5. DISCUSSION

Mangrove trees are fascinating study objects for the mycologists because, the base of their trunks and aerating roots are permanently or intermittently submerged. Whereas the upper parts of roots and trunks are rarely or never reached by the salt water, although they sometime may be subjected to saline spray. Thus, terrestrial fungi occupy the lower part. Marine fungi play an important role in nutrient generation cycles as decomposers of dead and decaying organic matter in the estuaries. Although, mangroves are dominant feature of the Indian coastline and provide niches and habitat for many marine organisms, very little is known about the fungi associated with them till recently. Hence the present investigation has been made to study the diversity of fungi from Ennore, Pichavaram, Muthupet and Thondi mangrove environments and their extracellular enzyme producing ability.

5.1. Environmental characteristics

Environmental factors including rainfall and other physicochemical characteristics influence the biological parameters to some extent. Hence many workers have carried out the ecobiological studies (Senthilnathan and Balasubramanian, 1997; Govindasamy and Azariah, 1999; Singh and Sarkar, 2003) and emphasized that, it would be important to understand

and to determine the ecological factors that influence the growth of organisms.

The population of fungi is generally influenced by the organic carbon, nitrogen and C:N ratio of the soil. The influence of these factors on the population of fungi in the soil has been reported and reviewed by many workers (Bocock, 1964; Singh and Gupta, 1977; Rai and Srivastava, 1982; Rai and Kumar, 1988; Umadevi and Manoharachary, 1991). The fecal matter of mangrove fauna, which is a source of nitrate, phosphate and other nutrients, contribute to the surface layer of sediments (Vennucci, 1989). Significant amount of nutrients carried into coastal zone by flood waters may be trapped by mangrove sediments and the marine environment. Ninety per cent of nutrients in marine sediments are believed to have come from the mangrove area and the remaining 10 per cent only from non-mangrove sites (Aksornkoae and Paphavasit, 1996). In the present investigation it was found that the carbon content of the soil samples collected from Ennore was maximum (3.3%) in January and minimum (1.5%) in the month of August; it was maximum (4.3%) and minimum (1.7%) respectively in the months of September and February in Pichavaram; maximum (4.2%) and minimum (1.7%) respectively in June and January; and it was maximum (3.4%) and minimum (2.3%) respectively in the months of February and August 2003 in Thondi.

Average as well as range of variation in the concentration of various heavy metals in the Pichavaram mangrove sediments have been studied and reported (Ramanathan, 1997). Fe was dominant heavy metal followed by Mn, Cr, Ni, Cu, Zn, Co, Pb and Cd during monsoon. It has been reported that there is accumulation of heavy metals in these sediments. This is perhaps because of the fact that there is enhanced organic matter content, abundance of fine particles with greater surface area (Forstner and Wittman, 1981; Salomons and Forstner, 1984), precipitation of metals as hydroxide coatings of Fe and Mn (Degroot and Allersma, 1975), especially in the marine environment. In the preset study it was found that iron content was a maximum of 5.35 ppm and a minimum of 4.11 ppm at Ennore during the months of March and January respectively; it was maximum of 5.12 and minimum of 3.15 ppm in March and January respectively at Pichavaram; maximum of 8.13 and minimum of 4.81 ppm in the months of June and March respectively at Muthupet and 5.63 and 4.21 ppm were recorded respectively in the months of March and December 2003 in Thondi.

Statistical analysis of the physicochemical characteristics of the soil samples revealed, significant positive correlation between rainfall and total number of colonies ($r = 0.524$; $P < 0.05$) and potassium and total number of species ($r = 0.527$; $P < 0.05$) collected from Ennore.

Similarly, positive correlation between pH and total number of species ($r = 0.679$; $P < 0.01$), rainfall and total number of species ($r = 0.573$; $P < 0.01$) and organic carbon and total number of species (r = 0.624; P < 0.01) was observed in the soil samples collected from Pichavaram.

There was a positive correlation between pH and total number of species (r = 0.511; P< 0.05), temperature and total number of species (r = 0.654; P < 0.01), organic carbon and total number of species (r = 0.745; P < 0.01), nitrogen and total number of species (r = 0.546; P < 0.05), and potassium and total number of species (r = 0.558; P < 0.05) in the soil sample collected from the Muthupet mangrove.

In Thondi soil samples, positive correlation between temperature and total number of species (r = 0.675; P < 0.01) and potassium and total number of species (r = 0.521; P < 0.05) was recorded. However, it has been reported that there was no correlation between major elements and fungi (Ramanathan *et al.*, 1988).

5.2. Soil mycoflora

In the present study, the fungal diversity of soil sediment of East coast environment revealed the occurrence of 42 species of fungi belonged to 15 genera. Soil sediment of Ennore showed rich in diversity (42) followed by Muthupet (39), Pichavaram (36) and Thondi (32). The occurrence of fungi was more during postmonsoon and monsoon than in summer and premonsoon. Ananda and Sridhar (2004) reported that the occurrence of terrestrial fungi *Arthrinium* sp. *Aspergillus* sp. and *Penicillium* sp. were competitive over marine fungi on leaves, and they were more dominant during monsoon than summer. In the present study, 12 species of *Aspergillus* namely *A. candidus, A. clavatus, A. flavus, A. fumigatus, A. japonicus, A. nidulans, A. niger, A. ochraceous, A. sydowi, A. terreus, A. ustus* and *A. wentii* were found dominant which were followed by *Penicillium camembertii, P. citrinum, P. exalicum, P. expansum, P. funiculosum, P. glabrum, P. janthinellam, P. spinulosum, Trichoderma harzianum, T. koningi, T. polysporum, T. pseudokoningi, T. reesi* and *T. viride* (Fig.5). Rare occurrence of Ascomycetes other than species of *Aspergillus* and *Penicillium* and the absence of Basidiomycetes and Phycomycetes in the mangrove swamps have also been reported from Portugese (Swart, 1958). The presence of *Aspergillus species* in abundance is an indication of tropical soils and *Penicillium* as indication of subtropical and temperate condition has been reported by Rai *et al.* (1969), which has also been emphasized by number of workers (Evans, 1971; Upadhyay, 1987; Rai and Kumar, 1988; Adnikari and Tiwari, 1991). The presence of *Trichoderma* species in mangrove mud has been reported (Swart, 1958; Rai *et al.*, 1969; Fell and Master, 1973).

Garrett (1951) classified soil fungi into ecological groups. Species of *Aspergillus* and *Penicillium* are included under saprophytic sugar fungi. Saito (1952) investigated the mycoflora of salt marsh and observed that the species *Penicillium* and *Trichoderma* were the common forms encountered in the surface mud. Swart (1958) recorded the dominance of the species of *Aspergillus* and *Penicillium* in the mangrove swamps of Inhyaca Island. Nicot (1958a) recorded the dominance of aspergilli and penicillia in the coastal soils of France. Roth *et al.* (1964) and Rai and Chowdhery (1976) reported that the species of *Aspergillus* were dominant over mucorales and penicillia in the mud of mangrove swamps. Chowdhery (1983a) studied the succession pattern of fungi colonizing wood in the mangrove swamps and reported that species of *Aspergillus* and *Penicillium* rank among initial colonizers along with mucoralean fungi.

Among the four different stations of East coast mangrove environments, the percentage frequency of mycoflora belonged to Deuteromycetes, Ascomycetes and Phycomycetes were in the order of 90.5, 2.4 and 7.1 respectively in Ennore. In Pichavaram, Ascomycetes were absent, but Deuteromycetes and Phycomycetes were in the order of 94.5 and 5.6 per cent respectively. On the other hand, Muthupet recorded Deuteromycetes, Ascomycetes and Phycomycetes in the order of 92.3, 2.6 and 5.1 per cent respectively. While in Thondi, Deuteromycetes were recorded with 93.8 per cent whereas Ascomycetes and Phycomycetes were 3.1 per cent each.

The salinity might be one of the factors, causing a reduction in Phycomycetes population. Swart (1963) has given a similar explanation for the absence of Phycomycetes in the soil of some mangrove swamp of Inhyaca Island. However, he has demonstrated that the seawater was inhibited by *Absida* sp, a Phycomycete in the mangrove habitats, which has been reported by many workers (Swart, 1958, 1963; Lee and Baker, 1973; Venkatesan and Natarajan, 1983). Thus the present investigation also concludes that Phycomycetes and Ascomycetes are poorly recorded when compared to Deuteromycetes in the mud. It is because of the presence of high level salinity, pH, temperature and fluctuation of the nutrients in the mangrove environments.

The temperature, moisture, carbondioxide, oxygen and porosity determine the fungal mycelium production in the soil. When organic matter or similar substrate is added to the soil, it becomes subjected to series of waves of colonization by fungi present in soil, and thus the nature of available energy materials largely determine the fungal flora. The active phase of the fungi has been reported to occur usually at the vicinity of energy rich organic substrates (Waid, 1960). For example the rhizosphere harbours rich mycoflora both quantitatively and qualitatively as it contains, energy rich substrates, vitamins and hormones. Hence the presence of actively growing fungi in the mangrove swamps could be justifiably due to the presence of rich organic substances.

Inspite of the fact that the fungi have wide distribution, they show variation in their population dynamics. In the present investigation it was found that there was significant correlation between environmental characters, physicochemical properties of soil and total fungal colony. It revealed that there was significant positive correlation between rainfall and total number of colony ($r = 0.524$; $P < 0.05$), and potassium and total number of species ($r = 0.527$; $P < 0.05$) in Ennore; pH and total number of areas of East coast mangrove environments by direct examination method. The distribution pattern of these fungi showed that 30 species (26 genera) belonged to Ascomycetes, 7 species (5 genera) belonged to Deuteromycetes and a single species to Basidiomycetes in the wood samples collected from Ennore, Pichavaram, Muthupet and Thondi.

Among the obligate marine fungi, the species diversity of Ascomycetes was more than other groups of fungi, as it has already been reported from mangroves (Ravikumar and Vittal, 1996; Aleem, 1980; Kohlmeyer, 1981), driftwood (Raghukumar, 1973; Prasannarai *et al.*, 1999; Prasannarai and Sridhar, 2001) and animal substrates (Ananda *et al.*, 1998). This is because of the fact that the Ascomycetes possess enzyme producing ability and hence they could potentially colonize the lignocellulosic wood substrates. Garrett (1970) rightly placed them as a separate group called cellulolytic fungi. In the present study also they were recorded from woody materials collected from the marine environments. Hence it could be concluded that these fungi are not only able to tolerate high salinity but also possess the enzyme producing mechanisms and they could possibly be exploited for the production of extracellular enzymes.

Among the fungi identified down to species level three species viz. *Dactylospora mangrovei*, *Nais glitra* (Ascomycetes) and *Zalerion varium* (Deuteromycetes) were recorded for the first time from the East coast of Tamil Nadu. Substrate availability and climate changes are the delimiting factors for the geographical distribution of fungi (Bebout *et al.*, 1987; Vrijmoed *et al.*, 1982) and therefore examination of more and more substrates is needed to understand the complete biodiversity status of marine fungi of India. Raghukumar (1996) pointed out that the areas of Indian mycological research should include thorough understanding of various niches occupied by marine fungi. In this context, the present investigation brought out the new distributional records of marine fungi to India and also emphasizes the need of intensive investigation of marine fungi in relation to spatial, temporal and functional aspects.

The present investigation also deals with the pattern of distribution of different species of obligate marine fungi. The pattern of distribution has been categorized into common, frequent, occasional and rare. In the present investigation, among the fungi isolated from wood samples, 8 were recorded in common, 37 as frequent, 69 as occasional and species such as, *Aigialus parvus*, *Antiptodera haispora, Anthostomella nypensis, Ascocratera manglicola, Dactylospora mangrovei, Kallichroma tethys, Lecanidion atratum, Leptosphaeria perviana, Marinosphaera mangrovei, Massarina ramunculicola, Nais glitra, Phaeosphaeria oraemaris, Savoryella lignicola, Zopfiella latipes, Halocyphina villosa, Helicorhoidon nypicola, Periconia prolifica, Trichocladium acrasporum* and *Zalerion varium* were rare. Similr type of results has also been reported by Ravikumar and Vittal (1996). Jones *et al.* (1988) recorded *Massarina velatospora, Savporyella lignincola, Zalerion varium* and *Rosellinia* as common species from Philippines. Hence, it could be concluded that there is no uniformity in the marine fungal species diversity and their distribution pattern in different geographical regions. They are dependent on the nature of the substrate and temporal regions that favour the colonization, growth and substrate possession of the fungi. However, the species diversity observed in the present study (38 species) was comparable with the study made by Prasannarai and Sridhar (1997) who reported 24 species of fungi from driftwoods collected along the West coast of India. There are number of reports available on the fungi recorded from the submerged wood samples collected from Indian mangroves (Ravikumar and Vittal, 1996; Sarma and Vittal, 2000, 2001; Sarma *et al.,* 2001; Maria and Sridhar, 2002, 2003). Randomly collected wood samples of *Rhizophora apiculata* along the East coast of India harboured the highest number of fungi (63 species) (Sarma and Vittal, 2000), while wood samples (*Avicennia* and *Rhizophora*) collected from Udayavara (West coast mangroves) showed 44 to 50 species (Maria and Sridhar, 2003).

5.4. Enzyme production

In the recent years, the potential of using microorganisms as biotechnological sources of industrially relevant enzymes has stimulated interest in the exploration of extracellular enzymatic activity in several microorganisms.

Mangrove environment is originally very rich due to the presence of high amount of dissolved and organic matter which offers diversity of species ($r = 0.679$; $P < 0.01$) and organic carbon and total number of species ($r = 0.624$; $P < 0.01$), in Pichavaram; pH and total number of species ($r = 0.511$; $P < 0.05$), temperature and total number of species ($r = 0.654$; $P < 0.01$), organic carbon and total number of species ($r = 0.745$; $P < 0.01$), nitrogen and total number of species ($r = 0.546$; $P < 0.05$), and potassium and total number of species ($r = 0.675$; $P < 0.01$) in Muthupet; temperature and total number of species ($r = 0.675$; $P < 0.01$), organic carbon and total number of species ($r = 0.523$; $P < 0.05$) and phosphorous and total number of species ($r = 0.521$; $P < 0.05$) in Thondi.

It has been reported that pH, salinity, temperature and nutrient contents of the soils have been correlated with fungal population (Waid, 1960; Chowdhery *et al*., 1982; Sudha, 2005). Carbon, nitrogen and phosphorous are necessary energy sources for the growth of microorganisms. Besides the energy sources the environmental factors such as pH and salinity also influence the growth and activity of microorganisms (Chowdhery *et al.,* 1982; Alexander, 1961). Hence, it could be concluded that inspite of the fact that the fungi are ubiquitous; their population dynamics are often influenced by the available nutrients and the physicochemical conditions of the ecosystem.

5.3. Driftwood fungi

In the present investigation, totally 38 species of obligate marine fungi belonged to 32 genera, were recorded form four different study niches and microorganisms. Microorganisms from these areas play an important role in the biodegradation of the enormous amount of dead plant materials through their extracellular enzyme producing ability (Venkatesan and Ramamurty, 1971). It is well known that the microorganisms possess a remarkable adaptive capacity and may develop resistance and/or degradative ability to any given organic compounds. Similarly the mangrove sediments are known to harbour heterogenous groups of microflora which potentially excrete degradative enzymes, such as cellulases, amylases and pectinases (Matondkar *et al.,* 1980b). In the present investigation among the 42 species of fungi screened for their enzyme producing ability, 30, 22 and 27 species showed amylolytic, cellulolytic and pectinolytic activities respectively. They were determined in terms of the diameter of the zone formed around the colonies. Among them, the maximum amylolytic activity (mm) was found with *A. niger* (34) followed by *T. viride* (33), *A. terreus* (29), *A. flavus*(27), *T. harzianum* (27) *and P. expansum* (26)*; cellulolytic* activity was with *A. niger* (37) followed by *A. fumigatus* (34), *A. terreus* (33), *T. viride* (31), *A. flavus* (30) and *P. expansum* (28) and pectinolytic activity was with *A. fumigatus* (39) followed by *A. flavus* (38), *P. expansum* (33), *A. niger* (32), *A. candidus* (32) and *T. harzianum* (31) (Table 14).

Rai and Chowdhery (1976) found that the fungal species isolated from the mangroves had a higher rate of cellulolytic activity when compared to their normal soil counterparts, and suggested that the unique prolonged impact of mangrove environment might have resulted in the higher amount of cellulolytic enzyme production. Highest cellulase activity of *A. terreus, C. globosum, C. nigricolar, C. lunata, F. oxysporum, F. solani* and *Trichoderma lignorum* facilitates their competitive life as saprophytes by increasing their ability to degrade cellulose. It has also been demonstrated that these fungi rapidly degraded the wood due to their enzymatic activities (Rai *et al.*, 1981). There is direct correlation between the cellulase activity and the degrading capability of fungi (Panneerselvam and Saravanamuthu, 1999; Kumar and Saravanamuthu, 2004; Muthukumar *et al.*, 2004). Velho and D'souza (1982) reported that among 52 filamentous fungi isolated from the mangrove swamps, 38 were found to be pectinolytic, which included various species of *Aspergillus, Mucor, Rhizopus, Trichoderma, Fusarium, Verticillium, Cladosporium, Botyotrichum, Penicillium* and *Geotrichum.* Kohlmeyer (1963) reported the presence of Deuteromycetes in the decomposing mangrove roots, whereas Fell and Master (1973) found a wide range of 53 genera of fungi associated with decomposing leaf litter of mangroves in South Florida which included Phycomycetes, Ascomycetes and Deuteromycetes. In the present study, it was found that species isolated from Ennore, Pichavaram, Muthupet and Thondi mangrove environments included *Aspergillus, Penicillium and Trichoderma* which showed significantly high amylolytic, cellulolytic and pectinolytic activities (Table 14).

The enzymatic activity of the fungi varied in relation to pH, salinity, temperature and nutrients. Obligate and facultative marine fungi were studied for the enzymatic activity in relation to pH, temperature, salinity and nutrients (Rohrmann *et al.*, 1992). Fungal activity in mangrove ecosystems is influenced by various factors such as nature of substrate, chemical composition of wood, presence or absence of bark, period of submersion, competition among fungi and salinity of water (Nakagiri, 1993; Hyde and Lee, 1995; Prasannarai and Sridhar, 1997, 2003; Jones, 2001).

The pH of the medium also plays an important role on the production of microbial enzymes (Garg, 1982). Cherry *et al.* (2004) studied the influence of pH on the production of extracellular enzymes such as cellulase and glucoamylase. The maximum production was achieved at pH 5.0. In the present study also it was found that the pH levels 5.0 and 6.0 were more favourable than other pH levels for the production of amylase, cellulose and pectinase. The influence of temperature on enzyme production is related to the growth of microbes. Optimum temperature for amylase was in the range between 25 and 37° C for the mesophilic fungi (Kundu *et al.*, 1973; Veno *et al.*, 1987; Gupta *et al.*, 2003). In the present study, it was found that 30 and 35°C for amylase, 35°C for cellulase and 40°C for pectinase as optimal temperature for enzyme production.

It has been reported that the cellulolytic activity of some fungi isolated from Sunderban mangroves was influenced by salinity (Garg, 1982). All the fungal isolates were capable of producing considerable amount of cellulase activity. Highest cellulolytic activity was recorded with *Chaetomium globosum* at zero per cent salt level, whereas *A. terreus* showed highest activity at 6 per cent salt level. The present study also showed that salinity influenced the production of extracellular enzymes such as amylase, cellulase and pectinase. It was found that the activity decreased with increase in the salinity content of the medium. Salinity at 10 to 20 per cent favoured the enzyme production by *A. candidus, A. flavus, A. fumigatus, A. niger, A. terreus, P. expansum, P. funiculosum, T. harzianum and T. viride.* It lends support to previously reported such findings (Rai and Chowdhery, 1976; Meyers and Reynolds, 1959a, b).

The production of fungal enzymes was maximum in the presence of different sources of carbon and nitrogen (Mandels and Reese, 1957; Raman, 1978; Cherry *et al.*, 2004; Kumar and Saravanamuthu, 2004; Muthukumar *et al.*, 2004; Singh and Raghukumar, 2004). The phosphorous is known to play a role in ATP generation and nucleic acid formation and therefore, helps in growth and propagation of fungi (Velho and D'Souza, 1982). Thus the potentiality of the enzyme production by fungi is not only influenced by different sources of carbon, nitrogen and phosphorous but also by other mineral nutrients.

In a nutshell the present study highlights the following aspects.

- Analysis of the biodiversity of fungi recorded indicates that there is first report of some fungi to India.

- The prevalent species belonged to *Aspergillus, Penicillium* and *Trichoderma* offer enough scope for biotechnological explorations and production of bioactive compounds.
- Studies on the colonization of the drift wood by common obligate marine fungi and facultative terrestrial fungi, their growth over the wood samples; their role in the decomposition of wood; and the influence of physicochemical factors on the enzyme production would pave the way for under standing the complexity of mechanisms involved in lignin degradation.