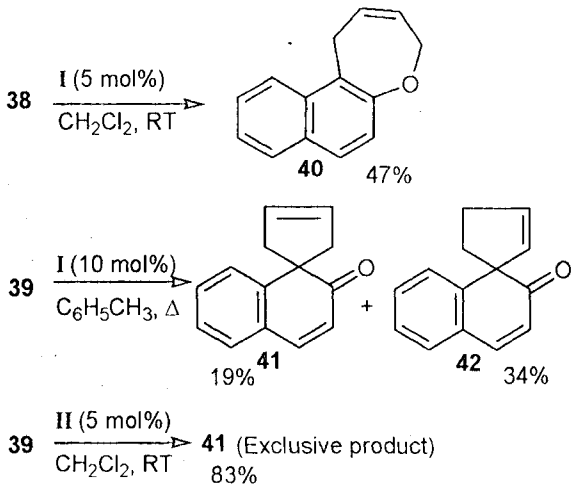
Table 3: Diallylated and RCM product of hetero β-dicarbonyl compound.

Entry	Substrate	Diallyl Product	Yield (%)	RCM Product	Yield (%)
1			55		69
2			60		79
3			65		75

In the absence of the Grubbs catalyst no such conversion was observed. When the compound **39** was subjected to RCM in presence of 2nd generation Grubbs catalyst only one product **41** was obtained.



Scheme 3: i) K_2CO_3, dry acetone, allyl bromide, RT, 4h; ii) microwave irradiation (power=100), 15 min.



Scheme 4:

In conclusion, microwave assisted Claisen rearrangement and RCM reaction was utilized as key steps to produce naphthoxepin ring system and double Claisen rearrangement approach has been used to generate the spiro skeleton. Non-metathetic behavour of Grubbs catalyst (*ie*, isomerization of the double bond) has been observed beyond the metathesis reaction. Some of the biologically active spiro analogues can be prepared by using the above methodologies.

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