STUDIES ON DIVERSITY, ECOLOGY AND ACTIVITY OF MICROFUNGI IN RELATION TO MOSQUITO DEVELOPMENTAL STAGES

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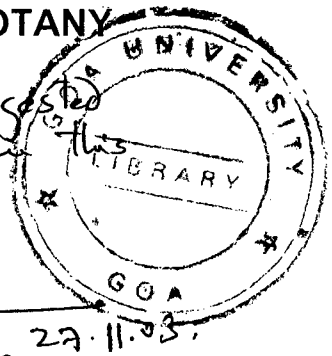
DOCTOR OF PHILOSOPHY IN BOTANY



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DECLARATION

I hereby declare that the Ph.D. thesis entitled "STUDIES ON DIVERSITY, ECOLOGY AND ACTIVITY OF MICROFUNGI IN RELATION TO MOSQUITO DEVELOPMENTAL STAGES" submitted to Goa University, forms an independent work carried out by me in the Department of Botany, Goa University, under the supervision of Dr. D. J. Bhat, Professor and Head, Department of Botany, Goa University and Dr. Ashwani Kumar, Officer-in-Charge, Malaria Research Centre, Panjim. The thesis has not formed previously the basis for the award of any degree, diploma, associateship or other similar titles.

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CERTIFICATE

We certifiy that the thesis entitled "STUDIES ON DIVERSITY, ECOLOGY AND ACTIVITY OF MICROFUNGI IN RELATION TO MOSQUITO DEVELOPMENTAL STAGES" submitted by Mr. T.S. Keshava Prasad, is a record of research work done by him during the period from 2001-2003 when he worked under our supervision. The thesis has not formed the basis for the award of any degree, diploma, or associateship to Mr. T.S. Keshava Prasad.

We affirm that the thesis submitted by Mr. T.S. Keshava Prasad incorporates the independent research work carried out by him under our supervision.

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Chapter I:

INTRODUCTION

1. Entomogenous fungi

1.1. Definition

Fungi are considered as the second largest group after insects, amongst living organisms. After careful analyses, Hawksworth (2001, 2002) suggested that his earlier proposition of 1.5 million fungi on earth surface (Hawksworth, 1991) is an underestimate. Fungi live on a variety of substrates, habitats and ecosystems. Those growing on insects are called as 'entomogenous fungi' or 'entomo-pathogenic fungi'.

The entomopathogenic fungi have been reported from all Divisions of Eumycota (Agarwal and Rajak, 1988). Over 700 species belonging to 90 genera of fungi were reported to be pathogenic to insects and mites. Several reviews dealing with their taxonomy, host range and economic potential are available (Burges, 1981; Evans, 1999; Boucias and Pendland, 1991; Evans and Hywel-Jones, 1997; Lacey and Goettel, 1995; Roberts and Humber, 1981; Goettel et al., 2000; Lacey et al., 2001; Samson et al., 1988; Tzean et al., 1997).

Many species of fungi are known to grow on and regulate insect population including mosquito larvae (Butt et al., 2001; Inglis et al., 2001; Pell et al., 2001). The potential of fungi as biocontrol agents in vector control programmes has been recognized since the time of Pasteur. Although not administered so far at measurable scale, mass production of fungal spores aimed at control of insects has been reported (Agarwal and Rajak, 1988).

Several reports on taxonomy, biology and ecology of entomogenous fungi were known from India (Narasimhan, 1970; Agarwal and Rajak, 1985a,b; Gupta and Sharma, 2002; Kamat and Rao, 1975; Srinivasan and Tirumalachar, 1964, 1967; Srinivasan et al., 1964; Yadav et al., 2000).

1.2. Biological control agents of vector mosquitoes

Mosquito-borne diseases continue to take heavy toll of mankind. Malarial parasites infect nearly 500 million people per year (Weiss, 2002; Greenwood and Mutabingwa, 2002). Other vector-borne diseases such as filariasis, dengue and Japanese encephalitis are also prevalent in many parts of India and elsewhere in the world and cause extensive morbidity, debility and mortality (Kumar et al., 1994). These diseases result with significant economic losses. Control of mosquito-borne diseases has therefore become a priority issue in national health-care agenda. This is organized by anti-mosquito, anti-parasitic and through integrated pest control activities of primary health care system in India.

Use of bio-control agents against mosquitoes has emerged as a thrust area of research in the recent years and is further gaining importance due to large scale and wide spread resistance in vectors of malaria, filariasis and Japanese encephalitis to synthetic insecticides (Kumar et al., 1994). Among various biological control agents of mosquitoes that exist in nature, larvivorous fishes and bacilli have been deployed in large-scale vector control operations (Kumar et al., 1996).

1.3. Taxonomy and diversity

In view of their utility value in the control of mosquitoes and other pests, search and recognition of entomogenous and entomo-pathogenic fungi has become an important component of medical entomology and mycology. Entomogenous fungi belong to genera such as *Aschersonia, Ascosphaerea, Aspergillus, Beauveria, Coelomomyces, Cordyceps, Culicinomyces, Entomophthora, Fusarium, Hirsutella, Metarhizium, Nomuraea, Paecilomyces, Septobasidium* and *Verticillium*. Amongst these, fungi pathogenic to mosquitoes belong to genera such as *Beauveria,*

Coelomomyces, Culicinomyces, Entomophthora, Lagenidium, Leptolegnia, Metarhizium and *Tolypocladium* (Federici, 1995; Rawlins, 1989; Lacey and Lacey, 1990). Besides these well-known entomogenous fungi, species of *Alternaria, Aspergillus, Fusarium, Mucor, Penicillium* and *Trichoderma* have been reported as 'insect-killing' (Miczulski and Macowicz-Stefaniak, 1977; Gillium et al., 1990; Christias et al., 2001).

Taxonomy of *Entomophthora* has been reviewed by Wolf (1981). King (1976a,b, 1977) reviewed *Conidiobolus* and recognized several species in the genus. De Hoog (1972) re-examined the biology and taxonomy of *Beauveria* and since then several new taxa have been described (Samson, 1995). The genus *Nomuraea* was resurrected by Samson (1974). Genus *Hirsutella* has been monographed by Minter and Brady (1980). Petch (1921b) monographed *Aschersonia* which was later updated by Evans and Hywel-Jones (1990) who recognized 53 taxa in the genus. Taxonomy of *Cordyceps* is now fairly well known (Kobayasi, 1941; Kobayasi and Shimizu 1983a,b). Sweeny (1975) described species of *Culicinomyces* pathogenic to chironomids and mosquitoes. Acknowledging the importance of these developments, Samson (1995) advocated urgent revision of entomogenous fungi belonging to genera such as *Aschersonia, Beauveria, Cordyceps, Metarhizium* and *Verticillium*.

1.4. Secondary metabolites of fungi

Wright et al. (1982) have reviewed the insecticidal activities of several metabolites, although so far only crude extracts of fungi have been tested against insects (Vijayan and Balaraman, 1991; Paterson and Kemmelmeier, 1989; Paterson et al., 1987, 1990). Knowledge on action of fungal secondary metabolites on insects is important to effectively use them as biocontrol principles.

1.5. Present work

In the absence of any detailed investigation on the biology of entomogenous fungi of the region and their potential as vector control agents, an effort was made in this thesis to study the taxonomic diversity and ecology of fungi found growing on insects of all kinds and their biocontrol potential against mosquito larvae, with following objectives.

- To collect, isolate, identify and document entomogenous fungi from infected mosquito larvae/pupae/adults, non-mosquito insects, arachnids and natural water bodies.
- To isolate fungal biocontrol agents of adult and larval mosquitoes directly and by using baits from different mosquito breeding habitats such as ponds, pools, streams, rivers, brackish waters, rice fields, lakes, polluted water habitats, etc. in Goa and surrounding regions in different seasons.
- To assess the activity of fungi on larval and immature stages of mosquito vectors of various diseases under laboratory conditions.
- To assess the activity of secondary metabolites of promising isolates on larvae of mosquito vectors under laboratory conditions.
- To conduct preliminary field trials of promising fungi for their action against vectors in field conditions.

The thesis has been organized into 5 Chapters. Besides a brief introduction in this chapter, a review of work so far done on diversity, ecology and activity of entomogenous fungi and studies conducted on development of mycopesticide is presented in Chapter II. Materials used and methods followed in the work have been elaborated in Chapter III. Results are presented in the Chapter IV of the thesis in several parts. A brief summary and a comprehensive list of bibliography are given at the end of the thesis. Research articles published during the tenure of this work are appended to the thesis at the end.

Chapter II:

REVIEW OF LITERATURE

2.1. Entomogenous fungi

Entomogenous fungi constitute mycota, which generally inhabit insects, mites, ants, spiders and so on. The word 'entomogenous', derived from Greek, means 'entomon' = insect and 'genesis' = arising in. Entomogenous fungi may be obligate commensals as in Trichomycetes or specialized pathogens, for example, *Coelomomyces* spp., *Lagenidium giganteum* and Entomophthorales, or facultative pathogens as in entomogenous Hyphomycetes (Cafaro, 2000; Misra, 1998; Agarwal and Rajak, 1988; Butt et al., 2001; Inglis et al., 2001; Pell et al., 2001) but in nature these cause mortality of many insect pests and are reported from different parts of the world. They constitute an efficient and important biocontrol factor (Butt et al., 2001, Inglis et al., 2001, Pell et al., 2001). The epizootic caused by fungi resulting with a check on insect population are well known (Steinhaus, 1949).

The potential of fungi as biocontrol agents in vector control programmes has been recognized since the time of Pasteur. Although not used so far at a recognizable scale, mass production of fungal spores aiming at control of insects has been reported in the last century (Agarwal and Rajak, 1988).

Entomogenous fungi have been reported from almost all divisions of 'Eumycota' (Table 2.1). Over 700 species in 90 genera of fungi are known to be pathogenic to insects and mites (Burges, 1981; Evans, 1999; Boucias and Pendland, 1991, Evans and Hywel-Jones, 1997; Faria and Wraight, 2001; Hajek and St Leger, 1994; Lacey and Goettel, 1995; Roberts and Humber, 1981; Goettel et al., 2000; Lacey et al., 2001; Samson et al., 1988; Tzean et al., 1997). Pell et al. (2001) have reviewed the potential of entomophthoralean fungi in insect control programme. Ferron (1985), Evans (1989), Boucias and Pendland (1998), Glare and Milner (1991), Roberts and Hajek (1992), Tanada and Kaya (1993), Wraight and Carruthers (1999), Ferron et al.

(1991) have reviewed entomogenous hyphomycetes and their development as microbial control agents. Butt and Goettel (2000), Goettel and Inglis (1997), Lacey and Kaya (2000) and Goettel et al. (2000) have reviewed methods and techniques involved in the study of entomogenous hyphomycetes.

2.2. <u>As biocontrol agents</u>

Use of living natural enemies or their products to control undesired, harmful organisms is called biological control or 'biocontrol'. It implies manipulation of naturally occurring biological phenomena in one way or other to achieve desired level of control of a particular species or organism (Evans, 1999). Comparative ease in handling, easy way of mass production of spores or conidia under favourable conditions, overwintering and aestivating in the form of resting stages in their life cycle in unfavourable conditions, exhibition of other features such as direct host invasion, enzyme production, physiological starvation, toxin production, etc. in target suppression, specificity towards hosts and safety towards non-target organisms, make fungi an ideal choice as biocontrol agent and score over other alternatives in biological control efforts (Pell et al., 2001). Unlike chemical insecticides, most of entomogenous fungi kill the host by direct invasion through the cuticle. Conditions of high humidity and optimum temperature are known to help entomogenous fungi in achieving desirable results. Action of fungi is much slower than chemicals. Formulations have been tried to overcome the difficulties associated with application as well as unfavourable environmental conditions. Earlier efforts on development of fungal biocontrol agents fell short because of lack of proper knowledge on factors such as mode of action, host response, environmental conditions, etc. Several successes in development of mycoinsecticides in the recent times have focussed the need for further positive approach in this area of research (Hall, 1979,1980).

2.3. Mode of action

2.3.1. Establishment on insect surface

The integument, composed of cuticle and epidermis, provides arthropods a rigid support for internal tissues. It offers a protective barrier from external environment and invading microbes. It is the first tissue on which the fungal spores would encounter. Besides acting as a substratum for attachment of propagules, cuticle provides with necessary chemical induction for germ tube formation. Surface topography and chemical properties of cuticle play a role in the adhesion of fungal propagules to host surface (David, 1967; Boucias and Pendland, 1991; Boucias et al., 1988).

The outermost layer of cuticle, epicuticle is comprised of proteins, lipoproteins, phenolic compounds and lipids. Epicuticle consists of outer cuticulin and an inner intermediate layer (Locke, 1984). Tanning with cellular phenols and polyphenoloxidases provides cuticulin hydrophobic character. Overlaid by lipids and waxes secreted by underlying epidermal cells, the intermediate layer acts as a barrier against dehydration (Hadley, 1981). Germination and appressorial formation of conidia are affected by free lipids and short–chain fatty acids (Kerwin, 1984; Koidusma, 1957; Smith and Grula, 1982; Latge et al., 1987). Extracts of cuticle have also shown to induce germ tube formation in fungi (Boucias and Latge, 1988; Boucias and Pendland, 1984; Latge et al., 1987; St Leger et al., 1989).

Fungal invasion of insects involves adhesion, germination, differentiation, penetration of cuticle and colonization of insect haemocoel. Penetration of cuticle involves a combination of mechanical force and enzymatic degradation (Deshpande, 1999). Conidia attach mosquito larvae more or less passively. Conidia of *Culicinomyces clavisporus* cling over entire surface of the mosquito foregut (Sweeney,

1975). *Metarhizium anisopliae* spores anchor to the epicuticle of breathing siphon tube of mosquito larvae (Lacey et al., 1988). *Tolypocladium cylindrosporum* preferentially attach the anterior region of mosquito larvae (Goettel, 1988a,b). The motile zoospores of *Leptolegnia chapmanii* encyst on inter-segmental regions of mosquito larvae (Lord and Fukuda, 1988; Zattau and McInnis, 1987). Motile haploid zoospores and diploid biflagellate zygotes of *Coelomomyces psorophorae* encyst on the inter-segmental membrane and head regions of copepod and mosquito hosts (Travland, 1979; Zeboid et al., 1979).

Germination of spores occurs in response to non-specific carbon or nitrogen source. The ability to utilize lipid components of epicuticle may be essential for pathogenesis in some cases (Charnley, 1984; Smith and Grula, 1981; St Leger et al., 1986a). *Nomuraea rileyi* responds to diacyl glycerol and polar lipids, and hence infects only lepidopterans. *Erynia variabilis* requires oleic acid, component of small dipterans' cuticle, for germ tube induction (Kerwin, 1984). Toxic substances present in the epicuticle may also be a stumbling block for spore germination (Charnley, 1989).

Penetration of epicuticle is accomplished by infection peg produced from underside of appressoria or by germ tubes (Perkul and Grula, 1979; Zacharuck, 1981). After entry by a thin penetration tube, fungus expands laterally to form penetration plates (Zacharuck, 1970) which rupture the layers of procuticle. Attaining a certain degree of lateral growth, the fungus reaches haemocoel through epidermis and switches over to yeast phase by formation of hyphal bodies called blastospores and fill the host haemocoel. Toxins and enzymes are secreted in the pre-penetrative phase itself and, in most cases, result with death of the insects. Entomopathogenic fungi also are known to produce lipolytic, chitinolytic and proteolytic enzymes (Butt et al., 1998).

Hyaline spores are difficult to locate on pale or deeply pigmented cuticle by conventional techniques of light microscopy. Several workers have described cuticular darkening at the point of fungal entry (Brobyn and Wilding, 1977). However, these lesions are difficult to locate on dark cuticle. Fluorescence microscopy with optical brightners is known to facilitate rapid localization of spores and penetration sites and enhance detection of lesions on host cuticle. Butt (1987) described a method for studying fungal spores on insect cuticle by fluorescence microscopy and briefly commented its effects.

2.3.2. Factors affecting entry into insect gut

Failure of most fungi to invade gut reflects the adverse conditions posed by the insect gut. Foregut and hindgut are of ectodermal origin and lined by the cuticle. Composition of gut cuticle is believed to be similar to that of external cuticle apart from absence of outer wax layer (Bignell, 1984). Most of the gut cuticle is unhardened. Cuticle is absent in the midgut (Bertram and Bird, 1961) where it is protected with a peritrophic membrane. Retention of fungal entities in the gut for sufficiently long time is a primary requirement for fungal invasion, which provides fungi an opportunity to increase the inoculum potential. In mosquitoes, the peritrophic membrane protects fungal entities from action of food in the gut of mosquito larvae (Stohler, 1961).

Mosquito larvae are non-selective filter feeders. They rapidly ingest large number of fungal spores resulting in the packing of gut (Agudelo-Silva and Wassink, 1984; Lacey et al., 1988). Crisan (1971) reported 89% conidia of *M. anisopliae* in the gut of *Culex pipiens*. Lacey et al. (1988) reported digestion of *M. anisopliae* in the gut of *C. quinquefasciatus*. Ravallec et al. (1989) observed disruption of ingested spores of *Tolypocladium cylindrosporum* in the gut of *Aedes albopictus*.

Gut of insect larvae is a dynamic environment with its physiology different from one region to another. Fungal spores may be inhibited by a number of interacting factors. The pH found in many insect guts is between 4 and 5, a range in which most conidial fungi grow (Bignell, 1984). Nonavailability of oxygen may limit germination of fungal conidia (Hall, 1981). Nutrient availability may be another determinant, as fungi require nutrients from an external source (Charnley, 1984). Iron assimilation by host gut microbes results in a fungistatis condition, as it is one of the essential micronutrients required for conidial germination (Dillon and Charnley, 1991). Presence of fungal cell wall degrading enzymes in the digestive tract of insects has important implications on viability of fungal spores. Antibiotics secreted by fungi may suppress bacterial growth in insect cadavers (Ferron, 1978; Kodaira, 1961).

Culicinomyces clavisporus infects *Culex fatigans* through digestive tract. The germ tube apex becomes tightly bound to epicuticle. Penetration of the cuticle is completed 6-18 hr after exposure. The fungus is also capable of adhering to the external cuticle and anal papillae (Sweeney, 1975; 1979). *Metarhizium anisopliae* invades mosquito larva of *Aedes aegypti* through the gut (Al-Aidroos and Roberts, 1978). Goettel (1988a,b) found that infection by *Tolypocladium cylindrosporum* via the gut of *Aedes aegypti* was rare and occurred only when the insect was moulting.

2.3.3. Cuticle Degrading Enzymes

Entomogenous fungi produce a wide range of extracellular cuticle degrading enzymes corresponding to major components of the insect cuticle, viz. protein, chitin and lipid (St Leger, *et al.* 1986a). Enzymes appear in order, with esterases, proteases followed by N-acetylglucosaminidase in 1-2 days. Chitinase and lipase are produced 3-4 days later. Chitinase is an inducible enzyme (St Leger, *et al.* 1986b). Cuticular chitin is

shielded by protein (St Leger et al., 1986c). Only after degradation of encasing proteins by proteases, the chitins become available for inducing chitinolytic enzyme secretion (Charnley and St Leger, 1991). Entomogenous fungi produce a variety of endo- and exo- proteases in culture. Proteases include collagenases (Hurion et al., 1977, 1979), chymoelastases, trypsins, (St Leger et al., 1987a,b) subtilsins (Segers et al., 1999) and chymotrypsins (Samuels et al., 1990). A variety of assays have been used to detect protease production by entomogenous fungi in culture (Hurion et al., 1979; St Leger et al., 1987b).

Leopold and Samsinakova (1970) quantified chitinase production in *Beauvaria bassiana*. Segretain et al. (1971) noted that there is no relation between protease production and virulence. Paris and Segretain (1975, 1978) postulated that lipase dissolved lipid wax layer of the cuticle. Hadley (1980) opined that action of these enzymes might not be the prerequisite for infection. St Leger and co-workers (1986a) have shown the nature of enzymes secreted by fungi and their role in pathogenesis, with evidence, in penetration of host cuticle (Charnley and St Leger, 1991). Urtz and Rice (2000) purified and characterized a novel extracellular protease from *B. bassiana*. Subtilsins are the major proteins secreted by entomogenous fungi to digest protein component of nematodes (Segers et al., 1996, 1999; St Leger et al., 1988).

2.3.4. Secondary metabolites

Entomogenous fungi secrete several low molecular weight secondary metabolites known to induce tetanic paralysis (Dumas et al., 1996), while others can be immuno-suppressive (Cerenius et al., 1990). These metabolites have been reported to possess antibiotic properties while others are noted to cause pathogenicity (Amiri et al., 1999; Bandani et al., 2000). Though some metabolites are toxins, little is known about

their production, spatial distribution and effect. Approximately 3000 fungal metabolites have been listed of which many have biological activities of commercial and/or pharmaceutical significance (Paterson, 1986; Turner, 1971; Turner and Aldridge, 1983). Thirty mycotoxins have been reported from *Penicillium* spp. (Betina, 1984). Paterson et al. (1987) analysed action of crude extracts of 7 isolates of *Penicillium* spp. against *Spodoptora littoralis* larvae on weight reduction and mortality. Paterson and Kemmelmeier (1989) analyzed purified extracts of 4 *Penicillium* strains which were active against insect pests by gradient high performance liquid chromatography for secondary metabolites using alkylophenone retention indices. Amiri et al. (2000) studied inter- and intra-specific variation in destruxin production by insect pathogenic species of *Metarhizium* and its significance in pathogenesis. Destruxins produced in sufficient quantities cause death of insects and the former also possess insecticidal, phytotoxic and antiviral activities. Malphigian tubules and midgut of insects were assumed to be site of attack for these metabolites (Bandani et al., 2001). Metabolites secreted by *Beauveria brongniartii* include oxalic acid, bassianin, beauvericin, oosporein and tenellin (Khachatourians, 1991; Roberts, 1981; Ganassi et al., 2002). Beauvericin has been shown to have antibacterial activity (Ovchinnikov et al., 1971) and moderate insecticidal properties (Suzuki et al., 1977; Quadri et al., 1989; Zizka and Weiser, 1993). Beauvericin produced by *B. bassiana, Paecilomyces fumosoroseus* and *Fusarium subglutinans* greatly affected fitness of cereal aphid *Schizaphis graminum* (Ganassi et al., 2002). Bassianin and tenellin inhibited erythrocyte membrane ATPase activity (Jeffs and Khachatourians, 1997). Efrapeptins produced by *Tolypocladium* spp. were toxic to insects including mosquito larvae *Culex pipiens* (Bandani et al, 2000). Oosporein oxidised proteins and amino acids by changing SH-groups resulting in

enzyme malfunctions (Wilson, 1971). It is an effective antibiotic against gram-positive bacteria but shown no effect on gram-negative bacteria, fungi and plants (Brewer et al., 1984; Cole et al., 1974; Taniguchi et al., 1984).

2.3.5. Spore surface antigens

In certain host-parasite interactions, the specificity is established by fungal recognition of specific surface topography and cell wall ultra-structure, whereas according to Manocha and Chen (1990), the specificity is determined through binding of complementary macromolecules on surface of both host and fungal principle. Guy and Rath (1990) developed antiserum to the spore surface antigens of *Metarhizium anisopliae*. By using enzyme-linked immunosorbent assays (ELISA) they discriminated two morphologically distinct isolates of *M. anisopliae* (one pathogenic and the other non-pathogenic). This indicated that spore surface antigens are a primary factor determining pathogenicity of isolates, production of enzymes during penetration and toxin production within the host. Rath et al. (1996) inferred that there are antigenic determinants on the surface of *M. anisopliae* spores correlated with pathogenicity to specific host. These macromolecules were found to be proteins or glycoproteins and carbohydrates. Host-specific antigens found on spore surface of virulent fungal isolates are a part of large class of related antigens, specific structure of which confers to them differing recognition for different host cuticular material. That is, fungal isolates avirulent to one insect may have other spore surface antigens, which make them pathogenic to another insect species. This feature undoubtedly is a desirable trait in biocontrol and important in restricting the activity of a particular fungus to a specific host thus making them environmentally safe and target specific.

2.4. Epizootics

Epizootic is the result of a complex interaction among the host, pathogen and environment over time. Epizootics of fungi can occur in the field population of insects, and they can be important in natural regulation of insect pests. The best-studied entomogenous hyphomycetes fungus from an epizootiological perspective is *Nomuraea rileyi* (Carruthers and Soper, 1987). The fungus causes natural epizootics in populations of noctuids such as soyabean loopers (*Pseudoplusia includens*) and velvet-bean caterpillars (*Anticarsia gemmatalis*) on soyabean. Timing of the initial infection of host population, developmental lag associated with disease incubation period, rate of spread of the pathogen and various environmental parameters are all known to control occurrence of the epizootics (Ignoffo, 1981; Kish and Allen, 1978; Ignoffo et al., 1977a). Ovipositing moths or overwintering fungal entities provide initial source of infection (Kish 1975; Ignoffo et al., 1977a). Movement of infected host larvae and the airborne conidia from cadavers form sources for dispersal of inoculum (Kish and Allen, 1978). Infected insects occur in limited loci and subsequently disease spreads very rapidly (Fuxa, 1984). Kish and Allen (1978) developed one of the first quantitative epizootiological models.

2.5. Factors influencing pathogenicity

Ability of a fungus to cause a disease and the physiology of host collectively determine the disease initiation. As a group, fungi have a wider host range, but individually a fungal isolate has a restricted host range. *Aschersonia* spp. can infect only white flies while only noctuid lepidopteran are susceptible to *Nomuraea rileyi*. *Metarhizium anisopliae* and *Beauveria bassiana* have much wider host range in several orders of insects, but each genotype of these species has a narrow host range.

Prolonged viability of fungi on the host is important in biological control. Prolonging conidial persistence until the conditions are favourable for disease development enhances efficacy of entomogenous fungi (Inglis et al., 1997b).

Dispersal and recycling ability of fungi on host is known (Kish and Allen, 1978). The behaviour of insects influences dispersal of an entomopathogen and thereby development of epizootics (Poulin, 1995). 'Summit disease syndrome' exhibited by insects infected by entomophthoralean fungi where insects climb to the top of the trees and die by firmly clasping the plant has been known. Conidia of *Metarhizium anisopliae* spread among individual termites by grooming (Kramm et al., 1982). Complex social behaviours among social insects, including the removal of infected individuals from the colony limit development of epizootics (Boucias et al., 1996).

2.5.1. The Environment

Solar radiation, temperature, precipitation, humidity and wind are the major factors affecting efficacy of entomopathogenic fungi. These often interact with each other and impact on fungal biocontrol agents.

2.5.1.1. Solar radiation

The UVβ range (285-315 nm) of sunlight affects conidia, hyphal bodies and hyphae of all taxa of fungi. Resistance to UV damage is identified as a criterion in the genotype selection for fungal biocontrol agents. Conidia of *Metarhizium anisopliae* var. *acridum* were most resistant to artificial sunlight followed by conidia of *Beauveria bassiana and Paecilomyces fumosoroseus* (Fargues et al., 1996). Conidia protected in locations such as within the plant canopies, abaxial surface of leaves can have better survival rate than that of those exposed to direct to sunlight (Inglis et al., 1993; Sopp et al., 1990). Fungus-host interaction is favoured by the fact that insects also prefer to stay

in humid microclimates. The insects move to the exposed surface once these concealed areas are sprayed with fungal biocontrol agents or their products (Amiri et al., 1999, Wraight and Carruthers, 1999). Indirect irradiance may kill fungi even within the shaded areas (Smits et al., 1996a,b). Irradiation at a higher wavelengths may be beneficial by stimulating photoreactivation (Inglis et al., 2001)

2.5.1.2. Temperature

Ambient temperature is known to affect the rate of infection and time of mortality of host insects treated with entomogenous fungi. Vestergaard and co-workers (1995) determined the optimum temperature for *M. anisopliae* infecting adult thrips as ~23°C, and a decrease of 3-5°C increases the time to death by a day. The optimum temperature for most entomopathogenic fungi is 20-25°C. Vegetative growth of most taxa is inhibited and growth usually cease at ~37°C (Goettel et al., 2001). Thermal characteristics of entomogenous fungus may vary among different genotypes (Fargues et al., 1997b). Several studies have reported that there is no correlation between the geographical origin of a fungal isolate and thermal characteristic (McCammon and Rath, 1994; Fargues et al., 1997a; Ouedrago et al., 1997). In contrast, a strong relationship of thermal characteristics *in vitro* and place of sourcing has been observed by others (Vidal et al., 1997a).

Infections of *B. bassiana* and *M. anisopliae* var. *acridum* in acridids are affected by optimization of body temperature to ~40°C via basking (Inglis et al., 1996b, 1997b, 1999). Insects exhibit behavioural fever in response to fungal infection (Inglis et al., 1996b). Blanford et al. (1998) observed that body temperature of Senegalese grasshopper (*Oedaleus senegalensis*) sprayed with *M. anisopliae* was 3°C higher than those of control grasshoppers. However, good suppression provided by fungi indicated

that the behavioural fever is unable to provide disease resistance. It only delays the death and decreases the inoculum potential. Behavioural response and its effect on disease initiation and progression in other insects are uncertain and thermal biology of insects on field is very complex. Selection of genotypes with higher temperature optima, use of behavioural modifiers and physiological stressors, use of compatible insecticides in combination with entomopathogens, use of novel targeting strategies and development of predictive models for deployment of entomopathogens under suitable conditions are the strategies proposed to overcome barriers posed by behavioural fever and basking (Arthurs and Thomas, 2001a; Hallsworth and Magan, 1999; Goettel et al., 2000; Inglis et al., 2001).

2.5.1.3. Relative Humidity

Conidia of *M. anisopliae* survive better at moderate temperatures coupled with high relative humidity (Daoust and Roberts, 1983). A relative humidity of 97% or more was essential for the conidiogenesis of *B. bassiana* on insect cadavers (Fargues and Luz, 2000). High humidity was not a prerequisite for conidiogenesis within the haemocoel of the cadavers (Hallsworth and Magan, 1999; Fernandes et al., 1989). Because of the microclimate of high humidity around the insect integument and high moisture content in the cuticular folds, efficacy of *B. bassiana* and *M. anisopliae* var. *acridum* was not influenced by ambient humidity (Ferron, 1997; Ramoska, 1984; Marcandier and Khachatourians, 1987; Fargues et al., 1997b). Bateman et al. (1993) reported enhanced infectivity of oil-formulated conidia on desert locust.

2.5.1.4. Rainfall

Rainfall can dislodge and disperse conidia from substrates as well as aid in the dispersion of propagules. Compared to the endurance of entomogenous viruses and

Bacillus thuringiensis on foliage, persistence of entomogenous fungi on insects and on foliage during rainfall is less studied. Conidia of hyphomycetous fungi strongly adhere to insect cuticles (Burges, 1998; Boucias et al., 1988). Studies pointed out that canopy density and architecture and insect behaviour may affect persistence (Inglis et al., 2001).

2.5.1.5. Soil factors

Soil type, moisture and microflora influence persistence and efficacy of entomogenous fungi. Many hyphomycetous fungi, which arise on insects, are soil-borne and have established potential against soil pests (Keller and Zimmermann, 1989). Entomogenous fungi withstand high and variable temperatures, high moisture and drought stress (Roberts and Campbell, 1977). Conidia applied directly on soil surfaces or incorporated into soil following application exhibit considerable persistence (Muller and Zimmermann, 1986; Gaugler et al., 1989; Inglis et al., 1997a; Rath et al., 1997; Storey et al., 1989). Soil moisture adversely influences the presence of *B. bassiana, B. brongniartii, M. anisopliae* and *Verticillium lecanii* in soil suggesting a physical loss of inoculum due vertical movement (Storey and Gardner, 1987, 1988). Clay-textured soil and soils rich in organic matter tend to retain more conidia (Keller and Zimmermann, 1989; Storey and Gardner, 1988; Ignoffo et al., 1977b). Rath and co-workers (1995) observed no relations between soil pH and distribution of fungi. However, fungistasis against *B. bassiana* was correlated with pH of the soil (Groden and Lockwood, 1991). Soil pH and nitrogen fertilizers had an impact on germination of conidia of *B. bassiana* (Groden and Dunn, 1996) but not on the infection of the Colorado potato beetle. Plant-derived phenolic compounds inhibit entomogenous fungi in soils (Inglis et al., 2001).

2.5.2. Biotic factors

Several entomogenous fungi are ubiquitous in soil though very little is known about saprotrophic ability of most taxa. Many entomogenous fungi are relatively weak competitors in soil. Restricted vegetative growth emanating from insect cadavers that have died from mycosis in soil are known. Reduced vegetative growth was observed in soils containing high to moderate levels of organic matter and in non-sterilized versus sterilized soils (Gottwald and Tedders, 1984; Studdert and Kaya, 1990; Pereira et al., 1993). Lingg and Donaldson (1981) postulated that *in vitro* fungistasis by *B. bassiana* in non-sterile soils might have been due to water-soluble inhibitors produced by other soil fungi. The complexity and variety of soils hinder researches on influence of soil microflora on persistence and efficacy of entomogenous fungi.

2.6. Biology of entomogenous fungi

2.6.1. *Coelomomyces* Keilin

While describing *Coelomomyces*, Keilin in 1921 suggested its affinity to Chytridiales. In 1945, Couch amending the genus, accommodated *Coelomomyces* in Blastocladiales. About 25 species are known in *Coelomomyces* (Hawksworth et al., 1995).

Life cycle of members of *Coelomomyces* alternates between mosquito larvae and copepods or ostracods (Whisler, 1985). In most species, the haploid gametes that emerge from copepods are isogametes of two mating types. Whisler (1979) confirmed meiosis in resting sporangia of *Coelomomyces* and presumed that segregation of different mating types occurs during meiosis. Isogametes fuse to form zygote, which eventually settles on the inter-segmental membranes of mosquito larva. Flagella are retracted in zygotes and adhesion vesicles attach it to the host. A cyst wall develops around the zygote. An appressorium from which a narrow penetration tube enters

through cuticle into epidermal cells of the host. The fungus ramifies the epidermis, depletes nutrients in the haemocoel and thereby kills the mosquito larva. Resting sporangia are produced at swollen ends of hyphae. Federici and Chapmann (1977) estimated 10000 to 60000 resting sporangia in a single 4^{th} instar larva depending on the species of host. The fungus over-winters as resting sporangia. Usually larvae die in the 4^{th} instar stage and spores are released from decomposing cadaver. Posteriorly uniflagellated meiozoospores emerging out from the resting spores, infect appropriate copepod or ostracod host and establish haploid heterothallic gametophytic stage, which develops in the haemocoel.

In the adult female *Aedes aegypti*, infection is mostly localized in ovaries. During enlargement of ovaries, hyphae in the haemocoel get transferred to interstitial spaces of ovaries and penetrate epithelial cells (Lucarotti, 1992). Fungal hyphae develop into resting sporangia in the ovaries (Lucarotti and Klein, 1988). Resting sporangia are laid down, in place of eggs when female mosquitoes (infected with *Coelomomyces stegomyiae*) attempt to oviposit. Meiozoospores emerged out from the resting spores infect copepod host and complete the life cycle (Padua et al., 1986).

Species of *Coelomomyces* have life cycle involving an intermediate crustacean host, which helps in maintaining a viable progeny. Development in an intermediate host provides the fungi a launching pad to invade mosquito larvae. The gametes are released simultaneously when copepods and mosquito larvae are both near the water surface (Apperson et al., 1992) which facilitate mating between two types of gametes and help to encounter mosquito larvae by the resultant zygote. Species of *Coelomomyces* have potential for use in natural control because they can cause epizootics. These can cycle in the environment and infect adult female mosquitoes and thereby effect potential dispersal (Lucarotti and Andreadis, 1995).

2.6.2. *Lagenidium giganteum* Couch

L. giganteum (Lagenidiales: Oomycetes) is a facultative parasite on mosquito larvae, first observed by Couch in 1935. Umphlett (1973) reisolated the fungus from the host and recognized its biocontrol potential. *L. giganteum* is the only fungus that attained operational stage in mosquito control. Biflagellate motile zoospores are effective phase of the fungus that adhere larval cuticle. Infection is initiated by mechanical and enzymatic activity of encysted zoospores allowing entry of the fungus into larva and resulting with death of the latter within 24-72 hours. On maturation, the fungus reproduces either sexually or asexually. Asexual reproduction amplifies infection, with a new round of zoospore release occurring every 24-72 hours depending upon the mosquito host and environmental conditions. Sexual reproduction results in the formation of oospores that can survive desiccation, environmental extremes and mechanical abrasion. Oospores can be stored for at least 7 years in viable condition in laboratory or field (Kerwin et al., 1994).

Three formulations of *L. giganteum* consisting of various combinations of sexual and asexual spores have been registered by the US Environmental Protection Agency (USEPA Regulation Nos. 56984-1, 56984-2 and 56984-3) with Department of Health Service as registrant (Kerwin et al., 1994). The fungus can be commercially grown in large scale and applied by ground or air appliances (Kerwin and Washino, 1986, 1987, 1988). Its host specificity and safety has been evaluated using with a variety of non-target organisms. (Kerwin et al., 1990) Temperature intolerance (>32oC) of zoospores, absence of any toxin production, safety against mammals and birds makes the fungus very lucrative for pest control management (Siegel and Shadduck, 1987; Kerwin et al., 1990).

2.6.3. Entomophthorales

Entomophthorales is placed in the Subdivision: Zygomycotina, Class: Zygomycetes. Six families, viz. Entomophthoraceae, Neozygitaceae, Completoriaceae, Ancylistaceae, Meristacraceae and Basidiobolaceae are recognized in the Order (Humber, 1989). Entomogenous fungi (Keller, 1997) are accommodated in Entomophthoraceae (200 species) and Neozygitaceae (15 species). Several of the Entomophthorales regulate host population through epizootics. They have narrow host range exhibiting close associations with foliar insect or mite hosts (Evans, 1989).

The members of Entomophthorales have complex life cycles with two or more types of spores. *Entomophthora muscae* is a typical example. To increase the efficiency of out-reaching insect hosts, they show deviation form basic pattern of life cycle. Conidia are infective units in ideal conditions. Conidiophores arise through membranous regions of host integument and large amount of primary conidia are actively discharged (Samson et al., 1979; Soper et al., 1976). The mitospores are fragile, short-lived and germinate instantly. The primary conidium can produce and actively discharge a secondary conidium and the latter may produce and discharge a tertiary conidium. The size of successive conidia diminishes while shape remains the same.

In species of *Neozygites*, primary conidia are not effective whereas secondary conidia are infective and differ in shape and size from the former. In *Zoophthora,* successive conidia are produced on fine capilli-conidiophores. The capilli-conidium is a sticky propagule borne at the tip of the conidiophore, some distance above the surface, and readily attaches the host. Spherical appressoria are produced for host penetration although it is not a prerequisite (Brobyn and Wilding, 1977; Lambiase and Yendol,

1977). Entomophthorales grow as protoplasts lacking sugar rich cell walls within the haemocoel. Lack of cell wall is presumed to help in escaping detection by the host immune system (Beauvais et al., 1989). Reduced feeding and negative geotaxis are some of the symptoms of infection. Physiological starvation and short-lived cells are factors reported as cause of host death (Hajek, 1997b).

Entomophthorales survive hostile periods as resting spores. The zygospores or azygospores are thick-walled, remain dormant for several months before germination (Hajek, 1997a). These germinate any time when hosts are present in the field and produce one to several actively ejected infective germ conidia or capilli-conidia, as in case of *Neozygites* spp.

2.6.3.1. *Entomophthora muscae* (C.) Fresen

E. muscae was the first Entomophthoralean fungus described (Cohn, 1855). The biology and biocontrol potential of this fungus against adult flies, especially housefly, *Musca domestica*, have been studied (MacLeod et al., 1976). The fungus is apparent as dead flies get attached to vegetation, walls, etc. by rhizoids emerging through the proboscis and by the legs of the dead flies. *E. muscae* is known from a range of dipteran hosts. Keller (1984, 1987) demonstrated that *E. muscae* is a complex of species. Recently *E. muscae sensu stricto* has been described (Keller et al., 1999). *E. muscae* is not confined to one host species but may be transmitted to other dipteran species and epizootiology of the fungus involves several hosts (Mullens, 1989; Jensen and Eilenberg, 2000; Kramer and Steinkraus, 1981; Eilenberg et al., 1990; Mullens et al., 1987). Entire life cycle, including production of resting spores in the field population, has been studied (Thomsen and Eilenberg, 2000; Wilding and Lauckner, 1974; Carruthers et al., 1985). In other species, resting spores were observed

occasionally (Steinkraus et al., 1993b). Natural epizootics caused by *E. muscae* are reported in the populations of *M. domestica, Delia* spp. and *C. rosae* (Eilenberg, 1987a; Moller, 1993; Carruthers and Haynes, 1985; Mullens and Rodriguez, 1985; Six and Mullens, 1996; Bellini et al., 1992).

Behavioral fever was observed in *E. muscae* infected *Musca domestica* in which they prefer higher temperatures than uninfected flies, with the result, the fungus dies and fly survives (Watson et al., 1993). Females of *C. rosae* infected with *E. schizophorae* do not recognize their hosts, though they are capable of depositing eggs (Eilenberg, 1987b). This behaviour influences the effect of *E. schizophorae* on *C. rosae* populations, since infected females do not contribute to population growth. Towards the end of infection, flies attach themselves to the vegetation with their abdomen exposed. Fungus discharges primary conidia after death of host. (Krasnoff et al., 1995).

2.6.3.2. *Neozygites fresenii* (Nowawoski) Remaudiere and Keller

Isolated from aphids, the fungus was originally recognized as *Empusa fresenii* Nowakowski (Nowakowski, 1883). Witlaczil (1885) described it as *Neozygites aphidis*. Thaxter (1888) placed *E. fresenii* in a new subgenus *Triplosporium*. Giard (1888) noted that the genera *Triplosporium* and *Neozygites* were synonymous. Remaudiere and Keller (1980) replaced *Triplosporium* in *Neozygites*. About 15 species are currently recognized under *Neozygites* (Keller, 1997). More undescribed species of *Neozygites* from Collembola have been discovered (Dromph et al., 2000; 2001). *N. fresenii* has a worldwide distribution with reports of infected aphids from Africa, Australia, Europe, India, Israel, the South Pacific and North America (Gustafsson, 1965; Keller, 1997; Kuntz, 1925; Ramaseshiah, 1968; Thaxter, 1888; Thoizon, 1970, Milner and Holdom, 1986; Silvie and Papierok, 1991; Bitton et al., 1979). The fungus

is considered as most common aphid pathogen of tropical regions (Remaudiere, 1977). *N. floridana* was reported from Brazil, India, Israel, Poland, USA and the West Africa (Ramaseshiah, 1971; Brandenburg and Kennedy, 1981; Kenneth et al., 1972; Smitley et al., 1986; Yaninek et al., 1996).

Members of Neozygitaceae are specialized pathogens of small arthropods such as mites, springtails, thrips and aphids (Keller, 1997). Since the hosts are small in size, *Neozygites* spp. have not been well studied. *N. fresenii*, *N. cinarae*, and *N. microlophii* attack only aphids, whereas *N. cucumeriformes* and *N. parvispora* are known only from Thysanoptera (Balazy, 1993) and *N. sminthuri* only from Collembola (Keller and Steenberg, 1997). *N. floridana* and *N. tetranychi* are restricted to mites in the Tetranychidae (Keller, 1997).

Neozygites spp. are recognized as important natural enemies of cassava green mite, *Mononychellus tanajoa*, and mites on groundnuts cotton and lima beans (Keller, 1997; Brandenburg and Kennedy, 1983; Carner and Canerday, 1968; Boykin et al., 1984; Elliot et al., 2000). Very short life cycle, ability to attack all stages except eggs of the hosts, production of a large number of primary conidia per host make them very effective as natural control agent (Steinkraus et al., 1993a). There are a number of published reports dealing with life cycle and epizootiology of *Neozygites* spp. (Keller, 1997; Steinkraus and Slaymaker, 1994; Bitton et al., 1979; McLeod et al., 1998; Steinkraus et al., 1991, 1993a, 1995, 1996, 1999).

2.6.3.3. *Entomophaga maimaiga* Humber, Shimazu, Soper & Hajek

Though said to be native of northern Asia, *E. maimaiga* has been reported from North America (Andreadis and Weseloh, 1990; Hajek et al., 1990). Molecular studies have disclosed that *E. aulicae* is a 'species complex', with *E. maimaiga* included in one

of four groups. Fungi in other three groups currently retain the name *E. aulicae* (Walsh, 1996). *E. maimaiga* was initially differentiated from the *E. aulicae* complex based on the fact that it is the only member able to infect *Lymantria dispar* (Soper et al., 1988). Host range of this species has been extensively studied in view of its potential as biocontrol agent (Bidochka and Hajek, 1996, 1998; Hajek et al., 1995a,b).

2.6.3.4. *Entomophaga grylli* Fresen.

E. grylli was first collected on a *Gryllus* sp. by Fresenius (1858) from Europe and has been referred as *Entomophthora grylli*, *Empusa grylli* and *Conidiobolus grylli* (Carruthers et al., 1997). *E. grylli* exists as a complex of pathotypes. *E. grylli sensu stricto* is distributed mainly in Europe. (Humber, 1989; Carruthers et al., 1989). All members of the '*E. grylli* species complex' are pathogens of grasshoppers and locusts (MacLeod, 1963; Carruthers et al., 1989). North American pathotypes of the two species have different host range (Carruthers and Soper, 1987; Pickford and Riegert, 1964; Streett and McGuire, 1990; Ramoska et al., 1988). Both pathotypes have resting spores, which produce infective germ-tubes to initiate infection cycle in their hosts. The pathotype 1, produces activcly discharged conidia or resting spores in the host whereas pathotype 2 has only resting spores in all infected hosts (Carruthers et al., 1997).

Infection with *E. grylli* is commonly called `summit disease' as infected and dying insects exhibit abnormal behaviour and climb to the top of vegetation, dying in a head up position, grasping the plant stems (Evans, 1989). Insect death occurs in the late afternoon and early evening, synchronizing sporulation and infection with optimal conditions of high humidity, cool temperatures and zero ultraviolet radiation during the night (Carruthers and Soper, 1987; Carruthers et al., 1988, 1992). *E. grylli* sporulates within hours of host death. If abiotic conditions are not favourable, the cadaver

desiccates but the fungus remains viable for extended periods of time, with ability to rehydrate, sporulate and desiccate repeatedly (Sawyer et al., 1997).

Grasshoppers and locusts are highly mobile insects, and infection has little impact on their mobility during early stages. Though dispersing grasshoppers are likely to carry infection with them, at least over moderate distances, Carruthers et al. (1997) suggested that long range migration by hoppers may be a way of disease escape, particularly when the fungus can survive at a site for several seasons as resting spores. They also noted that some species leave favoured feeding sites to lay eggs in more open areas, which may separate susceptible early instars from over-wintering sites of the fungus.

2.6.3.5. *Erynia neoaphidis* Remaud. et Henn.

E. neoaphidis has a wide distribution, being recorded from Europe, Asia, Africa, North and South America, and Australia, Asia (Glare and Milner, 1991; Wilding and Brady, 1984; Hatting et al., 1999). Besides *Erynia* (Keller, 1991), the species was earlier assigned to *Pandora* (Humber, 1989) and *Zoopthora* (Balazy, 1993). *E. neoaphidis* has been recorded from more than 70 species of aphids on annual and perennial crops, weeds and wild flowers (Pell et al., 2001). Epizootiology of *Erynia neoaphidis* has been the subject for several publications (Hemmati, 1999; Morgan, 1994; Roy, 1997; Brown et al., 1995; Dromph et al., 1997, 2001; McLeod et al., 1998; Milner et al., 1984; Morgan et al., 1995; Nielsen et al., 1998; Roy et al., 1998, 1999, 2001).

2.6.3.6. *Zoophthora radicans* (Brefeld) Batko

Z. radicans was originally described from *Pieris brassicae* (Brefeld, 1870) as *Empusa radicans*. With a worldwide distribution, it has been recorded from members

of Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Orthoptera, Thysanoptera and Trichoptera (Glare and Milner, 1991). Individual isolates are better adapted to infect taxonomically related insect hosts (Milner and Mahon, 1985; Goettel et al., 1990; Magalhaes et al., 1988; McGuire et al., 1987a; Mietkiewski et al., 1986; Papierok et al., 1984). Infection may have an impact on host behaviour (Reddy et al., 1998). Under certain circumstances, persistent resting spores (azygospores) are produced within the host in response to changing climatic factors, particularly low temperature and high humidity, high inoculum density, host age or inappropriate hosts (Ben-Ze'ev and Uziel, 1979; Glare et al., 1989; McCabe et al., 1984; McGuire et al., 1987b; Perry et al., 1982). Resting spore production has not been observed in all isolates (Glare et al., 1989; Pell et al., 1993). Literature regarding effects of temperature and circadian rhythm on production and discharge of conidia (Milner and Lutton, 1983; Yamamoto and Aoki, 1983; Glare et al., 1987; Milner et al., 1984; Leite et al., 1996b; Pell et al., 1993; Sawyer et al., 1994; van Roermund et al., 1984; Wraight et al., 1990), effects of host or substrate (Leite et al., 1996a,c; Wraight et al., 1990), and effects of nutrients and pH (Magalhaes et al., 1990, 1991a,b; van Roermund et al., 1984) on germination and subsequent development are available.

2.6.4. Ascomycetes

Several entomogenous fungi reported to date belong to Ascomycetes. The genera such as *Cordyceps* (Fr.) Link, *Hypocrella* Sacc., and *Torrubiella* Bound. of Sphaeriales and members of Laboulbenniales are discussed here.

2.6.4.1. *Cordyceps* Link

Typified by *C. militaris* (Fr.) Link, the genus *Cordyceps* accommodates more than 300 species (Mains, 1958). Host range comprises of Coleoptera, Hymenoptera,

Homoptera and Lepidoptera. *C. ophioglossoides* attacks deer truffle, *Elaphomyces* sp. The classic Japanese book on *Cordyceps* and related genera, by Shimizu and Kobayasi contains colour paintings of these fungi (cf. Kendrick, 2001). Tzean et al. (1997) described 6 species of *Cordyceps* from Taiwan. Stalked stromatic fruit body with a fertile head characterizes the genus. These arising from insect larvae or pupae are known as 'vegetable caterpillars' and used in traditional Chinese medicine for various ailments. Masses of hyphae usually fill the host body, emerging out in the form of stalk of hyphae through exoskeleton. The perithecia are formed either superficially or immersed in the stalks. Thin-walled asci with 8 ascospores are typical of the genus with ascospores being long, filiform and sometimes end as part-spores at maturity (Evans and Hywel-Jones, 1997). The development of part-spores can be seen as adaptation to increase targeting-efficiency. Both shooting of spores into air and oozing into water films are reported. Usually each of the 8 long ascospores break into 128 part-spores, often while within the ascus. The number of part-spores in *Cordyceps* varies. Kendrick (2001) estimated that a single stroma of *Cordyceps* species can produce as many as 614,400,000 propagules. Anamorphic genera of *Cordyceps* belong to *Akanthomyces, Hirsutella, Hymenostilbe, Paecilomyces, Metarhizium* and *Verticillium. Tolypocladium niveum* has been recently discovered as the anamorph of *Cordyceps subsessilis* (Kendrick, 2001).

2.6.4.2. *Torrubiella* Boudier

The genus, typified by *T. arachnida* Boudier (Petch, 1923), accommodates about 54 species (Kobayasi and Shimizu, 1982a). Fruiting body is distinguished from that of *Cordyceps* by absence of an erect stalk and the perithecium is borne directly on the host. Host range comprises of homoptera and spiders (Evans and Hywel-Jones,

1997). Peritheicial ascocarps, with wall covered by anamorphic conidial structures, long cylindrical asci with 8-spores, with thickened ascal apices penetrated by a fine canal, filiform multiseptate ascospores which break into several part-spores at maturity characterize the genus. Tzean et al. (1997) described 6 species of *Torrubiella* from Taiwan.

2.6.4.3. *Hypocrella* Saccardo

Typified by *H. discoidea* (Berk. & Br.) Sacc. (Petch, 1921b), *Hypocrella* accommodates 38 species (Petch, 1921b; Evans and Hywel-Jones, 1997). The genus is one of the most specific of entomopathogenic genera being restricted to the members of the Aleyrodidae and Coccidae (Evans and Hywel-Jones, 1997). Stromatic exosclerotium cusion-like, brightly coloured, irregular and undulating due to semi-erumpent perithecia. Asci are cylindrical to filiform, with prominent apical thickening, 8-spored. Ascospores are filiform and break into part-spores at maturity. Blackening of otherwise brightly coloured fruiting bodies is observed while drying herbarium collections. Anamorphs of *Hypocrella* belong to *Aschersonia*. Anamorph and teleomorph can be found on the same stroma adjacent to each other.

2.6.5. Laboulbenniales

Laboulbenniales are most interesting of all entomogenous fungi, named after French mycologist Alexandre Laboulbene. The group contains around 2000 described species. The fungi usually are ectoparasitic, superficially penetrating host cuticle and evidence for deleterious effect against host is wanting. Therefore, this group has received little attention as agents of biological control (Weir and Beakes, 1995).

The fungal body consists of fixed number of cells and organs arranged in a definite pattern. The main axis supporting reproductive organs is called receptacle.

Lower foot cell of the ascospore attaches to host cuticle and give rise to primary receptacle, which further extend to secondary receptacle to form secondary axis which may bear one or more peritheica. The structural complexity of receptacle is the key criterion in the taxonomy of Laboulbenniales. Upper spore segment gives rise to primary appendages. Secondary appendages are also formed in some genera. Sometimes antheridia are produced in the septate columns of these appendages. It is assumed that spermatia are produced either exogenously like conidia (eg. *Ceratomyces* sp.), endogenously in flask-shaped structures (e.g. *Laboulbenia* sp.) or compound antheridia in which antheridial cells discharge spermatia into common antheridial cavity (e.g. *Eucantharomyces* sp.). Perithecial ascocarp is an outgrowth of receptacle comprising of stalk cells and a procarp, which later develops into trichogyne, ascogonium and related cells. Arrangement of perithecial wall cells has importance in delineating families and subfamilies. Ascospores are usually 4 or rarely 8, elongated, spindle shaped and always tow-celled.

Exact nature of host specificity, position specificity on host body and sex of host specificity is poorly understood. Transmission of the fungus seems to be by direct contact among host individuals. The fungus can be gathered round the year by collecting exact hosts. Fungus appears as short bristles either darkly pigmented or pale-coloured on the host body which can be seen microscopically.

Species of several other genera of Laboulbeniales are known. These include *Acompsomyces, Autoicomyces, Ceratmyces, Corethromyces, Dioicomyces, Diplomyces, Eusynaptomyces, Hydrophilomyces, Laboulbenia, Misgomyces, Monoicomyces, Peyritschiella, Rachomyces, Rhyncophoromyces Stigmatomycetes Triceromyces* (Santamaria, 1993, 1995, 1997, 2000, 2001a,b, 2002; Santamaria and Rossi, 1999).

2.6.6. Deuteromycotina

Deuteromycetes are mostly facultative entomopathogens. Most of them are anamorphs of entomopathogenic ascomycetes. Salient features and teleomorph connections of these genera are given in Table 2.2.

2.6.6.1. Hyphomycetes.

2.6.6.1.1. *Akanthomyces* Lebert

The genus *Akanthomyces,* typified by *A. aculeata* Leb., was established by Lebert in 1858 (cf. Mains, 1950b). Anamorph of *Cordyceps*, the genus is diagnosed by cylindrical, terminally tapering synnemata, conidiogenous cells in a hymenium and unicellular conidia in chains (Seifert, 1985). The host range includes members of Lepidoptera, Coleoptera, Diptera, Orthoptera, and Arachnida (Mains, 1950b). Tzean and co-workers (1997) have described 13 species of *Akanthomyces* from Taiwan.

2.6.6.1.2. *Aspergillus* Micheli

Species such as *Aspergillus flavus, A. fumigatus, A. parasiticus* and *A. sclerotium* were reported from insects and spiders (Evans and Hywel-Jones, 1997; Lisansky and Hall, 1983; Tzean et al., 1997). Erect conidiophores with terminally swollen vesicles bearing uniseriate or biseriate phialides and 1-celled, catenate conidia characterize the genus *Aspergillus* (Gams and Samson, 1985). *A. fumigatus* is found to be infective to mammals and hence excluded from biocontrol studies (Lisansky and Hall, 1983).

2.6.6.1.3. *Beauveria* Vuillemin.

The genus, typified by *B. bassiana* (Bals.) Vuill., is important as biocontrol agent and as source of novel metabolites (Ferron, 1981), The type species is an insect

pathogen and recorded from more than 500 hosts (Dromph, 2001; Bruck and Lewis, 2002; Dromph and Vestergaard, 2002; Hall and Papierok, 1982; Hallsworth and Magan, 1999; Ekesi et al., 1998; Klingen et al., 2002a,b). So far 49 species of *Beauveria* were recorded. Seven of them were found to be synonymous with *B. bassiana,* 2 with *B. brongniartii* and one with *B. felina.* Many of the recorded species have been transferred to and accommodated in related genera; 5 in *Tolypocladium,* 2 each in *Acrodontium* and *Tritirachium* and 1 each in *Engyodontium, Nomuraea* and *Pleurodesmospora.* Sympodial conidiophores with a zigzag appearance, densely clustered conidiogenous cells, 1-celled, hyaline, smooth, thin-walled, globose conidia characterize the genus (Tzean et al., 1997). According to Samson (1995), although there are considerable variations among isolates, 6 species can be recognized in the genus based on morphology and biochemical analysis. Though there are reports of allergic response to *Beauveria bassiana* (Lisansky and Hall, 1983), several formulations of the fungus are available commercially from Mycotech, Troy Bioscience (USA), NPP (France), and Nitto Denko (Japan).

2.6.6.1.4. *Gibellula* Cavara

Though species of *Gibellula,* anamorph of *Torrubiella,* are generally encountered as parasites of spiders, they have not been tested for their biocontrol efficacy. The genus is typified by *G. pulchra* (Sacc.) Cavara. The species range from synnematous to mononematous with septate, rough-walled and pigmented, conidiophores bearing terminal vesicular, hyaline, smooth-walled zone with 1-celled metulae and phialides. Conidia are 1-celled, smooth-walled, ellipsoidal and hyaline. About 35 species have been recorded so far (Mains, 1950a; Petch, 1932; Evans and Samson, 1987; Samson and Evans, 1992; Tzean et al., 1997).

2.6.6.1.5. *Hirsutella* Pat.

The genus, typified by *H. entomophila* Pat., has been studied by Minter and Brady (1980). Altogether 87 records of species can be found in literature. Several species have their perfect stages in *Cordyceps*. Synnematous or mononematous conidiophores, with monophialidic conidiogenous cells that are swollen at the base and with a slender neck, 1-celled, ellipsoidal to cylindrical conidia covered with mucus characterize the genus. *H. thompsonii* attacks only mites and is well-known for its epizootics among eriophyid mites, particularly citrus rust mite (*Phyllocoptruta oleivora*), citrus bud mite (*Eriophyes sheldonii*) and coconut mite (*Eriophyes guerreronis*). It is known to have considerable potential as an acaricide (McCoy and Couch, 1978; Hall et al., 1980).

2.6.6.1.6. *Hymenostilbe* Petch

The genus was established by Petch in 1931 to accommodate *Hymenostilbe muscaria* Petch, an inhabitant on flies. Similar to *Akanthomyces, Hymenostilbe* produces synnema with phialides on lateral branches. *Hymenostilbe,* however, differs in having solitary conidia whereas *Akanthomyces* is characterized by catenate conidia (Mains, 1950b). In all, 25 species have been recorded under the genus (Hawksworth et al., 1995).

2.6.6.1.7. *Metarhizium* Sorokin

The genus, typified by *M. anisopliae* (Metschn.) Sorokin, is characterized by mononematous and penicillate conidiophores, phialidic conidiogenous cells, basipitally developing conidial chains and ovoid to cylindrical, aseptate, pale to olive green conidia in mass. *M. anisopliae* is one of the best-studied entomopathogenic fungi. It was the first fungus to be produced in a large scale from a biological control perspective. Besides mosquitoes, *Metarhizium* spp. infect a wide range of insects

including members of Coleoptera, Collembola, Lepidoptera, Diptera and Homoptera (Dromph, 2001; Prior, 1990; Arthurs and Thomas, 2001a,b; Dromph and Vestergaard, 2002; Hallsworth and Magan, 1999; Butt et al., 2001; Ekesi et al., 2001; Hunter et al., 2001; Klingen et al., 2002a,b). Recently, *M. anisopliae* has been licensed for indoor control of cockroaches (Ward et al., 2000a,b).

2.6.6.1.8. *Nomuraea* Maublanc

Nomuraea rileyi (Farl.) Samson (=*Botrytis rileyi* Farlow) typifies the genus. Conidiophores are mononematous to synnematous, with verticillately arranged cylindrical to oval conidiogenous cells, aseptate, hyaline to green, smooth, ovoid to cylindrical conidia (Samson, 1974). The members of the genus are well-known for their epizootics among Lepidoptera, though they were reported from Homoptera, Diptera and spiders. Currently 6 species are known in the genus, viz., *N. anemonoides* A.D. Hocking, *N. atypicola* (Yasuda) Samson, *N. cylindrosporae* (Q.T. Chen & H.L. Guo) Tzean, L.S. Hsieh, J.L. Chen & W.J. Wu, *N. prasina* Maubl., *N. rileyi* (Farl.) Samson and *N. viridula* Tzean, L.S. Hsieh, J.L. Chen & W.J. Wu. *N. prasina* was however recognized as a synonym of *N. rileyi*.

2.6.6.1.9. *Paecilomyces* Bainier

Typified by *P. variotii* Bainier, the genus is cosmopolitan in distribution as saprophytes in soil and pathogen on nematodes or insects. Although 119 species have been recorded, many are later synonyms of *Acremonium, Acrophialophora, Gabarnaudia, Mariannaea, Penicillium, Sagenomella, Scopulariopsis, Septofusidium, Sesquicillium* and *Verticillium.* The species have their teleomorphs in the genus

Byssochlamys. *Paecilomyces fumosoroseus* and *P. farinosus* were studied from the biocontrol utility point of view (Hallsworth and Magan, 1999; Ekesi et al., 1998).

2.6.6.1.10. *Verticillium* Nees

The genus, typified by *V. tenerum* (Nees. Ex Pers.) Link, has erect, slender, branched, tapering conidiophores, verticillately branched phialides, 1-celled, smooth, hyaline, ovoid to ellipsoid, conidia which are solitary or sometimes in chains. The genus is heterogeneous with species usually saprophytic or parasitic on plants but also mycoparasitic and entomopathogenic. Altogether 261 specific names have been recorded for the genus. Many have been later accommodated in genera such as *Acremonium, Acrostalagmus, Cladobotryum, Clonostachys, Gliocladium, Paecilomyces, Phaeostalagmus, Phialophora, Sesquicillium* and *Trichoderma.*

V. lecanii, originally described from scale insects, has a broad host range among Arthropoda. There are a great deal of variation among isolates of the fungus from different hosts making *V. lecanii* a 'species complex' (Yun et al., 1991). As a consequence, the species of *Verticillium* has an impressive list of synonyms (Evans and Hywel-Jones, 1997). *V. lecanii* has its teleomorph associated with *Torrubiella,* although remaining species have it with *Hypomyces*. *Verticillium lecanii* has been used successfully to control aphids and white flies in glass houses (Lisansky and Hall, 1983). It was the first fungus to be registered for commercial use and produced in a controlled and standardized conditions. Three products of *V. lecanii,* Vertilac against whiteflies, Mycotal against aphids and Thriptal against thrips, produced by Microbial Resources Ltd. were in the market in early 1980's (Evans and Hywel-Jones, 1997). *V.*

chlamydosporium is well-known for its efficacy against cyst nematode of cereals. Clamydospores of this fungus were able to infect the target in soil, whereas the conidia were helpful in survival and dispersal (Lisansky ad Hall, 1983).

2.6.6.1.11. *Tolypocladium* Gams

The genus contains anamorphs of *Cordyceps* and *Hypomyces*. *Tolypocladium* was established by Gams in 1971, typified by *T. inflatum* W. Gams. Sparingly branched conidiophores, swollen phialides, and small, one-celled conidia borne in slimy phialides (Bisset, 1983) are the features of the genus. *Tolypocladium* spp. are wide spread as soil fungi, some of which are pathogenic on insects (Bisset, 1983; Samson and Soares, 1984). Some strains of *T. cylindrosporum* are considered as potential candidate for control of mosquitoes and other insect pests (Samson and Soares, 1984; Klingen et al., 2002a,b; Lam et al., 1988). The fungus causes epizootics in mosquito larval population (Weiser and Pillai, 1981). The susceptible mosquitoes include species of *Aedes, Culex, Culiseta, Anopheles, Maorigoeldia, Opifex,* etc. (Goettel, 1987a,b; Weiser and Pillai, 1981; Gardner et al., 1986; Pinnock et al., 1973; Soars et al., 1979,1985; Yu et al.,1980). *Tolypocladium* spp. produce a wide range of metabolites including cyclosporins, efrapeptins, elvapeptins and the antibiotic LP237-F8 (Dreyfuss et al., 1976; Jackson et al., 1979; Krasnoff et al., 1991; Tsantrizos et al., 1996). However, effect of these compounds during infection of mosquitoes is not known so far. Laboratory studies have shown that relatively high dosages of *T. cylindrosporum* are required to elicit a response in mosquitoes (Goettel, 1987a; Pinnock et al. 1973).

2.6.6.2. Coelomycetes

2.6.6.2.1. *Aschersonia* Montagne

The genus, typified by *A. taitensis* Montagne (Petch, 1921b), is characterized by production of pycnidia containing conidiophores and/or paraphyses, formed in hemispherical or cushion-shaped centrum; conidiophores are slender, branched consisting of thin-walled usually awl-shaped conidiogenous cells with hyaline, mostly fusoid, smooth, one-celled conidia. Species of *Aschersonia* are parasitic on homopteran insects and have *Hypocrella* as teleomorph (Tzean et al., 1997). Rolf and Fawcett (1913) first described their entomogenous nature. Reports on their biocontrol value are given by Frasen et al. (1987), Ramakers and Samson (1984) and Rombach and Gillespie (1988). Petch (1921b) examined the herbarium specimens then available for *Aschersonia* and recognized 25 species. Mains (1959a,b) reviewed species of *Aschersonia* described from America. Recent works on ecology, *in vitro* growth and biocontrol aspects are by Evans (1994), Hywel-Jones and Evans (1993) and Meekes et al. (2002).

Besides the well-known genera of entomopathogenic fungi, species of *Trichoderma, Penicillium, Mucor, Fusarium* Link: Fries and *Cladosporium* Link: Fries were also reported to infect insects (Evans and Hywel-Jones, 1997; Miczulski and Macowicz-Stefaniak, 1977; Gillham et al., 1990). Christias et al., (2001) reported a new pathotype of *Alternaria alternata* as pathogenic to aphids with scanning electron micrographs. A new mosquito killing fungus belonging to the genus *Pythium* has been reported from China (Su et al., 2001). Entomopathogenic fungi reported from insects are given in Table 2.3. Effective doses of some of the fungi are given in Table 2.4.

2.7. Mosquito control perspective

Difficulties associated with use of synthetic chemicals in the control of vector mosquitoes such as induced resistance in vector population, detrimental effect on environment and human health, contamination of water and food and increase in production cost of new insecticides have led to intensified search of other safer and sustainable alternatives (Kumar et al., 1994, 1996). Development of integrated control programme for vector and nuisance mosquitoes since early 1960s also emphasized the need of viable alternatives for chemical control (Lucarotti et al., 1985). Larvivorous fishes, bugs, bacteria, etc. have proved their usefulness in this regard and are now being tested and employed against mosquito developmental stages in aquatic environment. Entomogenous fungi have been found to cause epizootics that result in high level of mortality in mosquito larval population. Several workers have predicted that fungi could be an effective control measure against mosquito development (Lisansky and Hall, 1983; Lucarotti et al., 1985).

To date, entomogenous species of seven genera of fungi, namely *Coelomomyces* Keilin, *Culicinomyces* Couch et al., *Entomophthora* Fresen., *Lagenidium* Couch, *Leptolegnia* de Bary, *Metarhizium* Sorokin and *Tolypocladium* Gams, are considered as possible biological control agents of mosquito larvae (Federici, 1995; Rawlins, 1989; Lacey and Lacey, 1990).

2.8. Development of fungal insecticides

Despite their proven advantages, many promising fungi have never been tried to a level of practical tools for public health sector. This is because the biopesticide development process is a complex one requiring a broad range of resources that are not always available.

2.8.1. Isolation of fungi

Lacey and Brooks (1997) advocated isolation of microfungi from insects and arachnids from as many geographical locations and climatic conditions as possible and maintenance in culture collections as a priority area of research. Baath (1991) observed a phenomenon of Cu tolerance among entomogenous fungi and recovered several of these directly from soil in Cu-amended media. Isolates of *Cordyceps militaris* and *Paecilomyces farinosus* were found to be tolerant (LD50 values >400mg of Cu L-1) to copper.

2.8.2. Screening and strain improvement

Mutation leads to altered antibiotic production, enhancement of enzyme activity and increased toxin synthesis, all of which were proved to be beneficial in the development of fungal biocontrol agents (Deshapande, 1999; St Leger and Joshi, 1997). A range of approaches, namely the use of heterologous DNA probes (Desjardins et al., 1992; Joshi et al., 1995), oligonucleotide probes or primers based on conserved regions of genes (Kusserow and Schafer, 1994), heterologous expression (Froeliger and Leong, 1991), the screening of expression libraries with antibodies (Osbourn et al., 1994) are in operation. Differential hybridization (St Leger et al., 1992a,b; Talbot et al., 1993; Pieterse et al., 1994) and differential display (Joshi et al., 1998) techniques allow isolation of infection regulated genes without making any assumptions about their products. Parasexual crosses between auxotropic mutants of *Metarhizium anisopliae* were made for higher production of amylases and proteases, which have role in degradation of insect cuticle during the process of invasion. However, due to incompatibility factors, the production of heterokaryons appeared to be difficult (Messias and Azevedo, 1980; Bello and Meirelles, 1998; Silveira and Azevedo, 1987).

Protoplast fusion of diauxotropic mutants of *Beauveria bassiana* and *B. sulfurescens* was used to produce hybrids (Viaud et al., 1998). Some of the hybrids were hypervirulents and maintained pathogenicity even after passage through insects. Additional copies of PR1 protease gene were incorporated for over expression of the enzyme in *Metarhizium anisopliae*, under regulation of constitutive *Aspergillus nidulans* promoter (St Leger and Roberts, 1997). Increased production of the enzyme has been found to reduce time of mortality of the challenged larvae.

2.8.3. Production of live propagules.

Mass production of live fungal propagules, useful as biocontrol agents, is known with principle that these can be applied using agrochemical tools and they would withstand extremities of temperature, moisture and aeration (Lisansky and Hall, 1983). Large scale production of spores has been achieved through surface culture in still liquid or semi-solid medium (Hall and Papierok, 1982; Roberts and Sweeney, 1982) and submerged fermentation (Jenkins and Prior, 1993). Higher conidiation of *Hirsutella thompsonii* has been achieved in submerged cultures (VanWinkelhoff and McCoy, 1984; McCoy et al., 1972). Jenkins and Prior (1993) have grown *Metarhizium flavoviride* in a simple liquid medium and shown that the fungus can produce sporogenous cells and spores morphologically indistinguishable from that of aerialy grown fungus, by altering conditions of the medium. Jackson et al. (1997) have produced desiccation tolerant blastospores of a bioinsecticidal fungus *Paecilomyces fumosoroseus* in liquid culture by carefully controlling nutritional status of the medium. Large-scale production of conidia of *Beauveria bassiana* by this method were practiced in erstwhile USSR (Goral, 1971; Kondryatiev et al., 1971; Thomas et al., 1987).

2.8.4. Processing and formulations

Once cultivated on a suitable substrate, spores have to be collected, concentrated and maintained until use. Table 2.5 shows the percentage viability observed under different conditions of temperature, humidity and formulations by different researchers. Desired physical characteristics can be brought about by adding wetters, stickers, dispersants, UV protectants etc. so as to enable easy and effective delivery at target sites and to maintain its *in situ* activity (Lisansky and Hall, 1983). Conidia, mycelial fragments or blastospores are administered in the field in the form of aqueous solutions (Butt et al., 1994; Li et al., 1993). Sunlight, temperature, humidity, substratum and chemical pesticides, factors either independently or collectively influence the stability, sensitivity and persistence of formulations (Ibrahim et al., 1999).

Oil-based preparations showed promise of control of some pests (Prior et al., 1988). Possible advantages on use of oil rather than water as a carrier of fungal propagules include infections at lower humidities (Bateman et al., 1993), stimulated germination (Winder and VanDyke, 1990), longer duration of viability (Prior et al., 1988), decreased sensitivity to high temperature in storage (McClatchie et al., 1994), decreased sensitivity to UV radiation (Moore et al., 1993) and enhanced attachment to hydrophobic surfaces of insect integument (Inglis et al., 1996a).

Several oil-soluble sunscreens significantly increased the survival of *B. bassiana* and *M. anisopliae* conidia exposed to artificial sunlight (Moore et al., 1993; Inglis et al., 1995). Formulation of *B. thuringiensis* in melanin provided excellent photoprotection (Liu et al., 1993) and increased the persistence of entomogenous hyphomycetes (Inglis et al., 2001). A number of formulations have been shown to influence the persistence of propagules in soil (Inglis et al., 1997a; Studdert et al., 1990). Several of these products have been commercialized (Shah and Goettel, 1999).

2.8.5. Persistence and its implications

The ability of an entomogenous fungus to persist on its host will have far-reaching implications in the effectiveness of naturally occurring and introduced pathogens (Jacques, 1983). Inglis et al. (1997a,b) demonstrated that the conidia of *B. bassiana* are relatively long-lived in prairie grassland soil. Protection of conidia from solar radiation and buffering from extremes of temperature and moisture are reasons for increased survival of conidia of *B. bassiana* when incorporated into soil (Gaugler et al., 1989). Storey et al. (1987) observed that conidia suspended in oil are less prone to leaching than those applied in water. Formulations of conidia and blastospores in clay have found to enhance their persistence in soil under controlled conditions (Fargues et al., 1983; Studdert et al., 1990). Inglis et al. (1997a) obtained densities of conidia in the range of 10^4-10^5 per gram dry weight of soil, which means that conidia of *B. bassiana* are deposited on soil surface.

Under suitable temperature and water quality, Jaronski and Axtell (1983) found persistence of *L. giganteum* even in the absence of mosquito larvae. Washino (1981) observed *L. giganteum* for at least 8 years in a drainage ditch. Jaronski and Axtell (1983) concluded that the fungus could be a self-perpetuating biocontrol agent of larval mosquitoes at least for an entire mosquito breeding season. Ability of conidia of *Culicinomyces clavisporus* to persist and remain infective could be prolonged by lowering the temperature (Frances et al., 1984, 1985b). They observed seasonal appearance of *C. clavisporus* and attributed this to recycling of the fungus by rainfall and flooding of rock-pools with river water. Fate of fungal entities following field application have also been examined (Goettel et al., 2000).

2.8.6. Bio-safety measures

Benefit of using a biological control agent on a target organism is linked with bio-safety of non-target organisms. Safety issues and concern of deployment of microorganisms for pest control are (i) pathogenicity to non-target organisms, (ii) toxicity to non-target organisms, (iii) competitive displacement of microorganisms and (iv) allergy to humans (Goettel and Jaronski, 1997). Austwick (1980), Evans (1998, 2000), Prior (1990) and Goettel and Johnson (1992) have reviewed safety of fungal biocontrol agents. Siegel (1997) reviewed guidelines for testing pathogenicity of entomopathogens to mammals. Measures for evaluating the safety for invertebrate nontarget organisms have been reviewed by Hajek and Goettel (2000). The safety concerns regarding fungal biocontrol agents are being addressed at many tier. Competitive displacement of nontarget organisms, allergenicity, toxigenicity and pathogenecity to non-target organisms are identified as thrust areas of potential hazards of biocontrol (Goettel and Hajek, 2001; Cook et al. 1996).

Fungi are known to secrete a variety of secondary metabolites that may be toxic to non-target organisms. Oosporein, beauvericin, bassianolide and beauveriolide produced by *Beauveria bassiana*, destruxins and cytochalasins by *Metarhizium anisopliae,* hirsutellin by *Hirsutella thompsonii* and peptaibols by *Trichoderma harzianum* are some examples. Not much is known on possible effect of mycotoxins present in the formulated products to the applicator, rate of degradation of mycotoxins in nature and accumulation in the food-web. *Fusarium nygamai,* once identified as a promising mycoherbicide for control of *Striga hermonthica* (witch-weed), is now known to produce many mycotoxins (Abbasher and Sauerborn, 1992; Capasso et al.,

1996) and threat of these towards human and other vertebrates reduced the use of this fungus in biocontrol research (Goettel et al., 2001).

Beauveria bassiana has been reported on American alligators under captivity (Heimpel, 1971; Saik et al., 1990; Semalulu et al., 1992). Embryos of silverside fish, *Menida beryllina* and grass shrimp *Palaemonetes pugio* were infected in the laboratory conditions by conidia of *B. bassiana* and *M. anisopliae. Paecilomyces lilacinus,* a nematode pathogen, is reported to cause human infections in both immune compromised and immune competitive individuals (Itin et al., 1998; Gutierrez-Rodero et al., 1999, Goettel et al., 2001). *Conidiobolus coronatus* causes lesions in both humans and horses (Saik et al., 1990). These indicate the importance of screening of biocontrol agents towards nontarget organisms, especially vertebrates, prior to their deployment as biopesticides. *B. bassiana* was listed as dermal sensitizer by the US Environmental Protection Agency (EPA) following reports of moderate to severe allergic reactions. Crude extracts of *M. anisopliae* was reported to contain one or more potent allergens (Goettel et al., 2001; Saik et al., 1990; Ward et al., 1998).

Allergenicity and potential pathogenicity are of concern. Most microbial agents used against pests are only accidentally consumed by humans. The risk of consumption of pathogens is addressed by standard maximum challenge tests that require acute mammalian oral toxicity/pathogenicity tests. Safety to vertebrates is evaluated by a series of laboratory tests (Burges, 1981; Betz et al., 1990). These protocols combine elements of maximum challenge testing with methods of exposure (Siegel and Shadduck, 1990). Among entomogenous fungi, only *Aspergillus fumigatus* and *Conidiobolus coronatus* are reported to be infective to mammals. Fungal entomotoxins are least toxic to mammals. Most regulatory authorities are following a 'tier' system of

biosafety testing (Goettel and Jaronski, 1997). In the USA, fungi are tested in 5 tiers and those that clear 1st tier are approved for sale. Examples include *Lagenidium giganteum* (Kerwin et al., 1994), *Hirsutella thompsonii* (Ignoffo et al., 1973), *Nomuraea rileyi* (Ignoffo, 1981) and *Verticillium lecanii* (Lisansky and Hall, 1983).

2.8.6.2. Instances of negative effects on non-target organisms

Beauveria bassiana, causative agent of muscardine disease in *Bombyx mori* (Bell 1974; Steinhaus, 1975). Adoption of modern practices and legislation have been responsible for control of muscardine disease (Anon, 1981a; Goettel et al., 1990). *Ascosphaera apis* causes chalkbrood disease of honey-bees (Steinhaus, 1975; Gilliam and Vandenberg, 1990) all over the world (Bradbear, 1988; Anderson and Gibson, 1998).

2.8.6.3. Regulation of fungi as microbial pesticides

The World Health Organization (WHO) proposed a tiered testing strategy to evaluate the hazard posed by microbial pest control agents to mammals (Anon., 1981b). Elements of this proposal are incorporated in the regulatory guidelines of Canada, USA, and the European Union. These tests replaced the long-term assays that are used in assessing chemical insecticides with short-term exposures that utilize invasive routes, such as intravenous and/or intraperitoneal injection, as well as feeding studies. Unfortunately, many of these data are unavailable to the public because they are considered as proprietary.

2.9. Mycoinsecticides

Some of the commercially available myco-insecticides are as follows. Several commercial formulations of *Beauveria bassiana* are available from Mycotech, Troy Bioscience (USA), NPP (France), and Nitto Denko (Japan). *Metarhizium anisopliae* is

produced by Ecoscience (USA), BioCare (Australia), BCP (South Africa) and a few Brazilian companies for use against termites, locusts and sugarcane froghoppers. CABI Bioscience launched an isolate of this fungus as GREEN MUSCLE® to control locusts and grasshopper pests in Africa (Evans, 1999).

Verticillium lecanii is available as MYCOTAL produced by Koppert Biologicals (www.koppert.com) packed as 500 g of wettable powder with 10E+10 spores/g targeted towards whitefly larvae, with some effect on thrips larvae. MYCOTAL is active at 18-28 °C and a minimum relative humidity of 75% for 10-12 hours a day for several days after application. Larvae and pupae die before the fungus is visible. Dead larvae and pupae are light to dark-yellow, wrinkled and no longer shiny. After some time and under favourable conditions (high R.H.), white fungal fluff appears on affected insects (known as 'fluffy bodies'). *V. lecanii* is also available as VERTALEC in the form of 500 g of wettable powder with 10E+9 spores/g (for UK: 5 x 10E+8 spores/g) targeted against many aphid species other than chrysanthemum aphid *Macrosiphoniella sanborni*. White fungal fluff appears on the affected aphids, which can be visible before the aphids are killed. VERTALEC requires a temperature of 18-28°C and a minimal relative humidity of 80% for 10-12 hours a day for several days after application.

AgraQuest has developed *Lagenidium giganteum* as LAGINEX to be used in mosquito pest management programs. LAGINEX provides long term control against larval stage of all species of mosquitoes, with no adverse effects against other insects, mammals, fish, birds, or plants (www.agraquest.com). The company boasts of its product's effectiveness in the control of mosquitoes for 3 to 4 weeks, or more with complete mortality of treated population in 5 to 7 days. LAGINEX controls mosquito

larvae of the following genera: *Aedes*, *Anopheles*, *Coquillettidea*, *Culex*, *Culiseta*, *Deinocerites*, *Eretmapodites*, *Haemagogus*, *Mansonia*, *Opifex*, *Orthopodomyia*, *Psorophora*, *Sabethes*, *Uranotaenia* and *Wyeomyia* in vernal pools, wetlands, freshwater marshes, and any other water source. LAGINEX is recommended for use on rice, soyabean and irrigated pasture.

2.10. Work done in India

While reviewing the hitherto work done on entomogenous fungi in India, Agarwal and Rajak (1988) grouped the available information into two distinct phases. They observed that from end of nineteenth to middle of twentieth century, most of the efforts were on study the occurrence and taxonomy of fungi associated with insects. In the later half of last century, work was centered around application and evaluation of entomogenous fungi such as *Beauveria bassiana*, *Metarhizium anisopliae* and *Nomraea rileyi* as agents of biological control against various agricultural pests.

2.10.1. The early work

In 1856, Berkeley recorded two species of *Cordyceps* from India (cf. Agarwal and Rajak, 1988). While Sydow and Butler reported *Verticillium lecanii* on scale insects of coffee from Karnataka, *Entomophthora muscae* infecting houseflies was recorded by Butler and Bisby (cf. Agarwal and Rajak, 1988). Petch (1931) reported *Hirsutella abietina* on *Pyrilla pusana* from Bihar. *Entomophthora brahminae* was described as a new species on *Brahmina* by Bose and Mehta (cf. Agarwal and Rajak, 1988). Nirula studied efficacy of *Metarhizium anisopliae* on *Rhinocerus* beetle of coconut. Ramamoorthi and coworkers reported an epizootic of sunhemp pest caused by *Beauveria bassiana* (cf. Agarwal and Rajak, 1988). Srinivasan and Tirumalachar (1961, 1962a,b, 1967, 1968a,b) worked on taxonomy of *Conidiobolus*. Srinivasan et al.

(1964) were able to culture a species of *Entomophthora* on synthetic medium. An account on entomogenous fungi and their potential in biocontrol of insect pests was published by Narasimhan (1970). A list of entomogenous fungi on insect pests occurring in Jabalpur, India was published by Agarwal and Rajak (1985b).

2.10.2. <u>The recent work:</u>

Khan et al. (1993) gave an account on use of muscardine fungi in biological control of termite *Odontotermes obesus*. Nehru and Jayarathnam (1993) evaluated the biological control strategies using entomogenous fungi against white grubs (*Holotrichia serrata*) infesting rubber seedlings. Rajak et al. (1993) evaluated susceptibility of teak defoliator (*Hyblaea puera*) and teak skeletonizer (*Eutectona machaeralis*) to *B. bassiana*. Ali and Varma (1994) reported *Beauveria bassiana as* a new insect pathogen on *Atteva fabriciella* and compared its efficacy with that of *Paecilomyces farinosus*. Ambetghar (1996a, 1997) reported *Beauveria bassiana* on rice leaf folder insects from Karaikal, Pondicherry. Ansari and Shukla (1996) recorded entomogenous fungi from Andaman. Balakrishnan et al. (1994, 1995) reported occurrence and association of entomopathogenic fungi on coffee pests in India. Hazarika and Puzari (1995) and Puzari et al. (1994) reported *Beauveria bassiana* as pathogenic to different stages of rice hispa (*Dicladispa armigera*). Kumar et al. (1999) described germination, penetration, and invasion of *B. bassiana* on silkworm, *Bombyx mori*. Narayanasamy (1999) published on infections of rice green leafhoppers in India. Padmaja and Kaur (1998) assessed relative susceptibility of brinjal spotted beetle, *Henosepilachna vigintioctopunctata* (Fabricius) to certain isolates of *Beauveria bassiana*. Kaur and Padmaja (2002) studied the relation between rate of germination to virulence of entomogenous fungi. Saikia (1998a) reported on colonization of nematode

eggs by *Beauveria bassiana.* Sardana (1997) reported entomogenous fungi on sugarcane root borer, *Emmalocera depressella.* Selvasundaram and Muraleedharan (2000) reported *B. bassiana* on the shot hole borer of tea. Viji and Bhagath (2001) evaluated potency of some plant products, synthetic insecticides and entomopathogenic fungi against black cutworm, *Agrotis ipsilon* larvae on maize.

Agarwala et al. (1998) evaluated effect of conidia of *Beauveria bassiana* against larvae of *Aedes aegypti.* Ambetghar (1996b) reported on biological control of brown plant hopper, *Nilaparvata lugens*, using entomogenous fungi. Devi et al. (2001a) evaluated efficacy of *Beauveria bassiana* on sorghum shoot borer *Chilo partellus, Swinhoe* (*Lepidoptera: Pyralidae*) and characterized the isolates by RAPD-PCR techniques. Geetha and Balaraman (1999) published potential of *Beauveria bassiana* in the control of mosquito larvae. Gloriana et al. (2000) determined pathogenicity of *B. bassiana* to larvae of *Spodoptera litura* and *Pericallia ricini.* Gurusubramanian et al. (1999) evaluated susceptibility of *Odontotermes obesus* to *B. bassiana.*

Haraprasad et al., (2001) confirmed that *B. bassiana* could be a potential mycopesticide for efficient control of coffee berry borer, *Hypothenemus hampei* in India. Haseeb and Murad (1997a,b) evaluated pathogenicity of *B. bassiana* to several insect predator *Coccinella septempunctata.* Haseeb and Srivastava (1998) assessed dose-mortality relationship of *B. bassiana* against mango mealy bug, *Drosicha mangiferae.* Hazarika and Puzari (1997) and Hazarika et al. (1998) evaluated field efficacy and seasonal and host-correlated variation in susceptibility of *B. bassiana* on rice hispa, *Dicladispa armigera.* Kak and Khan (1999) confirmed that *B. bassiana* could be a possible biocontrol agent against bamboo defoliator, *Crypsiptya coclesalis.* Mala and Solayappan (2001) screened microbial insecticides including entomogenous

fungi for control of sugarcane early shoot borer larvae *Chilo infuscatellus*. Mathew et al. (1998) evaluated efficacy of several entomogenous fungi on biological suppression of *Pentalonia nigronervosa* f. *caladii* of cardamom. Mohan et al. (1999) evaluated pathogenicity of 3 isolates of *B. bassiana* on American cockroach. Prabhakara et al., (1997) studied disease of silkworm caused by *B. bassiana* along with spread of the disease. Ramesh et al. (1999) studied usefulness of entomogenous fungus in cotton pest management. Reddy et al. (2001) studied efficacy of insecticides, biopesticides and their combinations against pod borers in pigeon pea. Saikia (1998b) investigated efficacy of *B. bassiana* on *Meloidogyne incognita* on tomato. Sandhu et al. (1993a) studied the dose response relationship of *B. bassiana* and *Metarhizium anisopliae* against mosquito larvae *Culex tritaeniorhynchus* and *Aedes aegypti*. Sandhu et al. (1993b) published larvicidal activity of *Beauveria bassiana*, *Metarhizium anisopliae* and *Aspergillus flavus* against *Culex pipiens*.

Keshavaprasad et al. (2003), based on preliminary bioassay conducted, reported several isolates of mosquito larvicidal and entomo-pathogenic fungi from Goa. Kumar et al (1997) studied epizootic potential of *Beauveria bassiana* on tobacco caterpillar. Varma and Tandan (1996) studied pathogenicity of entomogenous fungi against insect pests of sugarcane. Manjula and Padmavathamma (1999) studied effect of insect pathogens on larvae of *Helicoverpa armigera*. Padmaja and Kaur (2000, 2001a,b,c, 2002) worked on extracellular enzymes of entomogenous fungi against cotton pests. Pandit and Samantha (1995) evaluated the potential of *Beauveria bassiana* and *Metarhizium anisopliae* against polyphagous insects. Sardana (2000) evaluated the efficacy of biocontrol agents against sugarcane root borer, *Emmalocera depressella*. Saxena and Ahmad (1997) studied potency of *Beauveria bassiana* in the field against

Helicoverpa armigera (Hubner) infecting chickpea. Sheeba et al. (2001) studied efficacy of *B. bassiana* for control of the rice weevil *Sitophilus oryzae.*

Chavan et al. (1998) utilized silkworm pupae powder as an ingredient of culture medium for cultivation of *Beuveria bassiana.* Kaur et al. (1999) described mycopesticide formulations for control of insect pests on cotton. Mazumder et al. (1995) produced *B. bassiana* in bulk. Pramila et al. (1999) studied compatibility of entomogenous fungi with neem products used in pest suppression. Puzari et al. (1997) reported a medium for mass production of *Beauveria bassiana.* Rajendran (1997), Rajendran and Gopalan (1999) studied management of white fly *Aleurolobus barodensis* and egg plant spotted beetle, *Henosepilachna vigintioctopunctata* using *Bacillus thuringiensis* and *Beauveria bassiana.* Sandhu, (1995) studied effect of physical factors on germination of conidia of *B. bassiana.* Sharma et al. (1999a,b) studied mass production and formulation of entomopathogenic fungi and their efficacy against whitegrub, *Holotrichia consanguinea.*

Balavenkatasubbaiah et al. (1994, 1996,1999), Byrareddy et al. (1993) and Bhattacharya et al. (1997) evaluated efficacy of different disinfectants against entomogenous fungi including *Beauveria bassiana* attacking silkworm *Bombyx mori.* Javaregowda et al. (1994) reported on effect of sun drying on inactivation of *B. bassiana* infecting *Bombyx mori.*

Jayanthi and Padmavathamma (1996) and Manjula and Padmavathamma (1996) evaluated effect of microbial insecticides on control of *Maruca testulalis* and other natural enemies or predators of red gram pest complex. Devi (1994, 1995), Devi and Prasad (1996) and Devi et al. (2001b) studied conidia production of entomopathogenic

fungus *Nomuraea rileyi* and its evaluation for control of *Spodoptera litura* on *Ricinus communis*.

Keerthi et al. (1999) reported extracellular production of L-glutaminase by alkalophilic *Beauveria bassiana* isolated from marine sediment. Sandhu et al. (1998) developed an entomopathogenic hybrid through biotechnological approaches for biocontrol of *Aedes aegypti*. Sandhu et al. (2001a) developed benomyl resistant *B. bassiana* strains and studied their infectivity against *Helicoverpa armigera*. Sandhu et al. (2001b) transformed isolates of *Beauveria bassiana* and *Metarhizium anisopliae* using nitrate reductase gene of *Aspergillus nidulans*. Sandhu et al. (1999) studied usefulness of autolytic enzymes of entomopathogenic fungi to the protoplast isolation. Sharma et al. (1994) studied toxicology of metabolites of *Beauveria bassiana* in *Heliothis armigera*. Sharma et al. (1999b) studied effect of soil fungi on species of *Metarhizium* and *Beauveria* and their pathogenicity against *Holotrichia consanguinea*. Effect of temperature on growth, sporulation and bioactivity of entomogenous fungi against whitegrub (*Holotrichia consanguinea*) was also studied by them (Sharma et al. 1998). Sivasankaran et al. (1998) studied effect of temperature and relative humidity on growth, sporulation and pathogenicity of *Beauveria bassiana*. Suresh and Chandrasekaran (1998) studied the media preference for chitinase production by a marine isolate of *B. bassiana*. Deshpande (1999) reviewed intricacies involved in mycopesticide production.

Table 2.1. Entomopathogenic fungi in different fungal groups:

Sub-division/ Class/Order	Family	Genus	References
Mastigomycotina Oomycetes	Lagenidiaceae	*Lagenidium*	Couch, 1935; Federici, 1981; Brey & Remaudiere, 1985; Couch & Romney, 1973; Kerwin & Washino, 1988; Domnas et al., 1974.
Chytridiomycetes	Blastocladiaccae	*Coelomomyces*	Keilin, 1921; Lucarotti, 1987; Lucarotti, 1992; Lucarotti & Andreadis, 1995; Lucarotti & Klein, 1988.
Zygomycotina Entomophthorales	Ancylistaceae	*Conidiobolus*	Ben-Ze'ev, 1982; Drechsler, 1961; Humber, 1989; Keller, 1987; King, 1976a,b, 1977; Papierok, 1989; Waterhouse & Brady, 1982; Waters & Callaghan, 1989; Aruta et al., 1984; Srinivasan et al., 1964.
"	Entomophthoraceae	*Entomophaga*	Andreadis & Weseloh, 1990; Shimazu & Soper, 1986; Bidochka et al., 1996; Hajek et al., 1997; Soper et al., 1988.
"	"	*Entomophthora*	Ingold, 1987; Keller, 1987; Kenneth, 1977; Ben-Ze'ev & Zelig, 1987; Descals & Webster, 1984; Glare & Milner, 1987; Humber & Feng, 1991; MacLeod & Muller-Kogler, 1970; Remaudiere & Hennabert, 1980; Villacarlos & Wilding, 1994; Waterhouse & Brady, 1982; MacLeod et al., 1979; Samson et al., 1979; Srinivasan et al., 1964.
"	"	*Erynia*	Balazy, 1981; Ben-Ze'ev, 1982, 1986a,b; Humber, 1989; Keller, 1991, 1993; Li, 1986; Papiero k, 1989; Ben-Ze'ev & Kenneth, 1982; Descals & Webster, 1984; Glare & Milner, 1987; Humber & Ben-Ze'ev, 1981; Keller & Eilenberg, 1993; Li & Humber, 1984; Remaudiere & Hennabert, 1980; Villacarlos & Wilding, 1994; Waterhouse & Brady, 1982; Milner et al., 1983; Pena et al., 1990.
"	"	*Zoophthora*	Humber, 1989; Keller, 1991; Tyrell & MacLeod, 1975; Waterhouse & Brady, 1982; Fristencel et al., 1990; Glare et al., 1987.
"	Neozygitaceae	*Neozygites*	Humber, 1989; Keller, 1991; Papierok, 1989; Glare & Milner, 1987; Keller & Wuest, 1983; Remaudiere & Keller, 1980; Villacarlos & Wilding, 1994; Waterhouse & Brady, 1982; Dick et al., 1992.
Ascomycotina Clavicipitales	Clavicipitaceae	*Cordyceps*	Balazy, 1982; Chen, 1978; Eriksson, 1982; Ginns, 1988; Hywel-Jones, 1994, 1995a,b,c, 1996a; Kobayasi, 1941, 1982; Mains, 1951b, 1954; Petch, 1934, 1939; Rogerson, 1970; Shimizu, 1976a,b; Wright, 1993; Balazy & Bujakiewicz, 1986; Evans & Samson, 1982a, 1984; Hywel-Jones & Sivichai, 1995; Kobayasi & Shimizu 1976, 1980, 1981, 1982b,c, 1983a,b; Liu & Liang, 1993; Papierok & Charpentic, 1982; Samson et al., 1982; Liang et al., 1993; Zang et al., 1982, 1990.

"	"	*Torrubiella*	Hywel-Jones, 1995e; Kobayasi, 1941, 1982; Petch, 1923, 1937, 1944; Evans & Samson, 1982b; Humber & Rombach, 1987; Kobayasi & Shimizu, 1976, 1981, 1982a, 1983b; Samson & Evans, 1992; O'Donnel et al., 1977; Samson et al., 1989.
"	"	*Cordycepioideus*	Stifler, 1941.
Hypocreales	Hypocreaceae	*Hypocrella*	Hywel-Jones, 1993; Petch, 1921a, 1925b, 1939; Samson, 1995; Evans & Hywel-Jones, 1990; Evans & Samson, 1982b; Hywel-Jones & Evans, 1993.
Laboulbeniales	Laboulbeniocceae	*Laboulbenia*	Belazuc, 1982; Rossi, 1986; Thaxter, 1899; Weir, 1993.
"	"	*Zodiomyces*	Thaxter, 1931; Majewski & Sugiyama, 1989; Sugiyama & Phanichapol, 1984.
"	"	*Stigmatomyces*	Thaxter, 1901, 1905, 1908, 1917, 1931; Weir & Rossi, 1995.
"	Ceratomycetaceae	*Rhynchophoromyces*	Thaxter, 1900, 1908, 1931; Sugiyama & Phanichapol, 1984.
Pleosporales	Tubeufiaceae	*Podonectria*	Petch, 1927; Rossman, 1978; Kobayasi & Shimizu, 1983b.
Basidiomycotina Septobasidiales	Septobasidiaceae	*Septobasidium*	Couch, 1937, 1938.
		Uredinella	Couch, 1941.
Deuteromycotina Hyphomycetes		*Acremonium*	Balazy, 1973; Gams, 1971; Petch, 1925a.
"		*Akanthomyces*	Hywel-Jones, 1996b; Mains, 1950b, 1954; Papierock & Charpentie, 1982; Samson & Brady, 1982; Samson & Evans, 1974a; Vincent et al., 1988.
"		*Aspergillus*	Klich & Pitt, 1988; Raper & Fennel, 1965; Tzean et al., 1990.
"		*Beauveria*	Arx, 1986, 1988; Li, 1992; Limber, 1940; Petch, 1924; Samson, 1995; Benham & Miranda, 1953; Bissett & Widden, 1988; de Hoog & Rao, 1975; Samson & Evans, 1982; Samson & Soares, 1984; Glare et al., 1993.
"		*Culicinomyces*	Goettel et al., 1984; Frances et al., 1985a,b.
"		*Gibellula*	De Hoog, 1978; Gao, 1981; Mains, 1950a; Petch, 1932; Evans & Samson, 1987; Humber & Rombach, 1987; Kobayasi & Shimizu, 1976, 1981; Samson & Evans, 1973,1977, 1992.
"		*Hirsutella*	Fisher, 1950; Hywel-Jones, 1995c; Mains, 1951a; Pacioni, 1980; Speare, 1920; Balazy & Wisniewski, 1986; Evans & Samson, 1982a,b, 1984, 1986a; Minter & Brady, 1980; Rombach & Roberts, 1987; Samson & Evans, 1985, 1990; Samson & McCoy, 1982; Fernandez-Garcia et al., 1990; Samson et al., 1980, 1984; Strongman et al., 1990.
"		*Hymenostilbe*	Hywel-Jones, 1995a,d, 1996a; Mains, 1950b; Samson & Evans, 1974b.

"		*Metarhizium*	Shimazu, 1989; Tulloch, 1976; Gams & Rozsypal, 1973; Liu & Liang, 1993; Guo et al., 1986; Liang et al., 1991; Rombach et al., 1986, 1987; Yadav et al., 2000.
"		*Nomuraea*	Hocking, 1977; Samson, 1974; Shimazu, 1989; Hywel-Jones & Sivichai, 1995; Samson & Evans, 1977; Tzean et al., 1992, 1993.
"		*Paecilomyces*	Chen, 1991; Liang, 1981a,b, 1985; Samson, 1974; Brown & Smith, 1957; Jong & Davis, 1975; Pacioni & Frizzi, 1978; Samson & Evans, 1977; Liang et al., 1993.
"		*Sporothrix*	De Hoog, 1974; Moustafa, 1981; Taylor, 1970, 1977.
"		*Stilbella*	Benjamin, 1968; Mains, 1948, 1951a; Seifert, 1985; Samson & Evans, 1985; Morgan-Jones & McKemy, 1991; Samson et al., 1981, 1984.
"		*Tilachlidium*	Keissler, 1924; Mains, 1948, 1951a; Petch, 1939; Seifert, 1985; Evans & Samson, 1982b; Papierok & Charpentie, 1982; Samson et al., 1981; Stalpers et al., 1991.
"		*Tolypocladium*	Goettel, 1987b, 1988a,b; Weiser & Pillai, 1981; Lam et al., 1988.
"		*Verticillium*	Balazy, 1973; Gams, 1971; Petch, 1925a, Evans & Samson, 1986b; Gams & van Zaayen, 1982.
Coelomycetes		*Aschersonia*	Evans, 1994; Hywel-Jones, 1993; Petch, 1921a,b, 1925b, 1939; Samson, 1995; Evans & Hywel-Jones, 1990; Hywel-Jones & Evans, 1993.

Table 2.2: Diagnostic features of genera of entomopathogenic hyphomycetes and coelomycetes with information on teleomorph connections: (References in Chapter II)

Genus	Conidiophores	Conidiogenous cells	Conidia	Teleomorph
Akanthomyces	Synnematous, terminally tapering synnema	Arranged in a hymenial layer over synnema, denticulate	Catenate, aseptate, pleomorphic, ellipsoidal to elongated	*Cordyceps*
Aspergillus	Mononematous with swollen terminal vesicle	Arranged in uni or biseriate over the vesicle	Catenate, aseptate, globose to subglobose	*Emericella*
Beauveria	Mononematous	Clustered on lateral cells, sympodially denticulate	Solitary, aseptate, smooth, thin-walled, globose	*Cordyceps*
Gibellula	Synnematous to mononematous, rough-walled and pigmented, conidiophores with a terminal vesicle	One-celled metulae and phialides over the vesicle	Catenate or solitary, aseptate, smooth-walled and ellipsoidal	*Torrubiella*
Hirsutella	Synnematous to mononematous, terminally tapering	Arranged laterally, swollen at the base and with a long slender neck, monophialidic	Solitary, aseptate, ellipsoidal to cylindrical	*Cordyceps*
Hymenostilbe	Synnematous, terminally tapering	Arranged in a hymenial layer, denticulate	Solitary, aseptate, pleomorphic, ellipsoidal to elongated	*Nectria*
Metarhizium	Mononematous, penicillate	One-celled metulae and monophialides	Catenate, aseptate, pale to olive green, ovoid to cylindrical	*Cordyceps*
Nomuraea	Mononematous to synnematous	Verticillately arranged below the septum	Catenate to solitary, aseptate, hyaline to green, ovoid to cylindrical	-
Paecilomyces	Mononematous, penicillate or solitary	Terminal and intercalary	Catenate, aseptate, hyaline to green, globose to ellipsoidal	*Byssochlamys*
Verticillium	Mononematous, verticillately branched	Verticillately arranged monophialides	Solitary, aseptate, hyaline, ovoid to ellipsoidal	*Hypomyces* & *Torrubiella*
Tolypocladium	Mononematous, sparingly branched	Swollen phialides	Solitary in slimy balls, aseptate, globose to cylindrical	*Cordyceps* & *Hypomyces*
Aschersonia	Pycnidia in the stroma, slender, branched simple pycnidiophores	Slender, awl-shaped	Hyaline, mostly fusoid, smooth, one-celled	*Hypocrella*

Table 2.3. Entomogenous fungi employed to manage insect pests:

Fungus	Target/Host	References
Coelomomyces sp.	*Aedes trivittatus & Ae. sticticus*	Galloway et al., 2001.
C. stegomyiae	*Ae. aegypti*	Galloway et al., 2001.
Conidiobolus coronatus	*Myzus dirhodum*	Hatting et al., 1999.
C. obscurus	*Delia noxia, Myzus dirhodum, Rhopalosiphum padi, R. maidis & S. avenae*	Hatting et al., 1999.
C. obscurus	*Myzus cerasi*	Klingen et al., 2002b.
Conidiobolus spp.	*Bemisia* spp.	Gindin & Ben-Ze'ev, 1994.
C. thromboides	*Delia noxia, Myzus dirhodum, Rhopalosiphum padi, R. maidis & S. avenae*	Hatting et al., 1999.
Entomophaga grylli	*Camnula pellucida*	Erlandson et al., 2001.
Entomophthora muscae	*Musca domestica*	Floate et al., 2001.
E. muscae	*Delia foralis & D. radicum*	Klingen et al., 2002a,b.
E. planchoniana	*Aphis spiraecola, A. gossypii, Hyalopterus pruni, Macrosiphum euphorbiae, Myzus persicae, Sitobion fragariae & Delia noxia*	Hatting et al., 1999.
Erynia virescens	*Mamestra brassicae*	Klingen et al., 2002a.
Neozygites fresenii	*Aphis gossypii, Chaitophorus populialbae, Hyalopterus pruni & Myzus persicae*	Hatting et al., 1999.
Pandora neoaphidis	*Aphis gossypii, Metopolophium dirhodum, Diuraphis noxia, Sitobion avenae, Myzus* sp., *Macrosiphum euphorbiae, Rhopalosiphum padi, R. maidis, Ureleocon sonchi & Melanaphis sacchari*	Hatting et al., 1999.
Zoophthora (Erynia) radicans	*Bemisia* spp.	Silvie & Papierok, 1991.
Pythium carolinianum	*Aedes albopictus & Culex quinquefasciatus*	Su et al., 2001.
Aspergillus parasiticus	*Melanoplus bivittatus & M. packardii*	Erlandson et al., 2001.
Beauveria bassiana	Colorado potato beetle	Jacques & Laing, 1988; Drummond & Groden, 1996; Watt & LeBrun, 1984; Weber & Ferro, 1993; Anderson et al., 1988, 1989; Cantwell et al., 1986; Campbell et al., 1985; Hajek et al., 1987; Lacey et al., 1999a; Poprawski et al., 1997.
B. bassiana	European corn borer	Bing & Lewis, 1991, 1992a,b, 1993; Lewis & Bing, 1991; Wagner & Lewis, 2000; Hsiu et al., 1973; Cherry et al., 1999; Lewis et al., 1996.
B. bassiana	*Dendrolimus* spp. (Pine caterpillar)	Feng et al., 1994; Pan & Zheng, 1988.
B. bassiana	*Hypothenemus hampei* (coffee berry borer)	Reithinger et al., 1997.
B. bassiana	*Phlebotomus papatasi*	Reithinger et al., 1997.
B. bassiana	*Lutzomyia longipalpis*	Reithinger et al., 1997.
B. bassiana	*Melanoplus bivittatus*	Erlandson et al., 2001.
B. bassiana	*Strobilomyia* spp.	Sweeney et al., 2001.

B. bassiana	*Musca domestica*	Floate et al., 2001.
B. bassiana	*Aphis gossypii* & *Myzus persicae*	Gillespie et al., 2001.
B. bassiana	*Galleria melonella*	Klingen et al., 1998.
B. brongniartii	Common cockchafer	Aregger, 1992; Fornallaz, 1992; Strasser, 1999; Zelger, 1993; Keller et al., 1997.
B. brongniartii	*Folsomia fimetaria, Hypogastrura assimilis* & *Proisooma minuta*	Dromph 2001, Dromph & Vestergaard, 2002.
Cladosporium herbarum	*Pulvinariella mesembryanthemi*	Petch, 1935.
C. lauri	*Coccus hesperidum*	Petch, 1935.
Culicinomyces clavisporus	Mosquito larvae	Galloway et al., 2001.
Fusarium episphaeria	*Saissetia coffeae*	Gabriel, 1968.
F. merismoides	*Delia foralis* & *Galleria melonella*	Klingen et al., 1998.
Metarhizium anisopliae	*Adoryphorus couloni* (Red headed pasture cockchafer)	Shah & Goettel, 1999; Rath et al., 1995.
M. anisopliae	*Oryctes rhinoceros* (Coconut palm rhinoceros beetle)	Carruthers & Soper, 1987.
M. anisopliae	Cockroaches	Andis, 1994; Fehrenbach, 1993; Milner, 1994; Kaakeh et al., 1996, 1997; Mohan et al., 1999
M. anisopliae	*Folsomia fimetaria, Hypogastrura assimilis* & *Proisooma minuta*	Dromph, 2001; Dromph & Vestergaard, 2002.
M. anisopliae & *B. bassiana*	Termites	Quarles, 1999; Milner & Staples, 1996; Rath & Tidbury 1996; Rosengaus & Traniello, 1997; Almeida et al., 1998; Alves et al., 1995, 1998; Boucias et al., 1996; Kramm et al., 1982; Milner et al., 1998a,b; Ramakrishnan et al., 1999; Rosengaus et al., 1998a,b; Rosengaus et al., 1999
M. anisopliae var. *acridum*	Grasshoppers & locusts	Milner, 1997; Arthurs & Thomas, 2001a,b; Bateman et al., 1998; Inglis et al., 1997c; Jenkins et al., 1998; Kooyman et al., 1997; Lomer et al., 1997a,b; Magalhaes et al., 2000; Thomas et al., 1996, 1998.
M. anisopliae	*Maruca virata* (Legume pod borer) *Clavigralla tomentosicollis* (Pod sucking bug)	Ekesi et al., 2002.
M. anisopliae	*Strobilomyia* spp.	Sweeney et al., 2001.
M. anisopliae	*Delia foralis* & *Galleria melonella*	Klingen et al., 1998.
M. anisopliae	*Anastrepha ludens*	Gutierrez et al., 2000.
M. anisopliae	*Folsomia fimetaria, Hypogastrura assimilis* & *Proisooma minuta*	Dromph, 2001; Dromph & Vestergaard, 2002.
Nomraea rileyi	Lepidopterous (noctuidae) pests of soyabean	Devi, 1995;. Fuxa, 1984; Kish & Allen, 1978; Boucias et al., 1984; Ignoffo et al., 1976.
P. fumosoroseus & *B. bassiana*	Aphids, thrips, whiteflies & spider mites.	Shah & Goettel, 1999.
P. fumosoroseus	Whiteflies (*Bemisia argentifolii* & *B. Tabaci*)	Shah & Goettel, 1999; Wraight & Carruthers, 1999; Lacey et al., 1996, 1999b; Osborne et al., 1990; Poprawski et al., 1998; Vidal et al., 1997a,b; Wraight et al., 1998, 2000.
P. fumosoroseus	*Bemisia* spp.	Humber, 1992; Hall et al., 1994; Sosa-Gomez et al., 1997; Faria and Wraight, 2001.
Smittium sp.	Mosquito larvae	Galloway et al., 2001.
Tolypocladium cylindrosporum	*Simulium vittatum*	Nadeau & Boisvert, 1994; Mason et al., 2001.
T. cylindrosporum	*Aedes aegypti, Ae. vexans* & *Culiseta inornata*	Galloway et al., 2001.
Verticillium lecanii	*Myzus persicae*	Hall & Burges, 1979.
V. lecanii	Insect pests of glasshouse crops	Hall, 1981; Hall & Burges, 1979.

V. lecanii	A variety of aphid species	Milner & Lutton, 1986; Rombach & Gillespie, 1988; Hsiao et al., 1992.
V. lecanii	Aphids, thrips & white flies	Helyer et al., 1992.
V. lecanii	Mites, nematodes & rusts	Verhaar et al., 1996.
V. lecanii	Other fungi	Askary et al., 1998.
V. lecanii	*Melanoplus sanguinipes*	Erlandson et al., 2001.
V. lecanii	*Myzus persicae, Myzus euphorbiae, Nasonovia ribis-nigri*	Gillespie et al., 2001.
V. lecanii	*Bemisia* spp.	Drummond et al., 1987; Faria & Wraight, 2001.
V. lecanii	*Delia noxia*	Hatting et al., 1999.
Aschersonia aleyrodis	*Bemisia* spp.	Faria & Wraight, 2001.
A. andropogonis	*Bemisia* spp.	Frasen, 1990.
A. cf. *goldiana*	*Bemisia* spp.	Lourencao et al., 1999.

Table 2.4. Effective doses of some entomogenous fungi used to control insects.

Fungus	Host/Target	Stage	Effective dose	References
Beauveria bassiana	*Bemisia argentifolii*	Nymphs	1-2.5 × 10^3 spores/mm^2 of surfaces of cucurbit leaves	Wraight et al., 2000.
B. bassiana	*Blissus antillus* (Hemiptera: Lygaeidae)	Eggs	5 × 10^6 conidia/ml.	Samuels et al., 2002.
B. bassiana	*Clavigralla tomentosicollis*	Eggs	10^6 conidia ml^{-1} of spray	Ekesi et al., 2002.
B. bassiana	*Diatraea saccharalis*	Adult	5.6 x 10^6 blastospores/ml 4.8 x 10^6 conidia/ml	Alves et al., 2002.
B. bassiana	*Maruca vitrata*	Eggs	10^8 conidia ml^{-1} of spray	Ekesi et al., 2002.
B. bassiana	*Plutella xylostella*	Larvae	5.23 x 10^6 conidia/ml	Furlong & Pell, 2001.
B. bassiana	*Tetranychus urticae*	Adult	12.4 x 10^6 blastospores/ml 12.6 x 10^6 conidia/ml	Alves et al., 2002.
B. bassiana	*Plutella xylostella* (diamond back moth)	Third instar larvae	499 conidia/mm^2 of leaf surface	Furlong & Pell, 2001.
Metarhizium anisopliae	*Blattella germanica* (German cockroaches)	Adults	8.96 × 10^9 conidia/m^2	Zurek et al., 2002.
M. anisopliae	*Blissus antillus* (Hemiptera:Lygaeidae).	Eggs	10^4 conidia/ml.	Samuels et al., 2002.
M. anisopliae	*Boophilus microplus*	Females	10^7 spores/ml	Frazzon et al., 2002.
M. anisopliae	*Ceratitis capitata*	Adults	5.1 × 10^3 conidia/fly	Castillo et al., 2000.
M. anisopliae	*Megalurothrips sjostedti*	Adults	10^{13} conidia ha^{-1}	Ekesi et al., 1998.
M. anisopliae	*Musca domestica* (house fly)	Adult & larval stages	10^8 & 10^7 conidia/ml	Barson et al., 1994.
M. anisopliae	*Clavigralla tomentosicollis*	Eggs	10^8 conidia ml^{-1} of spray	Ekesi et al., 2002.
M. anisopliae	*Maruca vitrata*	Eggs	10^8 conidia ml^{-1} of spray	Ekesi et al., 2002.
Nomuraea rileyi	*Spodoptera litura*	Adults	2 × 10^{11} conidia/liter of spray solution	Devi, 1994.
P. fumosoroseus	*Ceratitis capitata*	Adults	6.1× 10^3 conidia/fly	Castillo et al., 2000.
P. fumosoroseus	*Bemisia argentifolii*	Nymphs	1-2.5 × 10^3 conidia/mm^2 of surfaces of cucurbit leaves	Wraight et al., 2000.
Tolypocladium cylindrosporum	*Musca domestica* (house fly)	Adult & larval stages	10^8, 10^7, 10^6, & 10^5 conidia/ml	Barson et al., 1994.

Table 2.5: Percentage viability of live propagules of entomogenous fungi under different conditions of storage.

Organism	Temp. (°C)	Relative Humidity (%)	Duration (months)	Percent Viability	References
Beauveria bassiana	8 18 25	0	12	99 53 29	Clerk & Madeline, 1965
B. bassiana with attapulgite clay	26	-	12	78	Ward & Roberts, 1981
B. bassiana with kaolinite clay	26	-	12	70	Ward & Roberts, 1981
B. bassiana unformulated	26	-	12	6	Ward & Roberts, 1981
Paecilomyces farinosus	8	0	12	14	Clerk & Madeline, 1965
Hirsutella thompsonii commercial formulation.	24-26 4	-	6 12	1 100	McCoy & Couch, 1978

Chapter III:

MATERIALS AND METHODS

This investigation aimed at studies on diversity and ecology of microfungi found growing on insect and mite hosts and associated substrates collected from a few urban and rural localities of Kerala, Karnataka, Maharashtra and Goa, besides forests of Western Ghats in Goa State (Fig. 3.1) and their activity in relation to control of mosquito developmental stages, was carried out for a period of over two years, from March 1999 to June 2002.

The materials used and methods followed are elaborated in this Chapter.

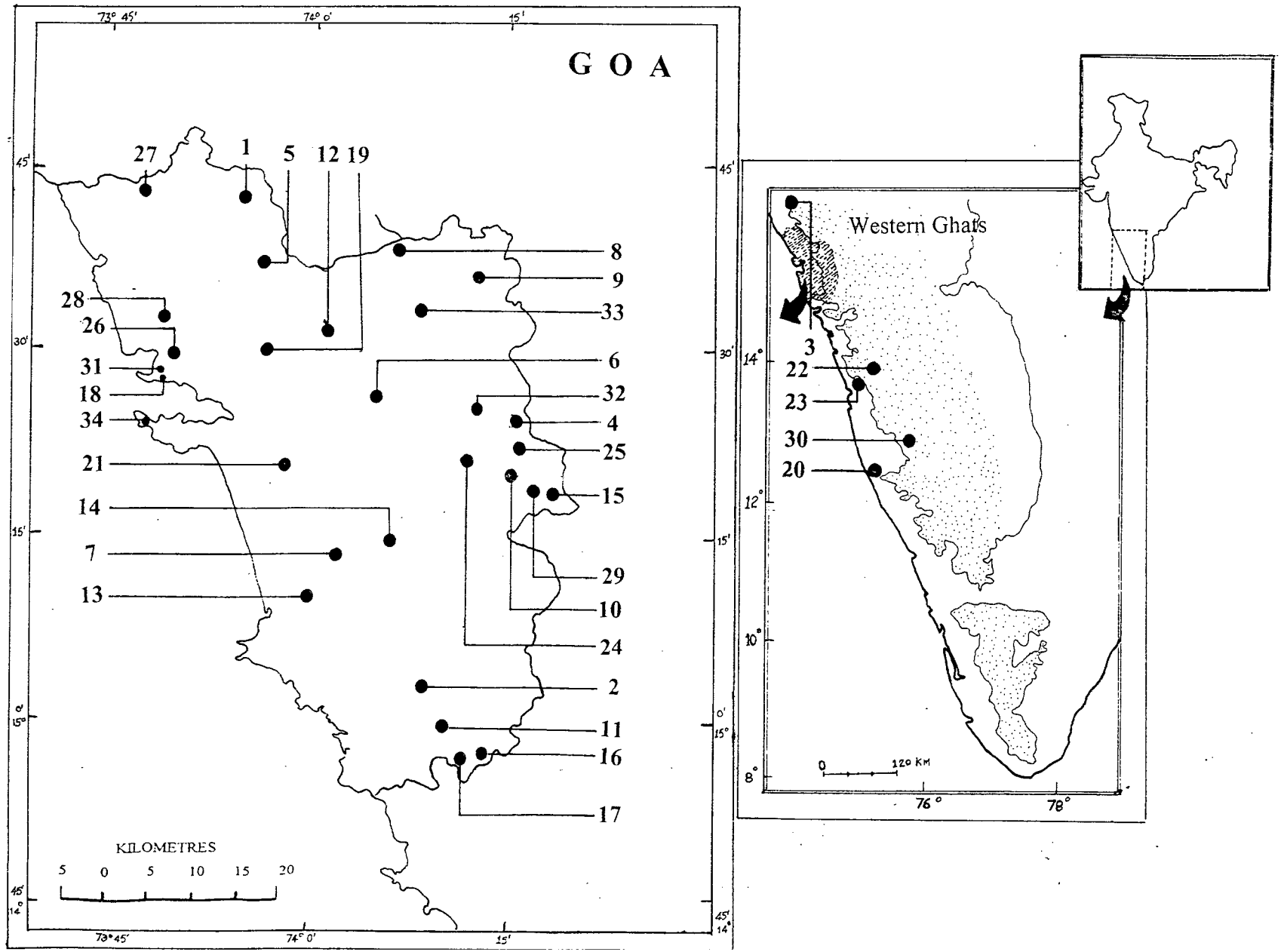
3.1. Sourcing and documentation of entomogenous fungi

3.1.1. Sampling sites and sample types

A variety of substrates collected from a wide range of habitats were scanned to recover maximum number and type of entomogenous fungi. The substrates included (i) live and dead larvae and dead adults of mosquitoes from breeding and resting sites from 8 localities in Goa (Bicholim, Cuncolim, Curchorem, Panaji, Pernem, Porvorim, Taleigao and Vasco), (ii) dead and live non-mosquito insects such as hemipteran, coleopteran, orthopteran, phasmida and dipteran and arachnids such as mites and spiders from foliage of forests and scrub-jungles of 24 localities in Goa (Alorna, Ambe, Anmod, Bondla, Chandreshwar, Chorlem, Codal, Colem, Cotigao, Cudnem, Curchorem, Dhudhsagar, Edda, Endrem, Goa University (GU) campus, Karmali, Kesarval, Melka, Molem, Pernem, Sonauli, Taleigao, Tambdi Surla and Valpoi), 1 locality in Maharashtra (Amboli), 3 localities in coastal Karnataka (Kodachadri, Kollur and Subrahmanya) and 1 site in Kerala (Kasaragod), (iii) healthy and later infected larval baits introduced, under laboratory set-up, into water samples collected from puddles, paddy fields and ponds from 7 sites in Goa

Fig. 3.1. Map of Western Ghats and Goa showing collection sites.

1. Alorna
2. Ambe
3. Amboli
4. Anmod
5. Bicholim
6. Bondla
7. Chandreshwar
8. Chorlem
9. Codal
10. Colem
11. Cotigao
12. Cudnem
13. Cuncolim
14. Curchorem
15. Dhudhsagar
16. Edda
17. Endrem
18. GU campus
19. Karmali
20. Kasaragod
21. Kesarval
22. Kodachadri
23. Kollur
24. Melka
25. Molem
26. Panaji
27. Pernem
28. Porvorim
29. Sonauli
30. Subrahmanya
31. Taleigao
32. Tambdi Surla
33. Valpoi
34. Vasco


GOA
73° 45'
74° 0'
15'
45'
30'
15'
14°
27
1
5
12
19
8
9
33
6
32
4
25
15
29
10
24
2
11
16
17
28
26
31
18
34
21
14
7
13
KILOMETRES
5 0 5 10 15 20
Western Ghats
3
22
23
30
20
14°
12°
10°
8°
0 120 KM
76°
78°

(Bondla, Cotigao, Cudnem, Curchorem, GU campus, Karmali and Taleigao), and (iv) healthy and later infected mosquito larval baits used in simulation float chambers maintained in ponds at GU campus and Panjim and slow running streams at Pernem. Baits used in the latter two exercises were 2nd instar larvae of *Culex quinquefasciatus*, reared in the laboratory. The mosquito breeding sites examined were well, pond, ditches, puddle, paddy field, slow flowing stream, curing water at construction site, overhead water-tank, sump, open septic tank, drain, abandoned container, barrel, abandoned tyres, etc. and transient rain water pools on building terraces (Plate: I-IV). The localities and habitats visited and substrates and samples scanned are given in Table. 3.1.

Typical of coastal belts in southern India, ambient temperature at the collection sites ranged between 22-35°C, the temperature seldom falling below 19°C. Mean annual rainfall was 200-300 cm and humidity ranged between 60-95% (Sourced from Mat. Dept., Panaji, Goa)

3.1.2. Sampling methods

Live mosquito larvae and pupae were collected from breeding sites following the methods described by Service (1976). A plastic container with 9 cm diam at the base, 11.5 cm wide in the middle and of a height of 7cm (Plate: VI-a,b,c & d) was used as a 'dipper' to collect mosquito larvae and pupae that appeared in small or large pool of water and other aquatic habitats. Wherever necessary, a 2 m long plastic stick was attached to the dipper. The dipper was gently scooped through surface water or lowered to subsurface level and lifted out. In very shallow waters, the dipper was pressed firmly to the bottom and lifted. From small pools three dipper-samples whereas in large-sized ponds, up to 20 dipper samples were taken.

Dead mosquitoes of *Culex quinquefasciatus, Anopheles stephensi* and *Aedes aegypti* were collected with help of an aspirator from adult mosquito cages in the insectory and from their resting sites. Dead and live non-mosquito insects such as hemipterans, coleopterans, orthopterans, dipterans and arachnids such as mites and spiders from litter and foliage were collected from forests at altitudes ranging from sea level up to approx. 650 m (Plate: IV). Humid places on either side of streams and rivers were scanned for infected insects or spiders. Water samples were also gathered from puddles, paddy fields and ponds without mosquitoes.

Healthy 2nd instar larva of *Culex quiquefasciatus* was used as bait in simulation float chambers to source entomogenous fungi in their natural habitats. A 500 ml plastic dish (9 cm diam at base and 11.5 cm diam in the middle and 7 cm height) with 2 windowsills of 6 x 3 cm covered with mesh and fitted to a wooden plank served as a float chamber (Plate: V-a). The float was tied to a stationary object in the field by a nylon rope and allowed to drift in water for 2-3 days. About 20 larvae were placed in each float chamber (Plate: V-b,c & d).

3.1.3. Collection of samples in the field

Live mosquito larvae collected using the dippers were concentrated by decanting excess water and subsequently transferred into small screw cap plastic jars (Plate: VI-a,b,c & d). Adult mosquitoes were transferred to 15 ml test tubes and plugged with non-absorbent cotton. Insects and arachnids were placed in clean polythene bags of 18 x 14 cm size or plastic boxes depending on size of the sample. Moribund insects found clasping to leaves and fungal fructifications were collected along with host foliage, twigs or litter in polythene bags. Larval baits used in simulation float chambers were transported to laboratory in plastic jars.

3.1.4. Processing in the laboratory

Mosquito larvae of different developmental stages were maintained in 250 ml water in small plastic containers. These were administered with larval feed (Baker's yeast and powdered dog-biscuit in 1:3 ratio) at regular intervals. The mouth of the container was covered by a mosquito net. A 2 cm diam hole was made in the centre of mosquito net so as to enable insertion of a dropper or aspirator to pick up larvae or adults. The hole was covered by a cotton plug (Plate: VI-e). The set up was observed every second day for sluggishness, mortality or associated symptoms until all the larvae/pupae either emerged as adults or dead. The sluggish or dead larvae/pupae were observed under a stereoscope (Zeiss Stemi 1000) and a light microscope (Olympus CH30) for signs of fungal infection. Infected material was used as source of entomogenous fungi.

Dead/live non-mosquito insects and arachnids were examined under the microscope for the signs of fungal infection. Infested hosts were used as source of entomogenous fungi.

Twenty healthy 2nd instar larvae of *C. quinquefasciatus*, reared in insectory, were introduced as baits into a small plastic container containing 300 ml of water sample. Larval feed was provided at intervals. For each water sample 5 replicates were maintained. The mouth of the container was covered with mosquito net so that emerged mosquito remained in the container itself. A 2 cm diam hole was made in the centre of the net to enable insertion of a dropper or aspirator and to pick up the larvae or adults as the case may be. The hole was plugged by non-absorbent cotton. The set up was observed every second day under the microscope for sluggishness, mortality or associated symptoms until all the larvae/ pupae emerged as adults. The material found infested was used as a possible source of entomogenous fungi.

The larvae from simulation float chambers were transferred to fresh water containers and fed with larval feed. The infested larvae were used as possible source of entomogenous fungi.

3.1.5. Culture media for isolation and maintenance fungi

Malt extract (5%) agar (MEA), Sabouraud dextrose (6.5%) agar (SDA) and Corn meal (1.7%) agar (CMA) media were used for isolation of fungi. A mixture of antibiotics consisting of bacitracin 0.02 g, neomycin 0.02 g, penicillin G 0.02 g, polymixin 0.02 g, streptomycin 0.02 g and terramycin 0.04 g dissolved in 10 ml of sterile distilled water added to 1 L of medium was used in isolation plates to prevent bacterial growth. Malt extract (2.5%) agar and Sabouraud dextrose (3%) agar were used for maintenance of cultures in slants.

3.1.5.1. Malt extract (5%) agar (MEA) medium

Five g of dehydrated malt extract (Himedia Laboratories Pvt. Ltd.) and 20 g of agar (E.Merck India Ltd.) were boiled in 1000 ml of distilled water to dissolve, pH adjusted to 6.0 and sterilized in an autoclave at 121°C for 15 minutes.

3.1.5.2. Corn meal (1.7%) agar (CMA) medium

Corn meal agar, 17 g (Himedia Laboratories Pvt. Ltd.) in 1000 ml distilled water boiled to dissolve completely and sterilized in an autoclave. It contained ingredients, viz., corn meal infusion from 50 gL^{-1}; Agar, 15 gL^{-1} and final pH of 6.0 ± 0.2.

3.1.5.3. Sabouraud dextrose (6.5%) agar (SDA) medium

Sabouraud dextrose agar, 65 g (Himedia Laboratories Pvt. Ltd.) was boiled in 1000 ml of distilled water to dissolve and sterilized in an autoclave. The ingredients included mycological peptone, 10 gL^{-1}; Dextrose, 40 gL^{-1} and Agar, 15 gL^{-1} adjusted to pH of 5.6 ± 0.2.

3.1.6. Isolation of fungi

Source materials were washed thoroughly in sterile distilled water and plated at equidistant in plates containing MEA, CMA and SDA with antibiotics. The plates were incubated at a temperature of 23-25°C and observed every second day for growth of fungi. Small portions of fungal colonies emerging out in different zones were aseptically transferred into fresh MEA plates cut into 9 equal sectors and incubated for 7 days (Plate:VII). Growing colonies were compared and dissimilar ones were transferred to MEA slants. Part samples were placed in sterile moist chamber and examined for sporulation.

3.1.7. Documentation of fungi

3.1.7.1. Microscopic observations

Description of each species was based on material collected and/or cultures established.

Semi-permanent slides of fungi from colonies with sporulating structures such as sporangiophores and sporangia (Zygomycetes), ascocarp, asci and ascospores (Ascomycetes), conidiophores and conidia (Hyphomycetes), pycnidia, conidiogenous cells and conidia (Coelomycetes) were prepared using water, lactophenol or lactophenol and cotton blue as mountant. The edges of cover slip were sealed with DPX mountant. The slides were numbered and maintained in the Herbarium of Botany Department, Goa University. Illustrations were made using camera-lucida drawing tube attached to a binocular microscope. Photomicrographs were taken using an automatic camera (Nikon Make) fitted to the microscope. Fungi growing on hosts were photographed using an automatic SLR camera fitted to the stereoscope (Plate: VIII-XIII).

3.1.7.2. Identification

Each fungal colony was considered as a 'morphotype'. The isolates were grouped into 'sporulating' and 'nonsporulating' forms. Sporulating structures were considered as features for identification of fungi. Using standard taxonomic keys and monographs (Carmichael et al., 1980; Domsch et al., 1980; Tzean et al., 1997), isolates were identified and assigned to respective genera and species. Along with morphological features, ecological and cultural characters of each taxonomic entity were compiled. Descriptions of fungi were written in a standard diagnostic form.

3.1.7.3. *Ex situ* maintenance and preservation of fungi

A representative pure culture of each identified species or morphotype was maintained in the collection of 'Goa University Fungus Culture Collection' (GUFCC).

3.1.7. 4. Herbarium preparation

Dried herbarium material of fungi in culture or on host/substrate was prepared by air-drying the specimen in a drier. The specimen was assigned with an accession number, well-packed in paper bags, sealed, labeled and maintained in the herbarium of Department of Botany, Goa University. Holotypes of new taxa were deposited at the International Mycological Institute, Cabiscience, U.K. Where the new taxon is based on a live fungus, dried and dead culture mats in herbarium sheets were maintained in the Herbarium of the IMI, U.K. and/or the GUBH, Goa University, so as to satisfy the nomenclatural rules.

3.2. Screening fungi for biocontrol potential against mosquito larvae

3.2.1. Composition of media used for mass production of fungal conidia

3.2.1.1. Malt Czapek agar (MCzA): Czapek solution A, 50 ml; Czapek solution C, 50 ml; sucrose, 30.0g; Malt Extract, 40g; Agar, 20.0g; in 900 ml distilled water. Malt Extract was dissolved by boiling in distilled water. Sucrose was added followed by Czapek solution A

& C. At the end agar was added and dissolved. pH was adjusted to 5.5. Medium was sterilized by autoclaving at 121°C for 15 minutes. [Cz solution A: Sodium nitrate $NaNO_3$, 4.0g; Potassium chloride, 1.0g; Magnesium sulphate, $MgSO_4.7H_2O$, 1.0g; Ferrous sulphate, $FeSO_4$. 20.0 mg; all dissolved in 100.0 ml of distilled water; Cz solution C: Dipotassium hydrogen phosphate, K_2HPO_4, 2.0g dissolved in 100.0 ml of distilled water].

3.2.1.2. Corn Meal agar: As in 3.1.5.2.

3.2.2. Preparation of spore suspension

Test fungus was grown in plates containing 1.7% CMA and MCzA at 22-25°C for 15-20 days. Spores were harvested by flooding the colony with 10ml of sterile 0.05% tween 80 saline solution and agitating with a glass rod or a sterile brush. The spore suspension was dispensed to a 30 ml sterile screw cap tube and vortexed for two minutes. Spore concentration was determined using a haemocytometer. In case of non-sporulating forms, mycelial mass was used without any estimation of dose.

3.2.3. Preliminary bioassay

Spore suspension was transferred to a 500 ml plastic container and made up to 50 ml with tap water. A final concentration of 10,000-100,000 conidia/ml was maintained. Twenty healthy late 2^{nd} instar larvae of *Culex quinquefasciatus*, raised in the insectory (Plate: XIV-a & b), were introduced into the container. Observations were made for features such as sluggishness, mortality and other associated pathogenic symptoms of mosquito for 5 days at intervals of 24 h. As in other toxicological investigations, effect produced in mosquito larvae was measured as 'all or none' principle, considering severely affected larvae also as dead. Since mosquito larvae being sensitive to starvation, larval feed was provided during the bioassay. Control was maintained with 50ml of tap water (Plate:

XIV-c). Experiment was done with three replicates and repeated twice. Fungi leading to more than 50% mortality were considered as possible biocontrol agents of mosquitoes and referred to as promising isolates.

3.2.4. Corrected mortality

When control mortality was more than 20%, the bioassay was discarded and repeated in order to correlate and confirm the results obtained. The control mortality when ranged between 5-20%, the 'corrected mortality' was calculated using Abbott's formula (Abbott, 1925)

$$\text{Corrected Mortality} = \frac{\text{Observed mortality (\%) - Control mortality(\%)}}{\text{100 - Control mortality (\%)}} \times 100$$

Corrected mortality was not calculated whenever the control mortality remained below 5%, (Plestina, 1984).

3.3. Assessment of mosquito-larvicidal activity of candidate species

Dose-response relationships of conidia of candidate isolates against mosquito larvae were determined in two successive steps. In the first, standardized bioassay were done in 250 ml water in 500 ml containers. Four representative promising isolates, viz. *Acremonium* sp. (E29), *Gliocladium* sp. (E16), *Penicillium* sp. (E9) and *Trichoderma* sp. (C54), were tested for dose-response relationship of mosquito-larvicidal activity against early 3rd instar larvae of *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti.* In the second step, standardized bioassay were done in 1500 ml water in 4000 ml containers. *Penicillium* sp. (E9) and *Gliocladium* sp. (E16) were evaluated for their potential against early 3rd instar larvae of *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti.*

3.3.1. Mass production of conidia

In vitro production of conidia in large quantity was done by modifying the still-shallow-liquid-culture technique of Jenkins and Goettel (1997). Sporulation occurred at the liquid-gas interface in this setup. Fifty ml of the medium was poured into culture bottles with a surface area of about 115 cm^2 when kept horizontally (Plate: XIV-d).

3.3.1.1. Media for production of conidia

Malt Czapek (MCz) and corn meal (CM) liquid media with and without sugar were used for mass production of conidia. For MCz broth, standard ingredients were used. In case of CM or CM sugar liquid media, locally available corn and sugar were used.

Malt Czapek (MCz) liquid medium: Malt extract powder-40g; sucrose-30g; Czapek Solution A-50ml; Czapek Solution C-50ml; distilled water-900ml. Malt extract was dissolved in 900ml of distilled water by boiling. Sucrose and Cz. solution A & C were added and pH adjusted to 5.5. Fifty ml of each medium was dispensed into culture bottles and sterilized in an autoclave.

Corn Meal Sugar medium: Whole corn was finely powdered in a blender and corn meal infusion was prepared by boiling the powder in water. Sugar was added to the infusion before autoclaving. One percent corn meal liquid medium: corn meal infusion from 10g of corn powder made up to 1000 ml with distilled water; 4% corn meal liquid medium: corn meal infusion from 40g of corn powder made up to 1000 ml with distilled water; 4% Corn meal Sugar liquid medium: corn meal infusion from 40g of corn powder made up to 1000 ml with distilled water with 30 g sugar. pH was adjusted to 5.5 in each case. Fifty ml of each medium was dispensed to culture bottles and sterilized in an autoclave.

3.3.1.2. 'Starter inoculum' and preparation of spore suspension

Using a sterile needle, candidate fungus was point-inoculated into the culture bottle and incubated for 7 days at room temperature. Pre-sterilized 0.05% tween 80 was flooded into the culture bottle and thoroughly shaken to harvest the conidia. The spore suspension was filtered through 250, 100 and 50 μm sieve plates and diluted to a rough concentration of 10,000 conidia/ml. This was used as 'starter inoculum' for mass production of spores.

One ml of starter inoculum was added to each culture bottle and incubated for 15-18 days. The bottle was flooded with 0.05% tween 80. Spores were harvested, thoroughly washed in sterile distilled water and concentrated by centrifugation (Plate: XIV-e). Spore concentration was estimated using a haemocytometer. Production of conidia in different media was compared.

3.3.2. Standard Bioassay in 250 ml of water

A spore concentration gradient of 1×10^2, 1×10^3, 1×10^4, 5×10^4 and 1×10^5 conidia/ml was prepared leading to a final volume of 50 ml in 500 ml containers. Twenty five healthy early 3rd instar larvae of *Culex quinquefasciatus* were introduced into each container. Control was maintained. Larval feed was provided. Mortality and sluggishness were observed at 24, 48, 72, 96 and 120 h. Range of concentration which provides 0-100 % mortality was determined. Similar experiments were also done with *Anopheles stephensi* and *Aedes aegypti*.

A 'dosage range' for spores was decided based on the results of 'dose range testing' so as to cover 0-100% mortality. Accordingly experiments were set up with 5 replicates for each dose planned, in a final volume of 250 ml in plastic containers with a surface area of

~70 cm^2. Five controls were maintained each with 250 ml of water. Twenty five healthy early 3rd instar larvae of *Culex quinquefasciatus* were introduced to each container. Larval feed was provided. Mortality and sluggishness were observed and noted down for 24, 48, 72, 96 and 120 h (WHO, 1992). LD_{50} values were determined using the Prism Demo Software from www.graphpad.com. The data were analyzed using sigmoid curve fitting with variable slope.

For *Penicillium* sp. (E9), standard bioassays were carried out against 3rd instar larvae of *Culex quinquefasciatus, Anopheles stephensi* and *Aedes aegypti* at the Microbial Containment Complex, National Institute of Virology, Pune. Susceptibility of Goa and Pune strains to the spores of *Penicillium* sp. (E9) were compared by linear regression using Prism Demo Software from www.graphpad.com.

3.3.3. Standard Bioassay in 1500 ml of water

As a next step towards effective candidate species, bioassays were done in large volume of water in shallow plastic trays. Except for a reasonable increase in the dosage, experiments were performed as described in the section 3.3.2. Two positive isolates, viz., *Penicillium* sp. (E9) and *Gliocladium* sp. (E16), were further evaluated against early third instar larvae of *Culex quinquefasciatus, Anopheles stephensi* and *Aedes aegypti.* LD_{50} values were determined using the Prism Demo Software from www.graphpad.com. The data were analyzed using sigmoid curve fitting with variable slope.

3.4. Larvicidal activity of culture filtrate of promising isolates

Cell-free extracts were tested for their insecticidal potential after 4, 7 and 20 day growth of fungal cultures.

3.4.1. Medium used for culturing fungi

Sabouraud dextrose liquid medium: Sabouraud dextrose broth powder (M033, Himedia Laboratories Pvt. Ltd.) 30 g was boiled in 1000 ml of distilled water. The medium contained ingredients, viz., special peptone, 10 gL^{-1}; Dextrose, 20 gL^{-1}; and final pH of 5.6 ± 0.2. Fifty ml of the medium was dispensed into culture bottles and sterilized in an autoclave.

3.4.2. Preparation of cell-free extract

Promising fungi were grown in plates containing 6.5% SDA. Culture blocks of approximately 1 cm^2 were inoculated into the broth. Four sets of cultures were maintained for each fungus. One bottle each was picked up at the end of 4^{th} day, 7^{th} day and 20^{th} day for further processing. The culture was macerated using mortar and pestle, sonicated in a sonicator (Bandelin Make) and centrifuged at 10000 rpm to get a clear supernatant containing mixture of metabolites.

3.4.3. Bioassay of cell-free extract

Ten ml of cell free-extract was diluted to 50 ml by adding water. Ten healthy *Culex quinquefasciatus* larvae were introduced into each container. Simultaneously, two controls containing 10 ml of medium diluted to 50 ml were maintained. Observations were made at 24 and 48 h. Results were tabulated in percentage mortality for cell-free extract from 4, 7 and 20 day old cultures.

3.5. Detection of enzymes of entomogenous fungi

All viable isolates were tested for production of enzymes by determining the ability of cultures to grow on specific substrates in defined and semi-defined media. (Paterson and Bridge, 1994). These included qualitative analysis for protease, esterase and chitinase.

3.5.1. Fatty acid esterase (presumptive test) activity (Sierra, 1957)

Principle: Medium contained Tween 80 as Carbon source, bromocresol purple as a pH indicator and a calcium salt. Tween 80 is a mixture of fatty acids, predominantly elaidic, linoleic and palmitic, and the final pH of the medium is acidic. Growth on fatty acids results in rise of pH, turning the medium blue-purple, and further in the formation of insoluble calcium salts such as calcium stearate which appear as a white ring in the medium around the colony. It was noted that this is not a confirmative assay for lipase activity.

Medium: Mycological peptone 10.0 g; NaCI 5.0 g; $CaCI_2.2H_2O$ 0.1 g; Bromocresol purple-25.0 mg; agar-15.0 g; distilled water to 1 L. pH was adjusted to 5.4 and medium dispensed into 90 ml aliquots.

Tween solution -A 10% (v/v) aqueous Tween 80 solution was prepared by slowly adding 10 ml Tween 80 to 90 ml warm (60- 70°C) distilled water. Agar and Tween solution were sterilized in an autoclave. When the media had cooled to 65-70°C, 10 ml Tween solution was added to each 90 ml basal medium and mix. The completed medium was poured into petri dishes (10 ml/plate). The plates were point-inoculated and incubated for up to 14 days.

3.5.2. Gelatin hydrolysis (presumptive protease) activity

Principle: Gelatin medium was used as a presumptive test for protease. Culture medium was solidified with gelatin. Gelatin is known to be hydrolyzed by proteolytic enzymes; leading to liquefaction of the medium. Gelatin was chilled (4°C for 30 minutes) after the incubation period before the test was read. Liquefaction of gelatin was read as positive for protease activity.

Medium: Czapek solution A-50.0 ml; Czapek solution C-50.0 ml; Zinc solution-1.0

ml, Copper solution-1.0 ml; sucrose-10.0 g; gelatin-120.0 g; distilled water to 1 L. The medium was prepared in warm water (up to 50-60°C). Gelatin was stirred slowly in small amounts until all dissolved. The warm medium was dispensed into test tubes (7 ml/test tube) and sterilized in an autoclave. On cooling, the tubes were inoculated and incubated up to 21 days.

3.5.3. Chitin utilization activity (presumptive chitinase)

Principle: Chitin was added as a sole carbon source replacing sucrose in CDA medium. Ability of the fungus to grow in the medium by utilizing chitin was read as positive chitinase activity.

Medium: Chitin-10g; Sodium nitrate, $NaNO_3$-2g; Dipotassium hydrogen phosphate, K_2HPO_4-1g; Magnesium sulphate, $MnSO_4$- 0.5g; Potassium chloride, KCl-0.5g; Ferrous sulphate, $FeSO_4$- 0.01g; agar-15g; distilled water to 1 L. Chitin was slowly stirred in small quantities of 1 molar acetic acid to get a pasty texture until all the chitin was suspended to colloidal consistency. Other components of the medium were dissolved separately. Chitin was added, total volume of the medium was made up to 1000 ml and pH was adjusted to 5.5 before autoclaving. The medium was poured into petri plates, point-inoculated and incubated for up to 14 days. Results of enzyme activity were tabulated as positive or negative for each promising culture.

3.6. Preliminary field trials of promising isolate

Experiment was conducted in curing water inside a construction site near Bhavishya Nidhi Bhavan, Patto, Panjim. Four litres of curing water from the breeding habitat was transferred to each of 6 containers (60 x 40 cm). One hundred 3rd and 4th instar larvae of *Anopheles stephensi* were sampled and transferred to each container and a dose of ~80

million spores (=20,000 spores/ml) of *Penicillium* sp. (E9) was sprayed to each except control. Observations were made at 24 and 48 hours and percentage mortality was determined. Dead larvae and fungal mass were transferred to laboratory in screw cap containers for microscopic observations.

3.7. *In situ* observation of life cycle of species of *Aschersonia* and *Hirsutella*

Sampling was done from a pre-defined site at Sri Bhagawan Mahaveer Wildlife Sanctuary, Molem, Goa, for a period of one year from June 2001 to May 2002 at regular intervals (Table 3.2). The vegetation at collection site ranged from moist-deciduous and semi-evergreen.

The collection site was a 2 km stretch of riparian habitat along a slow flowing stream. Vegetation composition in the proximity included (i) Tree species: *Abrus precatorius* Linn. (F: Fabaceae); *Alstonia scholaris* (Linn.) R.Br.(F: Apocynaceae); *Careya arborea* Roxb. (Barringtoniaceae), *Cinnamomum zeylanicum* Bl. (F: Lauraceae); *Dillenia indica* Linn. (F: Dilleniaceae); *Flacourtia montana* Grah. (F: Flacourtiaceae); *Hopea ponga* (Dennst.) Mabb. (F: Dipterocarpaceae); *Lagerstroemia parviflora* Roxb. (F: Lythraceae); *Mangifera indica* Linn. (F: Anacardiaceae); *Morinda citrifolia* Linn. (F: Rubiaceae); *Myristica malabarica* Lamk. (F: Myristicaceae); *Olea dioica* Roxb. (F: Oleaceae); *Pongamia pinnata* (Linn.) Pierre (F: Fabaceae); *Pterospermum acerifolium* Willd. (F: Sterculiaceae); *Saraca asoca* Roxb. Dc Wilde (F: Caesalpiniaceae); *Syzygium cumini* (Linn.) Skeels (F: Myrtaceae); *Terminalia bellirica* (Gaertn.) Roxb. (F: Combretaceae); *T. crenulata* Roth. (F: Combretaceae); *T. paniculata* Roth. (F: Combretaceae) and *Xylia xylocarpa* Taub. (F: Mimosaceae) (ii) Climbers and lianas: *Entada pursaetha* D.C. (F: Fabaceae) and *Gnetum ula* Brongn.(F: Gnetaceae) (iii) Shrubs: *Calotropis gigantea* (Linn.) R. Br. (F: Asclepiadaceae), *Calycopteris floribunda* (Roxb.) Lamk. (F: Combretaceae),

Cassia tora Linn. (F: Caesalpiniaceae), *Holarrhena antidysenterica* (Roth.) A. DC. (F: Apocynaceae); *Holigarna arnottiana* (Wt. & Arn.) Hook.f. (F: Anacardiaceae); *Ixora brachiata* Roxb. (F: Rubiaceae); *Ixora coccinia* Linn. (F: Rubiaceae); *Leea macrophylla* Roxb. Ex Hornem (F: Hornaceae); *Microcos paniculata* Linn. (F: Tiliaceae); *Psychotria dalzelli* Hook.f. (F: Rubiaceae); *Rauvolfia serpentina* (Linn.) Benth. ex Kurz (F: Apocynaceae) and *Strobilanthus ixiocephalus* Benth. (F: Acanthaceae), (iv) Cane and bamboos: *Calamus thwaitesii*.Becc. (F: Arecaceae); *Dendrocalamus strictus* (Roxb.) Nees (F: Poaceae) and *Bambusa arundinacea* (Retz.)Willd (F: Poaceae). Shrubs and herbs understory is abundant in this area.

Plant hosts such as *Calycopteris floribunda, Dillenia indica, Holarrhena antidysenterica, Hopea ponga, Leea macrophylla, Morinda citrifolia* and *Strobilanthus ixiocephalus* were found to harbour the fungi regularly and in sufficient numbers. Hence only these plants were observed for the fungi. *A. aleyrodis* and *A. badia* only occasionally seen in other plants. Equal number of leaves were collected randomly from a particular host plant in each collection. Ten individual plants were observed from each plant host species. Number of and diam. of colonies were recorded for each collection. Data were pooled together from all host plants to calculate the mean number of colonies per host plant, and mean colony diam (mm) of each fungus. Line graphs were plotted with mean number of colonies and mean colony diam (mm) against dates of collection for a year duration to observe variation along the season.

Sections of exosclerotium were done in a cryostat and mounted in lactophenol. Temporary mounts were prepared by sealing the coverglass with DPX. Representative herbarium specimens were prepared for each sampling. Illustrations of different stages of development of fruiting body were made.

Table 3.1. Localities and habitats visited and substrates and samples scanned.

Places	No of visits	Substrates/Host				Habitats
		i	ii	iii	iv	
Alorna	4		✔			ii) Scrub jungle and forest
Ambe	1		✔			ii) Forest
Amboli	1		✔			ii) Forest
Anmod	4		✔			ii) Forest
Bicholim	5	✔				i) Sump tank, cement tank, Overhead tanks, Curing water, wells, ponds, transient rainwater pools
Bondla	6		✔	✔		ii) Forest, National Park and water insects iii) Water from a pond in dense forest
Chandreshwar	1		✔			ii) Scrub jungle
Chorlem	2		✔			iii) Forest, foam trapped water insects from the stream
Codal	1		✔			ii) Cashew plantation
Colem	1		✔			ii) Forest, riparian habitat
Cotigao	7		✔	✔		ii) Wildlife Sanctuary, dense forest iii) Puddle in a scrub jungle, paddy field, stagnant water pool
Cudnem	1		✔	✔		ii) Degraded forest, scrub jungle in the mining area iii) Water from a spring
Cuncolim	5	✔				i) Curing water, masonry tank, barrels, containers.
Curchorem	5	✔	✔	✔		i) Curing water, paddy field, drain, cement tank, drain, abandoned tyres, bottles ii) Scrub jungle iii) Water from paddy field
Dhudhsagar	1		✔			ii) Dense forest, water falls, riparian habitat
Edda	1		✔			ii) Scrub jungle, dense forest.
Endrem	1		✔			ii) Scrub jungle, dense forest.
GU campus	5		✔	✔	✔	ii) University glass house, scrub jungle around a big water reservoir iii) Water from ponds, water reservoir iv) Ponds
Karmali	1		✔	✔		ii) Vegetable field and scrub jungle iii) Water from lake and ponds
Kasaragod	2		✔			ii) Mixed plantation
Kesarval	2		✔			ii) Mixed plantation, scrub jungle
Kodachadri	1		✔			ii) Dense shola forest
Kollur	1		✔			ii) Forest, riparian habitats
Melka	3		✔			ii) Dense forest
Molem	12		✔			ii) Dense forest, riparian habitats
Panaji	3	✔			✔	i) Curing water, asbestos tank, accumulated water over terrace iv) Water logged area in batlem

Pernem	6	✔	✔		✔	i) Tank, well, bottles, overhead tanks, abandoned tyres, open septic tank, waste disposal tanks of distilleries ii) Scrub jungle, paddy field, plantation iv) Slow flowing stream, paddy field
Porvorim	4	✔				i) Water collection, curing water, ditches
Sonauli	1		✔			ii) Dense forest
Subrahmanya	1		✔			ii) Dense shola forest
Taleigao	3	✔	✔	✔		i) Pond, puddle, paddy field, ditches etc. ii) Paddy field bunds, vegetable fields iii) Water from a pond
Tambdi Surla	3		✔			ii) Dense forest, riparian habitat
Valpoi	2		✔			ii) Plantation, forest
Vasco	7	✔				i) Drain, containers, barrel, abandoned containers, barrels, overhead tanks etc.

Note: i -Mosquito larvae and pupae; ii - other insects and arachnids; iii -larval baits in water samples; iv -larval baits used in simulation floats.

Table: 3.2 Collection dates for *In situ* observation of life cycle of species of *Aschersonia* and *Hirsutella*

13.06.2001	21.07.2001	05.08.2001
16.09.2001	07.10.2001	21.10.2001
18.11.2001	16.12.2001	13.01.2002
26.02.2002	24.03.2002	16.06.2002

Chapter IV:

RESULTS AND DISCUSSION

This investigation, carried out during March 1999 to June 2002, was aimed at collection of information on taxonomic diversity and ecology of entomogenous fungi found on a variety of insects, mites and other-related substrates from various localities in Goa, Karnataka, Kerala and Maharashtra of India, and elucidation of activity of these fungi on early developmental stages of mosquito vectors.

4. Results

Results obtained from the study are given under following Parts.

PART I: Diversity and taxonomy of entomogenous fungi.

PART II: Screening fungi for biocontrol potential against mosquito larvae.

PART III: Assessment of mosquito-larvicidal activity of candidate species.

PART IV: Activity of secondary metabolites of promising isolates.

PART V: Enzymes of entomogenous fungi.

PART VI: Preliminary field trials of a promising isolate.

PART VII: Field observations on *Aschersonia* and *Hirsutella*.

PART I:

4.1. Diversity and taxonomy of entomogenous fungi

From a large collection of samples, 286 isolates of fungi were recovered in pure culture (Table. 4.1; Fig. 4.1.1).

A total of 3672 mosquito larvae/pupae were collected and maintained in the laboratory till they emerged as adults. During the incubation, 389 larvae/pupae were found either passive or dead. Microscopic observation of altered larvae revealed opaque haemocoel in the body. In some cases ramification of fungal hyphae were observed. From the dead/inactive mosquito larvae/pupae, 57 fungal isolates were recovered in pure culture. Amongst these, 26 were from immature stages of *Anopheles* sp., 27 from *Culex* sp., 1 from *Aedes* sp. and 3 from exuviae of unidentified mosquito larvae (Table 4.1, Fig. 4.1.2). None of mosquito adults observed showed fungal infection.

A large number of aphids, ants, scale insects, other insects and arachnids were gathered from plant foliage and litter. Of the 19955 insects and arachnids, 2355 were sluggish, passive and moribund and yielded 222 fungal isolates in pure culture. Amongst these, immature stages of homopterans yielded a maximum number of 43 isolates (19.36%), followed by 32 from scale insects (14.4 %), 28 from unidentified insects (12.6 %), 26 from aphids (11.7 %) and so on (Table 4.1, Fig. 4.1.3).

Maximum number of insects infected by fungi were recovered from insects found on plant hosts such as *Hopea ponga, Chromolaena odorata* and *Holarrhena antidysenterica* accounting for over 27% of all fungi recovered in culture from non-mosquito insects and arachnids. Plant host-wise association of these isolates is presented in (Table. 4.2, Fig. 4.1.4)

Seven fungal isolates were obtained by challenging second instar larvae of *Culex quinquefasciatus* in water samples collected from 17 habitats containing decaying vegetation. However, larval baits used in simulation experiments did not yield any fungus (Table 4.1).

Entomogenous fungi were sourced from 34 localities. Amongst these, Molem was the most visited and yielded a highest number of 47 (16.43 %) fungal isolates followed by Tambdi Surla 25 (8.74 %), Taleigao 17 (5.94 %), Kollur 17 (5.94 %) and others (Table 4.3, Fig. 4.1.5).

4.1.2. Identification

Of the 286 fungal isolates obtained, 216 were sporulating and 70 were nonsporulating. In all, 188 isolates were assigned to 20 genera of fungi. They belonged to Zygomycotina (8), and Deuteromycotina (168). Sporulating fungi belonged to genera *Acremonium* (14 isolates), *Aschersonia* (7), *Aspergillus* (26), *Beltrania* (1), *Chaetomella* (1), *Cladosporium* (9), *Conidiobolus* (1), *Curvularia* (3), *Cylindrocladium* (3), *Fusarium* (17), *Gliocladiopsis* (2), *Gliocladium* (14), *Hirsutella* (5), *Mucor* (6), *Paecilomyces* (25), *Penicillium* (22), *Pestilotiopsis* (1), *Pleurothecium* (1), *Syncephalastrum* (1) and *Trichoderma* (29). Amongst the 28 undetermined taxa, 7 were pycnidial (Coelomycetes), 3 thallic and 18 phialidic (hyphomycetes). The identity of most fungi was confirmed using standard taxonomic keys and monographs. Where taxonomic identity was not confirmed, the fungus was recognized only as 'taxonomic' species. The description of fungi is given below in detail. Abbreviation given for a couple of repeatedly encountered words in the taxonomic description and these are as follows: Sri Bhagawan Wildlife Sanctuary = SBWS; Keshava Prasad = KP.

ZYGOMYCETES

1. *Conidiobolus obscurus* (Hall & Dunn) Remaudière & Keller 1980, *Mycotaxon* **11,** 331. **(Plate. IV-c, Fig. 4.1.6)**

Entomogenous zygomycete. Infected host attached to the lower side of leaf. *Hyphal bodies* filamentous; *Conidiophores* arising from host integument, rarely branched, terminally enlarged, 7.5-10 µm wide. *Primary conidia* spherical, with a distinct, medium-sized, conical papilla with rounded or pointed end, with numerous vacuoles and nuclei, 25-50 µm in diam. *Secondary conidia* resembling primary conidia in all respects, but produced on short conidiophores. Cultures obtained on SDA, with slowly spreading colonies, milky white, with large slimy balls, >9 cm diam./7days. Conidia ejected onto lid of petri plates, slow growing on repeated subculturing, revived by supplementing tween 80 in the medium.

Specimen examined: On an infected dipteran fly and a lepidopteran adult on leaves of *Hopea ponga* collected from SBWS, Molem, Goa State, 22.06.2001, 29°C, leg. KP, Isolation by projecting spores on lid of petri plates, Culture No.GU/BOT/MOENF/E172, Slide No. E172.

The genus typified by *C. utriculosus* Brefeld. *Conidiobolus obscurus* was reported to be parasitic on insects. Occasional infection on animals and humans were reported. The fungus was earlier reported from Taiwan. (Keller, 1987; Samson et al., 1988, Tzean et al., 1997).

ASCOMYCETES

2. *Hypocrella* sp. **(Plate. XI-d,e; Fig. 4.1.7)**

Entomogenous ascomycete. *Stromata* exosclerotium, consisting of densely interwoven hyphae, subspherical, holomorph flattened pulvinate to flattened conical, with teleomorph arising as small tuberculate outgrowths from thin periphery of anamorph, growing into long rounded or cylindrical, gloden yellow ascocarps with an

oily appearance. *Hyphae* septate, branched, colourless, thick-walled, smooth, hyaline with bright golden yellow, 2.5-4.7 µm wide. *Perithecia* stromatic, embedded in stroma, with ostiole scattered on the surface, flask shaped, with a long neck, 420-720 x 340-600 µm. *Asci* bitunicate, cylindrical, many-spored 150-375 x 7-17 µm, (up to 17 µm in the middle when matured), with a distinct thickened hyaline cap, 6-10 x 4-6 µm). *Ascospores* many in an ascus, released by opening of the cap, narrow cylindrical to widely fusoid tapering slightly towards ends, with ends rounded to obtuse, 1-celled, rarely 1-septate, guttulate with orange guttules, 9-15 x 3-5 µm.

Anamorph *Aschersonia indica* is represented by a centrally placed pycnidia with a sunken orifice. *Pycnidiophores* penicillate, mono- or bi-verticillate to irregular, paraphysate, with whorls of 2-4 conidiogenous cells. *Conidiogenous cells* phialidic, hyaline, smooth, slender, narrow cylindrical; *Paraphyses* hyaline, linear, filiform, up to 150 µm long; *Pycnidiospores* hyaline, fusoid, sometimes slightly curved, apiculate with acute ends, aseptate, amerosporous, smooth, guttulate, 8-16 x 2-3 µm.

Specimen examined: On immature stages of homopteran insect on fresh leaves of *Hopea ponga*, SBWS, Molem, Goa, India, 18.11.2001, 28°C, leg. KP, Herb. No. GUBH 6144. Slide No. GUBH 6146, 6154 & 6160. Additional specimen examined: i) On immature stages of homopteran insect on fresh leaves of *Hopea ponga*, SBWS, Molem, Goa, India, 13.06.2001, 29°C, leg. KP, Herb. No. GUBH 6101. Slide No. GUBH 6154 & 6160. ii) On immature stages of homopteran insect on fresh leaves of *Hopea ponga*, SBWS, Molem, Goa, India, 21.10.2001, 30°C, leg. KP, Herb. No. GUBH 6133 and 6134. iii) On immature stages of homopteran insect on fresh leaves of *Hopea ponga*, SBWS, Molem, Goa, India, 16.12.2001, 31°C, leg. KP, Herb. No. GUBH 6154. Slide No. GUBH 6154. iv) On immature stages of homopteran insect on fresh leaves of *Hopea ponga*, SBWS, Molem, Goa, India, 13.01.2002, 31°C, leg. KP, Herb. No. GUBH 6160. Slide No. GUBH 6160. v) On immature stages of homopteran insect on fresh leaves of *Hopea ponga*, SBWS, Molem, Goa, India, 16.02.2002, 30°C, leg. KP, Herb. No. GUBH 6162. vi) On immature stages of homopteran insect on fresh leaves of *Hopea ponga*, SBWS, Molem, Goa, India, 26.02.2002, 30°C, leg. KP, Herb. No. GUBH 6170. v) On immature stages of homopteran insect on fresh leaves of *Hopea ponga*, SBWS, Molem, Goa, India, 24.03.2002, 33°C, leg. KP, Herb. No. GUBH 6174.

With its brightly coloured stromata on scale insects, *Hypocrella* is an easily recognizable ascomycete. Typified by *H. discoidea* (Berk. & Br.) Sacc., the genus today accommodates about 38 species distributed in the tropics and subtropics (Petch, 1921b,

1925a,b, 1931; Mains, 1959a,b; Hywel-Jones and Evans, 1993; Evans and Hywel-Jones, 1997). *Hypocrella* is one of the specific of entomo-pathogenic genera restricted to members of Alcyrodidae and Coccidae (Evans and Hywel-Jones, 1997). Anamorphs of *Hypocrella* belong to *Aschersonia*. Anamorph and teleomorph can be found on the same stroma adjacent to each other at particular season during the development.

3. *Podonectria* sp. **(Plate. XII-a,b; Fig. 4.1.8)**

Entomogenous ascomycete. *Stromata* exosclerotium, consisting of densely interwoven hyphae, flattened pulvinate, circularly discoid, raised, with a smooth or circinate margin, yellowish orange to deep orange, 1.8-4.0 mm in diam., 0.4-1.5 mm high, upper margin almost plane, edges vertical or sometimes rounded, with a thin membranous hypothallus. When perithecia mature and upper plane surface becomes irregularly verrucose, ostiolar regions slightly project out and edges of perithecia become furrowed and distinctly de-marketed. Mature stroma forms undulating surface with dark brown raised ostiolar region and furrows marking the edges of each perithecium. *Hyphae* septate, branched, smooth, thick-walled, hyaline with bright orange coloured pigment, 4-7 µm wide. *Perithecia* stromatic, embedded in stroma, with the ostioles scattered on the surface, flask shaped, sometimes ovoid, with a long neck, 160-370 x 115-160 µm, *Asci* bitunicate, cylindrical, 8-spored, 125-225 x 4-6 µm, with an apical hyaline cap, *Ascospores* 8 in an ascus, filiform, slender, 4-9-septate, hyaline, tapering towards ends, distal end rounded, proximal end tapered, guttulate, 75-150 x 1.6-3.2 µm. *Anamorph Aschersonia badia*, represented by one or rarely two pycnidia, located centrally deep in the stroma with sunken orifices. *Pycnidiophores* penicillate, mono- or bi-verticillate to irregular, paraphysate, with whorls of 2-4 conidiogenous

cells. *Conidiogenous cells* phialidic, hyaline, smooth, slender, narrow cylindrical. *Paraphyses* hyaline, linear, filiform, up to 150µm long. *Pycnidiospores* hyaline, fusoid, sometimes slightly curved, apiculate with acute ends, aseptate, amerosporous, smooth, guttulate, 8-16 x 2-3µm.

Specimen examined: On immature stages of homopteran insect on fresh leaves of *Schizyzium* sp., SBWS, Molem, Goa, India, 13.01.2002, 31 C, leg. KP, Herb. No. GUBH 6168. Slide No. GUBH 6159, 6169, 6173 & 6176. Additional specimen examined: i) On immature stages of homopteran insect on fresh leaves of *Holarrhena antidysenterica*, SBWS, Molem, Goa, India, 13.01.2002, 31°C, leg. KP, Herb. No. GUBH 6159. ii) On immature stages of homopteran insect on fresh leaves of *Schizyzium* sp., SBWS, Molem, Goa, India, 26.02.2002, 30°C, leg. KP, Herb. No. GUBH 6169. iii) On immature stages of homopteran insect on fresh leaves of *Schizyzium* sp., SBWS, Molem, Goa, India, 24.03.2002, 33°C, leg. KP, Herb. No. GUBH 6173. iv) On immature stages of homopteran insect on fresh leaves of *Schizyzium* sp., SBWS, Molem, Goa, India, 27.04.2002, 36°C, leg. KP, Herb. No. GUBH 6176 and 6177.

The fungus was reported to be parasitic on scale insects and white flies (Petch, 1921b; Rossman, 1978). Reported anamorphic states were the members of *Tetracrium* or *Tetranacrium* (Tzean et al., 1997). However, in the present study only *Aschersonia badia* was encountered as its anamorphic associate. A detailed discussion on this association is given in the later part of this report.

The encountered *Podonectria* tax. sp. is closely related to *Podonectria coccorum* (Petch) Rossman, in shape and dimensions of exosclerotia, asci and ascospores. However, an anamorphic association with a member of *Aschersonia,* a hitherto unknown phenomenon for *Podonectria* keeps this species away from *Podonectria coccorum.*

Fungus	Ascocarp (µm)	Asci (µm)	Ascospores (µm)
Podonectria coccorum	Globose to ovoid, 200-230 x160-200	8-spored 80-176 x 6-10 hyaline cap present	Filiform, 4-9 septate 56-106 x 2-3.2
Podonectria tax. sp.	Flask shaped, 160-370 x 115-160	8-spored, 125-225 x 4-6 hyaline cap present	Filiform, 4-9 septate 75-150 x 1.6-3.2

COELOMYCETES

Aschersonia Mont.

The genus *Aschersonia*, typified by *A. taitensis* Montagne (Petch, 1921), is characterized by (i) globose pycnidia formed in hemispherical or cushion shaped stroma, (ii) slender, branched conidiophores with thin-walled usually awl-shaped conidiogenous cells, (iii) hyaline, mostly fusoid, smooth, one-celled conidia, and (iv) parasitic nature on homopteran insects (Tzean *et al.,* 1997). Petch (1921b) had examined large number of species till then described under *Aschersonia* and reduced them to 25 recognizable taxa. Mains (1959a,b) reviewed the species of *Aschersonia* described from America. Although CABI Biosciences, U.K., in FunIndex, displays 75 species under *Aschersonia*, according to Evans and Hywel-Jones (1997) little taxonomic revision has been done since the monumental work of Petch.

Species of *Aschersonia* Montagne (Montagne in *Ann. Sci. Nat., Ser. 3, Bot., X., p.122; 1848*) were once assumed to be either parasitic or superficial colonizers of green foliage. Webber, for the first time in 1897, observed abundant infections of larval and pupal populations of white flies by *Aschersonia* spp. (cf. Evans and Hywel-Jones, 1990). Rolf and Fawcett (1913) described the entomogenous habit of *Aschersonia* spp. and emphasized their biological control potential. A few reports have been published on biocontrol value of these fungi (Rombach and Gillespie, 1988; Ramakers and Samson, 1984; Frasen *et al.*, 1987).

Species of *Aschersonia* were not reported earlier from the Western Ghats. Three new records, viz. *Aschersonia aleyrodis, A. badia* and *A. brunnea* and *A. indica* sp. nov. are now described.

4. *Aschersonia aleyrodis* Webber, 1897. *U.S. Dept. Agr. Div. Veg. Phys. Path. Bull.* **13,** 20. **(Plate. VIII, Fig. 4.1.9)**

Entomogenous coelomycete. *Stromata* flattened to pulvinate, white to pale yellow, 1-4 mm diam., 0.4-0.5 mm high, with a thin hypothallus of 0.2-0.5 mm consisting of densely interwoven hyphae. *Hyphae* septate, branched, thick-walled, smooth, hyaline, 3-6 µm wide. *Pycnidia* embedded in stroma with the orifices located peripherally, ovoid to flattened globose, sometimes irregular, 100-300 x 150-300µm. *Pycnidiophores* penicillate, mono- or bi-verticillate, paraphysate, with whorls of 2-5 conidiogenous cells. *Conidiogenous cells* phialidic, narrow cylindrical, slender, smooth, hyaline. *Paraphyses* linear, filiform, hyaline, 30-100 µm. *Pycnidiospores* fusiform, sometimes slightly curved, apiculate with acute ends, smooth, non-septate (amerosporous), guttulate, hyaline, pale yellow to light orange, 6-13 x 1.5-2.5µm. *Colonies* in culture slow growing, with a wavy irregular, pulvinate to elevated white stroma, with smooth round margin, colourless on CMA and yellowish brown on SDA, 0.5-1 cm. Pycnidiophores exudate as creamy white.

Specimen examined: On homopteran insects on fresh leaves of *Hopea ponga,* SBWS, Molem, Goa, India, 17.10.2001, 28°C, leg. KP, Herb. No. GUBH 5053, Slide No. GUBH 5053. Additional specimen examined: i) From a spider cadaver on *Hopea ponga* leaves, SBWS, Molem, Goa State, 26.09.2000, 28°C, leg. KP, direct isolation, Culture No.GU/BOT/MOENF/E71, Slide No. E71. ii) On immature stages of homopteran insect on fresh leaves of *Dillenia indica*, SBWS, Molem, Goa, India, 13.06.2001, 29°C, leg. KP, Herb. No. GUBH 6100. iii) On immature stages of homopteran insect on fresh leaves of unidentified dicot shrub, SBWS, Molem, Goa, India, 29.06.2001, 30°C, leg. KP, Herb. No. GUBH 6102. iv) On immature stages of homopteran insect on fresh leaves of *Dillenia indica* , SBWS, Molem, Goa, India, 05.08.2001, 28°C, leg. KP, Herb. No. GUBH 6108. v) On immature stages of homopteran insect on fresh leaves of *Morinda citrifolia*, SBWS, Molem, Goa, India, 05.08.2001, 28°C, leg. KP, Herb. No. GUBH 6109. vi) On immature stages of homopteran insect on fresh leaves of *Calycopteris flioribunda*, SBWS, Molem, Goa, India, 16.09.2001, 27°C, leg. KP, Herb. No. GUBH 6112. vii) On immature stages of homopteran insect on fresh leaves of *Holarrhena antidysenterica*, SBWS, Molem, Goa, India, 16.09.2001, 27°C, leg. KP, Herb. No. GUBH 6113. viii) On immature stages of homopteran insect on fresh leaves of *Morinda citrifolia*, SBWS, Molem, Goa, India, 16.09.2001, 27°C, leg. KP, Herb. No. GUBH 6114. ix) On immature stages of homopteran insect on fresh leaves of *Calycopteris flioribunda*, SBWS, Molem, Goa, India, 07.10.2001, 29°C, leg. KP, Herb. No. GUBH 6117. x) On immature stages of homopteran insect on fresh leaves of *Morinda citrifolia*, SBWS, Molem, Goa, India, 07.10.2001, 29°C, leg. KP, Herb. No. GUBH 6118. xi) On immature stages of homopteran insect on fresh leaves of unidentified dicot shrub, SBWS, Molem, Goa, India, 07.10.2001, 29°C, leg. KP, Herb. No. GUBH 6119. xii) On immature stages of homopteran insect on fresh leaves of *Calycopteris*

flioribunda, SBWS, Molem, Goa, India, 21.10.2001, 30°C, leg. KP, Herb. No. GUBH 6126. xiii) On immature stages of homopteran insect on fresh leaves of *Dillenia indica*, SBWS, Molem, Goa, India, 21.10.2001, 30°C, leg. KP, Herb. No. GUBH 6127. xiv) On immature stages of homopteran insect on fresh leaves of *Holarrhena antidysenterica*, SBWS, Molem, Goa, India, 21.10.2001, 30°C, leg. KP, Herb. No. GUBH 6128. xv) On immature stages of homopteran insect on fresh leaves of *Morinda citrifolia*, SBWS, Molem, Goa, India, 21.10.2001, 30°C, leg. KP, Herb. No. GUBH 6129. xvi) On immature stages of homopteran insect on fresh leaves of unidentified dicot shrub, SBWS, Molem, Goa, India, 21.10.2001, 30°C, leg. KP, Herb. No. GUBH 6130. xvii) On immature stages of homopteran insect on fresh leaves of *Calycopteris flioribunda*, SBWS, Molem, Goa, India, 18.11.2001, 28°C, leg. KP, Herb. No. GUBH 6135. xviii) On immature stages of homopteran insect on fresh leaves of *Holarrhena antidysenterica*, SBWS, Molem, Goa, India, 18.11.2001, 28°C, leg. KP, Herb. No. GUBH 6136. xix) On immature stages of homopteran insect on fresh leaves of *Morinda citrifolia*, SBWS, Molem, Goa, India, 18.11.2001, 28°C, leg. KP, Herb. No. GUBH 6137. xx) On immature stages of homopteran insect on fresh leaves of unidentified dicot shrub, SBWS, Molem, Goa, India, 18.11.2001, 28°C, leg. KP, Herb. No. GUBH 6138.xxi) On immature stages of homopteran insect on fresh leaves of unidentified dicot shrub, SBWS, Molem, Goa, India, 18.11.2001, 28°C, leg. KP, Herb. No. GUBH 6139. xxii) On immature stages of homopteran insect on fresh leaves of unidentified dicot shrub, SBWS, Molem, Goa, India, 18.11.2001, 28°C, leg. KP, Herb. No. GUBH 6140.xxiii) On immature stages of homopteran insect on fresh leaves of *Calycopteris flioribunda*, SBWS, Molem, Goa, India, 16.12.2001, 31°C, leg. KP, Herb. No. GUBH 6147. xxiv) On immature stages of homopteran insect on fresh leaves of *Dillenia indica*, SBWS, Molem, Goa, India, 16.12.2001, 31°C, leg. KP, Herb. No. GUBH 6148. xxv) On immature stages of homopteran insect on fresh leaves of *Holarrhena antidysenterica*, SBWS, Molem, Goa, India, 16.12.2001, 31°C, leg. KP, Herb. No. GUBH 6149. xxvi) On immature stages of homopteran insect on fresh leaves of *Morinda citrifolia*, SBWS, Molem, Goa, India, 16.12.2001, 31°C, leg. KP, Herb. No. GUBH 6150. xxvii) On immature stages of homopteran insect on fresh leaves of unidentified dicot shrub, SBWS, Molem, Goa, India, 18.11.2001, 28°C, leg. KP, Herb. No. GUBH 6151. xxviii) On immature stages of homopteran insect on fresh leaves of *Calycopteris flioribunda*, SBWS, Molem, Goa, India, 13.01.2002, 31°C, leg. KP, Herb. No. GUBH 6156. xxix) On immature stages of homopteran insect on fresh leaves of *Morinda citrifolia*, SBWS, Molem, Goa, India, 13.01.2002, 31°C, leg. KP, Herb. No. GUBH 6157. xxx) On immature stages of homopteran insect on fresh leaves of *Dillenia indica*, SBWS, Molem, Goa, India, 26.02.2002, 30°C, leg. KP, Herb. No. GUBH 6163. xxxi) On immature stages of homopteran insect on fresh leaves of *Morinda citrifolia*, SBWS, Molem, Goa, India, 26.02.2002, 30°C, leg. KP, Herb. No. GUBH 6164. xxxii) On immature stages of homopteran insect on fresh leaves of unidentified dicot shrub, SBWS, Molem, Goa, India, 26.02.2002, 30°C, leg. KP, Herb. No. GUBH 6165. xxxiii) On immature stages of homopteran insect on fresh leaves of *Calycopteris flioribunda*, SBWS, Molem, Goa, India, 24.03.2002, 33°C, leg. KP, Herb. No. GUBH 6171. xxxiv) On immature stages of homopteran insect on fresh leaves of *Morinda citrifolia*, SBWS, Molem, Goa, India, 24.03.2002, 33°C, leg. KP, Herb. No. GUBH 6172. xxxv) On immature stages of homopteran insect on fresh leaves of *Dillenia indica*, SBWS, Molem, Goa, India, 16.06.2002, leg. KP, Herb. No. GUBH 6178. xxxvi) On immature stages of homopteran insect on fresh leaves of *Calycopteris flioribunda*, SBWS, Molem, Goa, India, 26.06.2001, leg. KP, Herb. No. GUBH 6180.

The fungus has so far been recorded from Florida, Trinidad, Taiwan (Petch, 1921b; Tzean et al., 1997).

5. *Aschersonia badia* Patouliard, 1897. *Jour. de Botanique* **11**, 370.

(Plate. IX, Fig 4.1.10)

Entomogenous coelomycete. *Stromata* an exosclerotium, composed of densely interwoven hyphae, flattened pulvinate, circularly discoid, raised, with a smooth or

circinate margin, yellowish orange to deep orange, 1.8-3.0 mm in diam., 0.4-1.0 mm high. *Hyphae* smooth, septate, branched, thick-walled, hyaline, 4-7 µm wide. *Pycnidia* embedded in stroma with the orifices circular or scattered over the surface, ovoid to flattened globose or spherical, 250-350 x 150-300µm. *Pycnidiophores* penicillate, mono- or biverticillate to irregular, paraphysate, with whorls of 2-4 conidiogenous cells. *Conidiogenous cells* phialidic, hyaline, smooth, slender, narrow cylindrical; *Paraphyses* hyaline, linear, filiform, up to 150µm long. *Pycnidiospores* hyaline, fusoid, sometimes slightly curved, apiculate with acute ends, aseptate, amerosporous, smooth, guttulate, 8-16 x 2-3µm. *Colonies* in culture with irregular wavy, yellow to orange stroma, 0.5 cm in diam. 0.5 cm height on SDA, slow growing, attaining a diam. of 2-2.5 cm in 2 months, with many ostioles containing ozzing slimy conidial mass. Ostiolate pycnidia appear in culture after one month.

Specimen examined: On homopteran insects over fresh leaves of *Hopea ponga,* SBWS, Molem, Goa, India, 17.10. 2001, leg. KP, Herb. No. GUFC 5050. Additional specimen examined: i) From a pupa of homopteran insect on leaves of *Dillenia indica*, SBWS, Molem, Goa State, 29.05.2001, 30°C, leg. KP, direct isolation, Culture No. GU/BOT/ MOENF/E196, Slide No. E196. ii) On immature stages of homopteran insects on fresh leaves of *Morinda citrifolia*, SBWS, Molem, Goa, India, 21.07.2001, leg. KP, Herb. No. GUBH 6103. iii) On immature stages of homopteran insect on fresh leaves of *Leea macrphylla*, SBWS, Molem, Goa, India, 07.10.2001, 29°C, leg. KP, Herb. No. GUBH 6120. iv) On immature stages of homopteran insect on fresh leaves of *Schizyzium* sp., SBWS, Molem, Goa, India, 07.10.2001, 29°C, leg. KP, Herb. No. GUBH 6121. v) On immature stages of homopteran insect on fresh leaves of unidentified dicot shrub, SBWS, Molem, Goa, India, 07.10.2001, 29°C, leg. KP, Herb. No. GUBH 6122. vi) On immature stages of homopteran insect on fresh leaves of unidentified dicot shrub, SBWS, Molem, Goa, India, 21.10.2001, 30°C, leg. KP, Herb. No. GUBH 6131. vii) On immature stages of homopteran insect on fresh leaves of unidentified dicot shrub, SBWS, Molem, Goa, India, 21.10.2001, 30°C, leg. KP, Herb. No. GUBH 6132. viii) On immature stages of homopteran insect on fresh leaves of *Maesa indica*, SBWS, Molem, Goa, India, 18.11.2001, 28°C, leg. KP, Herb. No. GUBH 6141. ix) On immature stages of homopteran insect on fresh leaves of *Leea macrphylla*, SBWS, Molem, Goa, India, 16.12.2001, 31°C, leg. KP, Herb. No. GUBH 6152. x) On immature stages of homopteran insect on fresh leaves of *Schizyzium* sp., SBWS, Molem, Goa, India, 16.12.2001, 31°C, leg. KP, Herb. No. GUBH 6153. xi) On immature stages of homopteran insect on fresh leaves of unidentified dicot shrub, SBWS, Molem, Goa, India, 13.01.2002, 31°C, leg. KP, Herb. No. GUBH 6158. xii) On immature stages of homopteran insect on fresh leaves of unidentified dicot shrub, SBWS, Molem, Goa, India, 26.02.2002, 30°C, leg. KP, Herb. No. GUBH 6166. xiii) On immature stages of homopteran insect on fresh leaves of unidentified dicot shrub, SBWS, Molem, Goa, India, 26.02.2002, 30°C, leg. KP, Herb. No. GUBH 6167.

The fungus has so far been known from Sri Lanka, Philippine, Pegu, Taiwan (Petch, 1921b; Tzean et al., 1997).

6. *Aschersonia brunnea* Petch, 1921b. *Ann. Roy. Bot. Gard., Peradèniya* **7,** 251.

(Plate. X, Fig 4.1.11)

Entomogenous coelomycete. *Stromata*, an exosclerotium, consisting of densely interwoven hyphae, circular, discoid, dark orange to brown, 2.0-3.0 mm in diam., 1.2-1.5 mm high. *Hyphae* hyaline, smooth, thick-walled, 3-9µm wide. *Pycnidia* embedded in stroma, peripheral, circular, ostiole depressed, inconspicuous, pycnidia spherical to flattened globose, 600-700 x 500-650µm. *Pycnidiophores* penicillate, mono- or biverticillate, paraphysate, with whorls of 2-6 conidiogenous cells; *Conidiogenous cells* phialidic, hyaline, smooth, slender, narrow cylindrical; *Paraphyses* hyaline, linear, filiform, up to 100 µm long. *Pycnidiospores* hyaline, fusoid, sometimes slightly curved, apiculate with acute ends, aseptate, amerosporous, smooth, guttulate and 6-13 x 1.5-2.5µm.

Specimen examined: On homopteran insects over fresh leaves of *Hopea ponga*, SBWS, Molem, Goa, India, 17.10. 2001, leg. KP, Herb. No. GUFC 5051. Slide No. GUFC 5051.

The fungus has so far been described from Brazil and Taiwan (Petch, 1921b; Tzean et al., 1997).

7. *Aschersonia indica* Keshava Prasad, A. Kumar et Bhat sp. nov.

(Plate. XI-a,b,c; Fig. 4.1.12)

Entomogenous Coelomycete. *Stroma* subspheroidea, aplannatus pulvinata ad aplannatus conica, subinde externus augmentum tuberculinus periphericus, flavus ad splendens aureus with an oleosum aspectum, 1.25-3 mm in diametrum, and 0.6 mm alta, dense intricatum hyphae compositum. *Hyphae* septata, ramosa, hyalina, crassitunicata, 2.5-3 µm lata. *Pycnidia* circumdare intusstroma, plerumque solitaria et centrale, globosa, comprimere ad perpendicum vel irregulare, et 120-500 x 120-250 µm, crassiusculus alinquantulum depressum ostiolum, pluries inductum ad pycnidiosporae,

in massis splendens aureus ad aurantius. *Conidiophores* mononematosa, exilise, recta vel flexuosa, solitaria, enata internus superficies de pycnidium, monoverticillata, genitum verticillus de 5-12 phialidis, septata, tenuitunicata, laevia, hyalina, 20-30 x 2-2.5 µm. *Conidiogenous cells* monoblastica, phialidica, descreta, terminale, angusta cylindrica, aliquando exigue curvata, hyalina, tenuitunicata, 10-22.5 x 2-2.5 µm, paraphysisis innascor deconidiophori, lineare, filiforme hyalina, usque 240 µm longa. Pycnidiosporae solitaria, fusoidae, with ambae terminale abrupte attenuata, aseptata (amerosporus), laevia hyalina with splendens aureus guttulae, et 12-16 x 3.5-4.5 µm.

HOLOTYPUS, In insecta homoptera in folis viridulus *Hopea ponga,* Sri Bhagawan Mahaveer Wildlife Sanctuary, Molem, Goa, India, 17.10.2001, 28°C, leg. Keshava Prasad, Herb. No. IMI 389315 (GUFC 5052)

Entomogenous coelomycete. *Stromata*, an exosclerotia, consisting of densely interwoven hyphae, subspherical, flattened pulvinate to flattened conical, some times with tubercle-like outgrowths form the periphery of main stroma, pale to shining golden yellow with an oily appearance, 1.25-3 mm in diam. and 0.6 mm high. *Hyphae* septate, branched, colourless, thick-walled and 2.5-3 µm wide. *Pycnidia* embedded in stroma, usually single and central, when many lie scattered, spherical, vertically compressed or irregular, and 120-500 x 120-250 µm, with a broad some what depressed ostiole, orifice often overlaid by pycnidiospores, in mass light shining yellow to orange with the age; *Conidiophores* mononematous, slender, straight or flexuous, solitary, arising from the lining of pycnidia, monoverticillate bearing a whorl of 5-12 phialides, septate, thin-walled, smooth, hyaline, 20-30 x 2-2.5 µm. *Conidiogenous cells* monoblastic, phialidic, discrete, terminal, narrow cylindrical, occasionally slightly curved, thin-walled, hyaline, 10-22.5 x 2-2.5 µm. *Paraphyses* originating from the conidiophores, linear, filiform hyaline, up to 240 µm long; *Pycnidiospores* solitary, fusoid, with both ends abruptly

tapering, aseptate (amerosporous), smooth, and hyaline with golden yellow guttules, 12-16 x 3.5-4.5 µm.

Aschersonia indica resembles *A. basicystis* Berk & Curtis and *A. goldiana* Sacc. & Ellis in the shape of spores i.e., wider in the middle and abruptly tapering towards both ends. However, presence of paraphyses in the pycnidia of *A. indica* distinguishes it from *A. basicystis* and *A. goldiana*, both of which do not possess paraphysis. Conidiophores in *A. indica* sp. nov. resemble that of *A. aleyrodis*, *A. badia* in having long filiform linear paraphyses but the fungus differs in the conidial shape and in the shape, colour and size of the stroma (Tzean et al., 1997). Presence of paraphyses and conidial shape are important criteria in the taxonomy of *Aschersonia*.

Additional specimen examined: i) On immature stages of homopteran insect on fresh leaves of *Hopea ponga*, SBWS, Molem, Goa, India, 21.07.2001, leg. KP, Herb. No. GUBH 6104, 6105 and 6106. ii) On immature stages of homopteran insect on fresh leaves of *Hopea ponga*, SBWS, Molem, Goa, India, 05.08.2001, 28°C, leg. KP, Herb. No. GUBH 6110 iii) On immature stages of homopteran insect on fresh leaves of *Hopea ponga*, SBWS, Molem, Goa, India, 16.09.2001, 27°C, leg. KP, Herb. No. GUBH 6115. iv) On immature stages of homopteran insect on fresh leaves of *Hopea ponga*, Dhudhsagar water Falls, Goa, India, 16.09.2001, 27°C, leg. KP, Herb. No. GUBH 6116. v) On immature stages of homopteran insect on fresh leaves of *Hopea ponga*, SBWS, Molem, Goa, India, 07.10.2001, 29°C, leg. KP, Herb. No. GUBH 6123 and 6124. vi) On immature stages of homopteran insect on fresh leaves of an unidentified dicot shrub, SBWS, Molem, Goa, India,1 8.11.2001, leg. KP, Herb. No. GUBH 6142. vii) On immature stages of homopteran insect on fresh leaves of an unidentified dicot shrub, SBWS, Molem, Goa, India, 18.11.2001, 28°C, leg. KP, Herb. No. GUBH 6143.

8. *Chaetomella* sp.

Entomogenous coelomycete. *Colonies* circular, hyaline, margin smooth, growth median attaining a diam. of 3-4.5cm in CMA in 7 days, with wet, slimy, fruiting bodies in concentric rings. *Mycelium* comprising of immersed, septate, freely branched, thin-walled, smooth, hyaline to olivaceous, 2-4µm wide hyphae; *Pycnidia* reniform to ovate, superficial, dark brown, without ostiole, with curved setae on all sides, 140-180µm; *Conidiophores* smooth, hyaline, 13-15 x 2-3µm; *Conidia* thick-walled, aseptate, ellipsoidal to cylindrical, hyaline 4-5 x 2-3 µm.

Specimen examined: From a spider cadaver found on the leaf of *Dillenia indica*, moist deciduous, SBWS, Molem, Goa State, 13.09.1999, leg. KP, direct isolation, Culture No.GU/BOT/ICMR/E35, Slide No. E35.

HYPHOMYCETES

9. *Acremonium charticola* (Lindau) Gams, 1971. *Cephalosporium-artige Schimmelpilze (Hyphomycetes),* p. 46. **(Plate. XII-d,f; Fig. 4.1.13)**

Entomogenous hyphomycete. Infested homopteran larva covered with white to yellow mycelia. *Colonies* on CMA circular and dome-shaped, white, margin smooth, cottony, slimy wet, reverse pale yellow, attaining a diam. of 1-1.5 cm in 7days. *Mycelium* partly immersed, partly superficial, composed of smooth, septate, branched, thin-walled, hyaline, 1.5-2.5 µm wide hyphae. *Conidiophores* smooth, septate, branched, thin-walled, hyaline, with many solitary conidiogenous cells attached, with conidia in slimy globules. *Conidiogenous cells* phialidic, integrated to discrete, slender, awl-shaped, hyaline, 15-50 µm long, 2.4-3.2 µm wide at the base, 1-2 µm at the tip. *Conidia* slimy, smooth, solitary, 1-celled, globose to ovoid, hyaline, 1.6-2.4 µm.

Specimen examined: On lepidopteran larvae on fresh leaves of *Hopea ponga*, riparian forest, undershrub, low canopy, Ambe Ghats, Goa State, 29^{0}C, 31.7.2001, leg. KP, direct isolation, Culture No.GU/BOT/Moenf/E187 & E192, Slide No. E187 and E192. Additional Specimen examined: i) On a spider on fresh leaves of *Holarrhena antidysenterica,* riparian forest, undershrub, low canopy, Ambe Ghats, Goa State, 29^{0}C, 31.7.2001, leg. KP, direct isolation, Culture No.GU/BOT/Moenf/E189, Slide No. E189.

The species resembles *Acremonium charticola* reported from Kaohsiung County, Taiwan by Tzean et al. (1997) in shape of conidiogenous cells and conidia. Although the size of conidia are much smaller, it is within the range. *A. charticola* was reported as a new species on homopteran larvae from Taiwan whereas in the present study it was recovered from lepidoptera and arachnida.

10. *Acremonium* sp. 1.

Entomogenous hyphomycete. *Colonies* on CMA circular, cottony white, raised in the middle with smooth margin, growth median, 3mm high at centre, cottony, slimy, reverse of the colony colourless, attaining a diam. of 4 cm in 7days. *Mycelium* partly

immersed partly superficial, composed of smooth, septate, branched, thin-walled, hyaline, 1.5-2.5µm wide hyphae. *Conidiophores* mononematous, smooth, septate, branched, thin-walled, hyaline, with many whorls of solitary to 2-4 conidiogenous cells, 40-300 x 2-3 µm with conidia in slimy globules at the tip of conidiophores. *Conidiogenous cells* phialidic, discrete, awl-shaped or cylindrical, tapering abruptly into a very narrow tip, 8-12 x 2-3 µm. *Conidia* slimy, solitary, 1-celled, subglobose or ovoid, smooth, hyaline, 2.5-4 x 1.5-3µm.

Specimen examined: On a spider cadaver found on the leaf of *Ixora brachiata*, moist deciduous dense forest, SBWS, Molem, Goa State, 29°C, 13.09.1999; leg. KP, direct isolation, Culture No. GU/BOT/Moenf/E29, Slide No. E29.

11. *Acremonium* sp. 2.

Entomogenous hyphomycete. *Colonies* on CMA, circular, cottony white, raised in the middle with smooth margin, growth median, cottony, slimy, reverse of the colony colourless, attaining a diam of 2.5-3.5 cm in 7days. *Mycelium* partly immersed, highly superficial, composed of smooth, septate, branched, thin-walled, hyaline, 1.5-2.5µm wide hyphae. *Conidiophores* mononematous, smooth, septate, branched, thin-walled, hyaline, with terminally attached many whorls of solitary or 2-4 conidiogenous cells, 80-140 x 2-3 µm, with conidia in slimy globules at the tip. *Conidiogenous cells* phialidic, discrete, cylindrical with a distinct neck, hyaline, 12-20 x 2.5-4 µm. *Conidia* slimy, solitary, 1-celled, globose, smooth, hyaline, 3-4 µm.

Specimen examined: On *Drosophila* sp., on fruits of *Ixora coccinea*, undershrub, scrub jungle, GU campus, Goa State, 29^{0}C, 05.07.2000, leg. KP, direct isolation, Culture No. GU/BOT/Moenf/E51, Slide No. E51.

12. *Acremonium* sp. 3. **(Fig. 4.1.14)**

Entomogenous hyphomycete. *Colonies* on CMA circular, white, raised in the middle with smooth margin, growth median, cottony, slimy, reverse of the colony

colourless, attaining a diam of 2-3 cm in 7days. *Mycelium* partly immersed, highly superficial composed of smooth, septate, branched, thin-walled, hyaline, 1.5-2.5 µm wide hyphae. *Conidiophores* mononematous, smooth, septate, branched, thin-walled, hyaline, with many whorls of solitary or 2-4 conidiogenous cells, 40-300 x 2-3 µm, with conidia in slimy globules at the tip. *Conidiogenous cells* phialidic, discrete, awl-shaped or cylindrical tapering abruptly into a very narrow tip, 8-12 x 2-3 µm. *Conidia* slimy, solitary, 1-celled, subglobose or ovoid, smooth, hyaline 2.5-4 x 1.5-3 µm.

Specimen examined: On an infected homopteran larva on the abaxial surface of the unidentified dicot plant, undershrub, forest, Tambdi Surla, Goa State, 28^{0}C, 25.07.2000, leg. KP, direct isolation, Culture No.GU/BOT/Moenf/E57, Slide No. E57.Additional specimen examined: i) On a homopteran larva on the abaxial surface of the unidentified dicot plant, undershrub, forest, Tambdi Surla, Goa State, 28^{0}C, 25.07.2000, leg. KP, direct isolation, Culture No.GU/BOT/Moenf/E58, Slide No. E58. ii) On a homopteran larva on the abaxial surface of the unidentified dicot plant, undershrub, forest, Tambdi Surla, Goa State, 28^{0}C, 25.07.2000, leg. KP, direct isolation, Culture No.GU/BOT/Moenf/E53, Slide No. E53. iii) On a homopteran larva on the abaxial surface of the unidentified dicot plant, undershrub, forest, Tambdi Surla, Goa State, 28^{0}C, 25.07.2000, leg. KP, direct isolation, Culture No.GU/BOT/Moenf/E54, Slide No. E54.

13. *Acremonium* sp. 4. **(Fig. 4.1.15)**

Entomogenous hyphomycete. *Colonies* on CMA circular, cottony white, raised in the middle, with smooth margin, with moderate growth, cottony, slimy, reverse of the colony colourless, attaining a diam. of 2 cm in 7days. *Mycelium* partly immersed, highly superficial, composed of smooth, septate, branched, thin-walled, hyaline, 1.5-2.5 µm wide hyphae. *Conidiophores* of various length, mononematous, smooth, septate, branched, thin-walled, hyaline, with many conidiogenous cells, 40-100 x 2-3 µm, with conidia in slimy globules. *Conidiogenous cells* phialidic, discrete, awl-shaped or cylindrical tapering into a fine tip, 12-40 x 2-3 µm. *Conidia* slimy, solitary, 1-celled, variedly shaped from globose, subglobose, ovoid, reniform to cylindrical, smooth, hyaline, 4-15 x 2-4 µm.

Specimen examined: On infested ants in a decaying spathe of coconut frond, near the paddy field, Taleigao, Goa State, 27^{0}C, 30.09.2000, leg. KP, direct isolation, Culture No.GU/BOT/Moenf/E91, Slide No. E91.

In its condial shape, the fungus has some similarities with *Acremonium crassum* Petch (Petch, 1931). The fungus was also reported from Taoyuan county, Taiwan by Tzean et al. (1997).

Fungus	*Host*	*Condial shape*	*Condial size (μm)*
Acremonium crassum	aphids	Oval, cylindrical, slightly curved, both ends rounded, rarely one septate	5.2-10.3 x (1.6) 2.4-3.2
Acremonium tax. sp. 4.	ants	Globose, subglobose, ovoid, reniform to cylindrical	4-15 x 2-4

14. *Acremonium* sp. 5. **(Fig. 4.1.16)**

Entomogenous hyphomycete. Infested homopteran larva covered with white to yellow mycelia. *Colonies* on CMA circular, white, margin smooth, growth median, attaining a diam of 1.5-2 cm in 7days, cottony, slimy, reverse of the colony colourless. *Mycelium* partly immersed, partly superficial, composed of smooth, septate, branched, thin-walled, hyaline, 1.5-2.5 μm wide hyphae. *Conidiophores* indistinct, smooth, septate, branched, thin-walled, hyaline, with many solitary conidiogenous cells attached, with conidia in slimy globules. *Conidiogenous cells* phialidic, discrete, slender, awl-shaped or cylindrical tapering towards tip, hyaline, 35-90 μm long, 4-6 μm wide at the base, 2-3 μm at the tip. *Conidia* slimy, solitary, 1-celled, smooth, elongated, curved in the middle, with a truncate base and rounded apex, hyaline, 8-22 x 4-6 μm.

Specimen examined: Infested homopteran larva located on the abaxial surface of *Holigarna arnottiana* leaves near soil level, Bondla National Park forest, Goa, India, 28^{0}C, 22.10.2000, leg. KP, direct isolation, Culture No.GU/BOT/Moenf/E115, Slide No. E115.

15. *Acremonium* sp. 6. **(Fig. 4.1.17)**

Entomogenous hyphomycete. *Colonies* on CMA circular, white creamish, with rhizoidal margin, fast growing, attaining a diam. of 3-3.5 cm in 7days, often with

concentric zones on dome-shaped superficial mycelia, cottony, with water droplets, reverse of the colony colourless. *Mycelium* partly immersed, partly superficial, composed of smooth, septate, branched, thin-walled, hyaline, 1.5-2.5 µm wide hyphae; *Conidiophores* erect, septate, branched, smooth, thin-walled, hyaline, 1.5-3 µm with many solitary conidiogenous cells, with conidia in false slimy heads. *Conidiogenous cells* phialidic, discrete, slender, awl-shaped or subulate, hyaline, 12-60 µm long, 1.6-3.2 µm wide at the base, up to 1 µm wide at the tip. *Conidia* slimy, solitary, 1-celled, oval to cylindrical, sometimes slightly curved with rounded ends, smooth, hyaline, 2.5-5 x 1-2 µm.

Specimen examined: On lepidopteran larvae on fresh leaves of *Hopea ponga*, riparian forest, undershrub, low canopy, Ambe Ghats, Goa State, 29^{0}C, 31.7.2001, leg. KP, direct isolation, Culture No.GU/BOT/Moenf/E188, Slide No. E188. Additional Specimen examined: i) On unidentified insect on fresh leaves of *Careya arborea* Roxb., forest, Sonauli, Goa State, 28^{0}C, 08.11.2001, leg. KP, direct isolation, Culture No.GU/BOT/Moenf/E216, Slide No. E216.

16. *Aspergillus clavatus* Dezm. 1834. *Annals Sci. nat., Bot., Ser.* **2,** 71. **(Fig. 4.1.18)**

Entomogenous hyphomycete. *Colonies* on CMA with wavy circular, light green conidial heads, margin hairy, fast growing, attaining a diam. of 5-6 cm in 7 days, dry, with watery exudates, with radiating green sectors, reverse of the colony white. *Mycelium* composed of septate, branched, smooth, thin-walled, hyaline, 2-4 µm wide hyphae. *Conidiophores* mononematous, long, 1-2-septate, unbranched, smooth, thin-walled, hyaline, 325-2500 x 25-50 µm, terminating into a clavate, 45-70 µm wide vesicle at the tip. *Conidiogenous cells* on uniseriate metula, phialidic, discrete, bottle-shaped, on the surface of vesicle, 5-7 x 2-3.5 µm. *Conidia* catenate, ellipsoidal, smooth, thick-walled, 1-celled, white to pale green, 3-4 x 2.5-3 µm.

Specimen examined: On *Anopheles* sp. 4th instar larva, Vasco, Goa, India, 29°C, 22.07.1999; leg. KP, direct isolation, Culture No.GU/BOT/ICMR/C33, Slide No. C33.

Aspergillus clavatus, characterized by blue-green clavate conidial heads which split into divergent columns and conidiophores up to 3 mm long, has been reported from earlier India, Bangladesh, Sri Lanka, Hong Kong, Jamaica, Brazil, Argentina, South Africa, Ivory Coast, Egypt, Libya, Turkey, Greece, Italy, USA, Japan, USSR and Taiwan. Habitats reported include cultivated soils under cotton, potatoes, sugar cane, legumes and paddy; desert soil, rhizosphere of banana, wheat, rice, clover and ground nuts; stored food products such as sorghum seeds, paddy rice, milled rice, flour and dough products, nuts and dried products. It has also been known from dead adult bees and honeycombs, feathers and droppings of free living birds (Domsch et al., 1980; Tzean et al., 1990).

17. *Aspergillus fumigatus* Fres. 1863; *Beitr. Mykol.* 81. **(Fig. 4.1.19)**

Entomogenous hyphomycete. *Colonies* on CMA circular, hyaline, with dark bluish green compact column of dry spores on erect conidiophores, smooth, with hairy margin, fast growing, attaining a diam. of 6-6.5 cm in 7days, with watery droplets on erect macronematous conidiophores, reverse of the colony colourless. *Mycelium* mostly immersed, composed of smooth, septate, branched, thin-walled, hyaline, 2-3µm wide hyphae. *Conidiophores* mononematous, aseptate, unbranched, smooth, hyaline with slight tinge of gray green, thin-walled, 100-150 x 4-6 µm, terminating into a pyriform vesicle of 12-25 µm diam. *Conidiogenous cells* uniseriate, phialidic, discrete, smooth, cylindrical, hyaline, 5-7 x 1.5-2 µm; *Conidia* catenate, subglobose, verrucose, 2.5-3 µm in diam.

Specimen examined: On pupa of a *Culex* sp. collected from Curing water at construction sites, Curchorem, Goa, India, 31°C, 23.04.1999, leg. KP, direct isolation, Culture No.GU/BOT/ICMR/C15, Slide No. C15. Additional specimens examined: i) On an *Anopheles* sp. 4th instar larva, collected from curing waters at construction sites, Panjim, Goa, India, 29°C, 20.04.1999, leg. KP, direct isolation, Culture No. GU/BOT/ ICMR/C1, Slide No. C1. ii) From an *Anopheles* sp. 2nd instar larva, collected from curing waters at construction sites, Panjim, Goa, India, 29°C, 20.04.1999, leg. KP, direct isolation, Culture No.GU/BOT/ICMR/C8, Slide No. C8. iii) From an *Anopheles* sp. 2nd instar larva, larvae collected from drainage near construction sites, Porvorim, Goa, India, 29°C, 23.04.1999, leg. KP, direct isolation, Culture No.GU/BOT/ICMR/C18, Slide No. C18.

Aspergillus fumigatus, a thermo-tolerant, ubiquitous fungus, is reported from air flora of India, British Isles and France. It also occurs in mountainous, marine, coastal, forest and peaty soil habitats. It is also known from litter, mineral horizon, mull and humus, sand blows, sand beaches, saline soils and mangrove swamps. Besides, sewage, activated sludge, slime of paper mills, freshwater, alpine soil with long snow coverage, desert soils of Israel, Egypt, Africa, and USA, soils under vegetation, caves and mines were reported as habitats of *A. fumigatus*. It has a high competitive saprophytic ability. In rhizospheric studies, *A. fumigatus* has been isolated from soils of pine, coffee, flax, clover, groundnuts, onion, grass, rice, corn, barley, oat, wheat, sand dune plants, steppe plants peat bog plants, ferns, moss, roots of strawberry, broad beans, leaves of peas, grasses during senescence, bolls and green and aging leaves of cotton. It was also known from decaying plant materials, including *Pteridium aquilinum* and *Adiantum,* barley, cabbage, potato, *Ammophila*, pine, *Abies grandis*, *Picea sitchensis*, sclerotium of *Sclerotinia sclerotiorum*, plant debris from birds' nest, roosts, feathers and droppings, chicken pens, alligator nesting materials, garden compost, mushroom compost, dung of cattle and horses, self heated hay, self heated corn, seeds of wheat, barley, sorghum, oats, rice, and corn, grasses, cotton, groundnuts, castor beans, cacao beans, stored sweet potatoes and tobacco. The fungus can serve as food for Collembola and mites (Domsch, 1980; Tzean et al., 1990).

A. fumigatus is one of the causal agents of systemic mycosis attacking lungs and respiratory tracts, resulting in acute or chronic infections in cattle, lambs, rodents, piglets, poultry and minute lesions in pregnant cows. In humans, breathing spores of *A. fumigatus* was known to cause aspergillosis, in which the fungus grows on the walls of lungs. Immuno-suppressed people are susceptible to infection by *A. fumigatus*. Farm

workers who handle animal feed infected by the fungus suffer from a milder form of aspergillosis- farmer's lung- in which a ball of fungus grows in their lungs. Some are allergic to *A. fumigatus* resulting in breathing difficulties in people with asthma. The fungus is known to produce compounds called gliotoxins that can inhibit macrophages, thus causing immuno-deficiency in infected vertebrates. *A. fumigatus,* therefore, has been excluded as potential commercial insecticide (Lisansky and Hall, 1983).

18. *Aspergillus japonicus* Saito var. ***aculeatus*** (Iizuka) Al-Musallam. 1980. *Revision of Aspergillus species*. Ph. D. Thesis, University of Utrecht. **(Fig. 4.1.20)**

Entomogenous hyphomycete. *Colonies* on CMA circular, hyaline, with blackish brown conidial heads, margin smooth, hairy, growth median to fast, attaining a diam. of 3-3.55 cm in 7 days, with watery exudates, with scanty aerial mycelium, with black semi- divergent columnar aspergillia on erect conidiophores, reverse of the colony colourless. *Mycelium* composed of septate, branched, smooth, thin-walled, hyaline, 2-4 µm wide hyphae; *Conidiophores* mononematous, aseptate, unbranched, smooth, thick-walled, (1.5-3 µm), light brown 1300-1700 x 11-12 µm, terminating in a globose, 25-60 µm in diam vesicle. *Conidiogenous cells* uniseriate, phialidic, discrete, bottle-shaped, broader towards distal end, 8-9 x 2.5-3 µm. *Conidia* catenate, globose, thick-walled, verrucose, 1-celled, dark brown, 3-4 µm in diam.

Specimen examined: On a *Culex* sp. 2nd instar larva, from an asbestos tank, Panaji, Goa, India, 31°C, 19.04.1999, leg. KP, direct isolation, Culture No.GU/BOT/ICMR/C3, Slide No. C3.

A. japonicus var. *aculeatus* and *A. japonicus* var. *japonicus* share common characteristics in having uniseriate phialides and conspicuously echinulate conidia. These traits differ from all other black-spored species of *Aspergillus*. *A. japonicus* var.

aculeatus with larger vesicles (15-68 µm) differs from *A. japonicus* var. *japonicus* which has small-sized vesicles (8-38 µm).

A. japonicus is reported from soils in Japan, India, Pakistan, Brazil, the Bahamas, Tahiti, Sierra Leone, Ghana, South Africa and Turkey. It has been isolated from soils of forest, cultivated grassland, rhizosphere of wheat and ferns and leaf litter of *Eucalyptus maculata, Polypodium* sp., *Cyclosorus* sp. and fruits of *Theobroma cacao, Anisophyllea laurena*, stored sweet potatoes, rice seeds and milled rice (Domsch et al., 1980; Tzean et al., 1990).

19. *Aspergillus niger* van Tieghem var. ***awamori*** (Nakazawa) Al-Musallam. 1980. *Revision of the black Aspergillus species*. Ph.D. Thesis, University of Utrecht.

(Fig. 4.1.21)

Entomogenous hyphomycete. *Colonies* on SDA circular, flat, black, with concentric rings of erect conidiophores possessing globose black aspergillia, attaining a diam. of 4-5 cm in 7 days, reverse of the colony colourless. *Mycelium* composed of smooth, septate, branched, thin-walled, hyaline, 2-4 µm wide hyphae. *Conidiophores* mononematous, smooth, aseptate, unbranched, thin-walled, hyaline, 400-600 x 5-7.5 µm, with pyriform, 25-50 µm in diam vesicles. *Conidiogenous cells* phialidic, on biseriate metulae, discrete, bottle-shaped, covering entire vesicle, 4.5-6.5 x 2.5-3.5 µm. *metulae* cylindrical, smooth, brown, 5-7 x 3-4.5 µm. *Conidia* catenate, globose to subglobose, verrucose, thick-walled, 1-celled, black, 3-5 µm.

Specimen examined: On a *Culex* sp. 3rd instar larva, from curing water at construction sites, Cuncolim, Goa, India, 29°C, 26.07.1999; leg. KP, direct isolation, Culture No.GU/BOT/ICMR/C46, Slide No. C46. Additional Specimen examined: i) On infested coccids found on the leaf of *Holarrhena antidysenterica* , forest, Molem, Goa, India, 22.06.2001, 29°C, leg. KP, direct isolation, Culture No. GU/BOT/MOENF/ E174, Slide No. E174. ii) On infested spider on the leaf of *Dillenia indica*, forest, Ambe, Goa India,30°C, 31.07.2001, leg. KP, direct isolation, Culture No. GU/BOT/MOENF/ E195, Slide No. E195.

20. ***Aspergillus oryzae*** (Ahlburg) Cohn var. ***oryzae*** 1884. *Jahresberichtschles. Ges. Vaterl. Kult.* **61,** 226. **(Fig. 4.1.22)**

Entomogenous hyphomycete. *Colonies* on SDA circular, yellow with concentric rings of erect conidiophores possessing columnar yellowish green aspergillia, attaining a diam. of 4-5 cm in 7 days, reverse of the colony colourless. *Mycelium* submerged composed of septate, branched, smooth, thin-walled, hyaline, 2-4 µm wide hyphae. *Conidiophores* mononematous, aseptate, unbranched, verruculose, thin-walled, hyaline, 90-680 x 9-17 µm with pyriform, 45-70 µm in diam. Vesicles. *Conidiogenous cells* uniseriate, phialidic, discrete, bottle-shaped, covering upper 3/4 of the vesicle, 7-14 x 3.5-6 µm. *Conidia* catenate, globose to subglobose, smooth to verruculose, thick-walled, 1- celled, yellowish green, 3-5 µm in diam.

Specimen examined: On infested lepidopteran eggs on leaves of *Holarrhena antidysenterica*, Tambdi Surla, Goa, 28^{0}C, 02.12.2000, leg. KP, direct isolation, Culture No.GU/BOT/Moenf/E135, Slide No. E135. Additional specimen examined: i) On aphids on leaves of *H. antidysenterica*, forest, Tambdi Surla, Goa, India, 28^{0}C, 02.12.2000, leg. KP, direct isolation, Culture No.GU/BOT/Moenf/E129, Slide No. E129.

The fungus was reported from India, USSR, Czechoslovakia, Japan, Tahiti, Peru, Syria, Italy, USA, British Isles and Taiwan. Reported habitats included grassland, forest and cultivated soils; air, rhizosphere of wheat, cotton; milled rice, seeds of wheat, paddy rice, pods of groundnut, fermented food, dried cereals, and legumes, dried fruits and nuts, nesting materials of birds, coarse fodder, wood pulp and cotton fabrics (Raper and Fennel, 1965; Klitch and Pitt, 1988; Kozakiewicz, 1989; Tzean et al., 1990).

21. ***Aspergillus restrictus*** Smith. 1931. *J. Textile Inst.* **22,** T115. **(Fig. 4.1.23)**

Entomogenous hyphomycete. *Colonies* on CMA irregular, with bluish green conidial heads, with rhizoidal margin, slow growing, attaining a diam. of 1.5-2 cm in 7 days, with watery exudates, with scanty aerial mycelium, reverse of the colony

colourless to pale yellowish brown. *Mycelium* composed of smooth, septate, branched, thin-walled, hyaline, 2-4 μm wide hyphae; *Conidiophores* mononematous, smooth, aseptate, unbranched, thin-walled, hyaline, 120-450 x 3-6 μm, terminating in ellipsoidal to pyriform, 7-16 μm in diam. vesicles. *Conidiogenous cells* uniseriate, phialidic, discrete, bottle-shaped, arranged on upper part of the vesicle, 5-10 x 2.5-3.5 μm. *Conidia* catenate, doliform to pyriform, verruculose, thick-walled, 1-celled, bluish green, 3.5-6.5 x 2.5-4 μm.

Specimen examined: On *Culex* sp. 2nd instar larva, from curing water at construction site, Curchorem, Goa, India, 30°C, 20.04.1999, leg. KP, direct isolation. Culture No.GU/BOT/ICMR/C9, Slide No. C9. Additional specimen examined: i) on infested scale insect found on dead wood log, scrub jungle, GU Campus, Goa, India,15.09.2000, leg. KP, direct isolation. Culture No.GU/BOT/Moenf/E64, Slide No. E64.

Distinguishing characteristics of the species are slow growth on MEA, with long columnar, sometimes twisted pale green conidial heads, uniseriate aspergillia, elongated, doliform, ellipsoidal to pyriform conidia, rough to spinose. *Aspergillus restrictus* is very much similar to *A. penicillioides* but differs by fast growth on M40Y and with doliform conidia (Tzean et al., 1990). *A. restrictus* has been reported from Antarctic, Northern Ireland, USA, France, Australia, Honduras, India, Pakistan, Kuwait, Finland, the British Isles, Brazil, Argentina, Burma, Malaysia, Nigeria, Japan, Taiwan and Tanzania. The reported habitats include ice-free lake, organic detritus in a stream, arable land, acid mine drainage streams, soils of cultivated land, forest, salt-marsh, seeds of Graminae, cotton goods, food stuffs, bird nests, dried sheep meat, ground pepper, stored soyabean seeds and air. (Raper and Fennel, 1965; Klitch and Pitt, 1988; Kozakiewicz, 1989; Tzean et al., 1990).

22. *Aspergillus* sp. 1 **(Fig. 4.1.24)**

Entomogenous Hyphomycete. *Colony* on SDA circular, flat, yellow, with concentric rings of erect conidiophores possessing columnar yellowish green aspergillia, fast growing, attaining a diam. of 4-5 cm growth in 7 days, reverse of the colony colourless. *Mycelium* composed of smooth, septate, branched, thin-walled hyaline, 2-3 µm wide hyphae. *Conidiophores* mononematous, finely verruculose, aseptate, unbranched, thin-walled, hyaline, 200-350 x 5-9 µm, terminating in pyriform, 20-40 µm in diam. vesicles. *Conidiogenous cells* biseriate, phialidic, discrete, bottle-shaped, covering the vesicle completely, 7-9 x 4-5 µm, with broadly obovate, smooth, hyaline, 6-10 x 6-8 µm metulae. *Conidia* catenate, globose to subglobose, smooth to verruculosc, 1-cellcd, thick-walled, yellowish brown, 4-6 µm.

Specimen examined: On a *Culex* sp. 2nd instar larva, from curing water at construction sites, Cuncolim, Goa, India, 26.07.1999; leg. KP, direct isolation. Culture No.GU/BOT/ICMR/C45, Slide No. C45.

23. *Aspergillus* sp. 2

Entomogenous hyphomycete. *Colonies* on CMA circular, hyaline with reddish brown divergent conidial heads, with hairy margin, attaining a diam. of 4cm in 7 days, with watery exudates on the surface, with reddish brown divergent columnar aspergillia on erect conidiophores in concentric rings, with scanty aerial mycelium; reverse of the colony colourless. *Mycelium* composed of smooth, septate, branched, thin-walled, hyaline, 1.5-2.5 µm wide hyphae, *Conidiophores* mononematous, smooth, aseptate, unbranched, thin-walled, hyaline, 120-150 x 8-10µm, terminating in pyriform, 20-25µm in diam. vesicles. *Conidiogenous cells* uniseriate, phialidic, discrete, bottle-shaped,

arranged on the upper half of the vesicle, 10-12 x 2-3 μm. *Conidia* catenate, globose, 1-celled, verrucose, thick-walled, brown, 4-6μm diam.

Specimen examined: On aphids found on leaves of *Chromolaena odorata,* scrub jungle mixed with plantation, Curchorem, Goa, India, 27° C, 22.07.1999, leg. KP, direct isolation. Culture No.GU/BOT/ICMR/E21, Slide No. E21.

24. *Aspergillus* sp. 3

Entomogenous hyphomycete. *Colonies* on CMA circular, brownish black, margin smooth hairy, median to fast growing, attaining a diam. of 4.5-5cm in 7 days, with watery exudates on the surface, with scanty aerial mycelium containing black semi-divergent columnar aspergillia on erect conidiophores; reverse of the colony colourless. *Mycelium* composed of smooth, septate, branched, thin-walled, hyaline, 1.5-2.5 μm wide hyphae; *Conidiophores* mononematous, smooth, aseptate, unbranched, with a tinge of black, thick-walled (2.5.3μm), 2000-2500 x 11-14μm, terminating in globose, 50-70 μm in diam. vesicles. *Conidiogenous cells* uniseriate, phialidic, discrete, bottle-shaped, arranged fully on the vesicle, 8-12 x 2-4μm. *Conidia* catenate, globose, 1-celled, verrucose, thick- walled, black, 5-7μm in diam.

Specimen examined: On a *Culex quinquefasciatus* 3rd instar larvae used as bait in a pond water, Bondla National Park, Bondla, Goa, India, 27°C, 15.10.1999, leg. KP, isolation using baits. Culture No. GU/BOT/ICMR/W5, Slide No. W5.

25. *Aspergillus* sp. 4.

Entomogenous hyphomycete. *Colonies* on CMA circular, hyaline, yellowish brown conidial heads, margin smooth, median to fast growing, attaining a diam. of 3.5-4.5 cm in 7 days, with watery exudates, with scanty aerial mycelium containing yellowish brown compact columnar aspergillia on erect conidiophores; reverse of the colony colourless. *Mycelium* composed of smooth, septate, branched, thin-walled,

hyaline, with 1.5-2.5 μm wide hyphae. *Conidiophores* mononematous, aseptate, unbranched, thin-walled, smooth, hyaline, 250-400 x 4-14 (mostly 8-12) μm, terminating in ellipsoidal, 8-35 x 7-25 μm, vesicles. *Conidiogenous cells* uniseriate, phialidic, discrete, bottle-shaped, arranged on the upper 4/5th of the vesicle, 4-7 x 3-4μm. *Conidia* catenate, sub-globose to doliform, smooth, 1-celled, 3.5-5 μm in diam.

Specimen examined: From a mosquito larva (*Anopheles* sp. 2nd instar larva) in a sample of 8 mosquito larvae in the curing water at construction sites, Porvorim, Goa State, 29°C; 23.04.1999; leg. Keshava Prasad, direct isolation, Culture No.GU/BOT/ICMR/C11, Slide No. C11.

26. *Aspergillus* sp. 5 **(Fig. 4.1.25)**

Entomogenous hyphomycete. *Colonies* on CMA irregular, hyaline to grayish white, conidial heads with a purple tinge, with rhizoidal margin, attaining a diam. of 1.5-2.5 cm in 7 days, with watery exudates; reverse of the colony colourless. *Mycelium* composed of smooth, septate, branched, thin-walled, hyaline, 1.5-2.5μm wide hyphae; *Conidiophores* mononematous, septate, unbranched, thin-walled, smooth, hyaline, 50-300 x 2.5-6 μm, terminating in variously shaped ranging from sub-globose to ellipsoidal, 6-15 μm diam. vesicles. *Conidiogenous cells* uniseriate, subglobose to inflated with papilla-like neck, phialidic, discrete, 4-5 μm in diam. *Conidia* catenate, globose to subglobose, 1-celled, smooth, pale bluish green, 2.5-3.5 μm in diam.

Specimen examined: On an *Anopheles* sp. 2nd instar larva, water collected from by road side near a construction site, Porvorim, Goa, India, 29°C, 23.04.1999; leg. KP, direct isolation. Culture No. GU/BOT/ICMR/C14, Slide No. C14. Additional specimen examined: i) On infested ants found on dead wood log, scrub jungle, GU campus, Goa, India, 27°C, 05.07.2000, leg. KP, direct isolation. Culture No.GU/BOT/Moenf/E50, Slide No. E50. ii) On an infested scale insect found on the leaf of *Microcos paniculata,* scrub jungle, GU campus, Taleigao plateau, Goa, India, 27°C, 15.09.2000; leg. KP, direct isolation. Culture No.GU/BOT/Moenf/E65, Slide No. E65.

The species in culture produces variations in morphology of the conidiophores, ranging from penicillous to aspergilliform, which also are variously branched.

Conidiophores are septate. Conidiogenous cells are unusually swollen becoming globose.

27. *Aspergillus* sp. 6 **(Fig. 4.1.26)**

Entomogenous hyphomycete. *Colonies* on CMA circular, with brown conidial heads, margin smooth, hairy, median to fast growing, attaining a diam. of 3-4.5 cm in 7 days, with watery exudates, with scanty aerial mycelium containing brown loosely columnar aspergillia on erect conidiophores; reverse of the colony colourless. *Mycelium* composed of smooth, septate, branched, thin-walled, hyaline, with 1.5-2.5 µm wide hyphae. *Conidiophores* mononematous, verruculose, aseptate, unbranched, thick-walled, hyaline, 300-550 x 7-10 µm, terminating in globose, 20-30 µm in diam. vesicles. *Conidiogenous cells* uniseriate, phialidic, discrete, cylindrical to bottle-shaped, 6-9 x 2.5-5 µm. *Conidia* catenate, globose to sub-globose, smooth, 1-celled, 4-5 µm in diam.

Specimen examined: On aphids found on leaves of *Chromolaena odorata,* scrub jungle, Curchorem, Goa, India, 27°C, 22.07.1999, leg. KP, direct isolation, Culture No. GU/BOT/ ICMR/E22, Slide No. E22.

28. *Aspergillus* sp.7

Entomogenous hyphomycete. *Colonies* on CMA wavy circular, with green conidial heads, margin hairy, growth median, attaining a diam. of 1.5-2.5 cm in 7 days, with blackish-brown watery exudates, scanty aerial mycelium with green radiating aspergillia on erect conidiophores in concentric rings and radial lines apparent; reverse of the colony colourless in CMA and brown on SDA. *Mycelium* composed of septate, branched, thin-walled, hyaline, smooth, with 1.5-2.5 µm wide hyphae. *Conidiophores* mononematous, aseptate, unbranched, smooth, thick-walled, hyaline, 150-350 x 5-7 µm, terminating in ellipsoidal, 9-13 µm wide, vesicles. *Conidiogenous cells* biseriate,

phialidic, discrete, cylindrical to bottle-shaped with a distinct neck, 6-9 x 2-3 μm; *metulae* cylindrical to obglobose, smooth, hyaline, 4-6 x 2.5-3 μm. *Conidia* catenate, globose, 1-celled, verrucose, green, 3-3.5 μm in diam.

Specimen examined: On infested scale insect found on the leaf of *Psychotria dalzellii*, Cotigao Wildlife Sanctuary; Goa, India, 28°C, 30.09.2000; leg. KP, direct isolation, Culture No.GU/BOT/Moenf/E86, Slide No. E86. Additional specimen examined: i) On infested scale insect found on the leaf of unidentified grass, Taleigao, Goa, India, 30°C, 02.10.2000; leg. KP, direct isolation. Culture No.GU/BOT/Moenf/E97, Slide No. E97.

29. *Beltrania sp.*

Entomogenous hyphomycete. *Colonies* on CMA circular, light brown, margin smooth, fast growing, attaining a diam. of 7.5-8 cm in 7 days, cottony, wet, brown at fructifications, with concentric rings; reverse of the colony colourless on CMA, brown on SDA. *Mycelium* composed of smooth, septate, freely branched, thin-walled, hyaline, 1.6-3.2 μm wide hyphae. *Conidiophores* mononematous, erect or flexuous, smooth, septate, thick-walled, pale brown above, hyaline below, 5-6 μm wide. *Conidiogenous cells* polyblastic, discrete, terminal, ellipsoidal, hyaline, denticulate, denticles cylindrical, form a penicillium of 3-5, each bearing 4-5 conidia, 7-10 x 3.2-4.8 μm. *Conidia* solitary, obconical, smooth, 0-septate, free end spicate, olive brown, 20-28 x 8-11 μm; appendages straight, 6-20 μm.

Specimen examined: On infested scale insects on the leaves of the *Microcos paniculata*, Molem, Goa, India, 28°C, 29.09.2000, leg. KP, direct isolation. Culture No.GU/BOT/Moenf/E81, Slide No. E81.

30. *Cladosporium* sp. 1. **(Plate. XII-c,e)**

Entomogenous Hyphomycetes: *Colonies* on CMA circular, dome shaped, brown, dry, smooth margin, growth median, attaining a diam. of 1,5-2.5 cm in 7 days; reverse of the colony pale brown. *Mycelium* superficial and submerged composed of smooth, septate, freely branched, thin-walled, brown, 3-5 μm wide hyphae.

Conidiophores mononematous, erect, smooth to verruculose, septate, branched, dark brown, thick-walled, 1.6-3.2 µm wide. *Conidiogenous cells* polyblastic, discrete, denticulate, dark brown. *Ramoconidia* variously shaped, usually cylindrical, with a single scar at the base and 3-5 at the distal end, 0-1 septate, dark brown, 8-30 x 1.6-3 µm. *Conidia* catenate, subglobose to ellipsoidal with one or two marks of attachment, aseptate, verrucose, brown, 4-10 x 3-5 µm.

Specimen examined: On infested homopteran larva on a leaf of *Hopea ponga*, forest, Anmod, Goa, India, 30^{0}C, 07.05.1999, leg. KP, direct isolation, Culture No. GU/BOT/ Moenf/E143, Slide No. E143.

Cladosporium Link: Fries is a large genus, members of which are reported as pathogens of Coccidae (Evans and Hywel-Jones, 1997; Petch, 1935). *Cladosporium* sp. (nr. *oxysporum*) was believed to be a primary pathogen and responsible for epizootics of homopteran insects in South African guava orchards (Evans and Hywel-Jones, 1997). Evans and Hywel-Jones (1997) however have cautioned against considering *Cladosporium* sp. as primary pathogen of insects.

31. *Cladosporium* sp. 2.

Entomogenous Hyphomycetes: *Colonies* on CMA circular, dome shaped, blackish brown, dry, smooth margin, with growth median, attaining a diam. of 1.5 cm in 7 days; reverse of the colony black; *Mycelium* superficial and submerged, composed of smooth, septate, freely branched, hyaline to dark brown, thin- to thick-walled, 1.6-3.2 µm wide hyphae. *Conidiophores* mononematous, erect, smooth, septate, branched, dark brown, thick-walled, 2.4-4 µm wide. *Conidiogenous cells* polyblastic, discrete, denticulate, dark brown; *Ramoconidia* variedly shaped, usually cylindrical, with a single scar at the base and 3-5 at the distal end, 0-1 septate, dark brown, 8-25 x 3.2-4 µm;

Conidia catenate, subglobose to ellipsoidal, with one or two marks of attachment, aseptate, verrucose, brown, 4-5 x 1.6-2.4 μm.

Specimen examined: On infested unidentified insect on a leaf of *Hopea ponga*, forest, Dhudhsagar, Goa, India, 30^{0}C, 25.10.2001, leg. KP, direct isolation. Culture No. GU/ BOT/Moenf/ E210, Slide No. E210.

32. *Cladosporium sp.* 3.

Entomogenous hyphomycete. *Colonies* On SDA circular, 1.5 cm diam in 7 days, with white hairy mycelium at the periphery, green dry spore mass in central region, pale buff green; reverse concentric green rings. *Mycelium* verruculose, septate, branched, brown, thin-walled, 2.4-6.4 μm wide hyphae. *Conidiophores* mononematous, erect, verruculose, septate, unbranched, dark brown, thick-walled, 600-1500 x 4-4.8 μm. *Conidiogenous cells* polyblastic, denticulate, integrated, cylindrical, dark brown, 40-70 x 3.2-4 μm; *Ramoconidia* variedly shaped, oval to cylindrical, with a single scar at the base and 3-5 at the distal end, 0-septate, dark brown, 4-20 x 3-5 μm. *Conidia* catenate, subglobose to ellipsoidal, with one or two attachment scars, aseptate, verrucose, brown, 2.4-4 x 2.4-3.2 μm in diam.

Specimen examined: On infested unidentified insect on a leaf of *Chromolaena odorata*, Pookala, Kasaragod, Kerala, India, 30^{0}C, 07.05.1999, leg. KP, direct isolation. Culture No.GU/BOT/Moenf/E1, Slide No. E1.

33. *Cladosporium* sp. 4.

Entomogenous hyphomycete. *Colonies* on SDA circular, with slightly elevated in the centre, attaining a diam of 3.0 cm in 7 days, margin smooth, flat, dry, green to black without white hyphae; reverse of colony black. *Mycelium* verruculose, septate, freely branched, brown, thick-walled, 4-6.4 μm wide hyphae. *Conidiophores* mononematous, erect, verruculose, septate, branched, dark brown, thick-walled, 4- 7 μm. *Conidiogenous cells* polyblastic, denticulate, discrete, cylindrical, dark brown, 8-14

x 4-6 µm; *Ramoconidia* variedly shaped, cylindrical, with a single scar at the base and 3-5 at distal end, verrucose, 1-septate, dark brown, 15-25 x 3.2-4 µm. *Conidia* catenate, subglobose to ellipsoidal, aseptate, verrucose, brown, 3.2-4.8 µm in diam.

Specimen examined: On infested lepidopteran eggs on leaves of an unidentified herb in a plantation, Kodar, Goa, India, 28°C, 30.11.2000, leg. KP, direct isolation. Culture No.GU/BOT/Moenf/E121, Slide No. E121. Additional specimen examined: i) On infested aphids on leaves of *Cassia tora*, forest, Alorna, Goa, India, 28°C, 16.12.1999, leg. KP, direct isolation. Culture No.GU/BOT/Moenf/E46, Slide No. E46.

34. *Cladosporium* sp. 5.

Entomogenous hyphomycete. *Colonies* on SDA circular, with slight elevation at the centre, attaining 3 cm diam in 7 days, margin smooth, flat, dry, green to black without white hyphae; reverse of the colony black; *Mycelium* verruculose, septate, branched, olive brown, thick-walled, 3.2-4 µm wide hyphae. *Conidiophores* mononematous, erect, verruculose, 1-4 septate, unbranched, dark brown, thick-walled, 30-80 x 2.4-4 µm; *Conidiogenous cells* polyblastic, denticulate, integrated, cylindrical, dark brown, 15-30 x 2.4-4 µm; *Ramoconidia* cylindrical, with a single scar at the base and 3-5 at the distal end, verrucose, aseptate, dark brown, 12-20 x 2.4-3.2 µm. *Conidia* catenate, subglobose to ellipsoidal usually with long cylindrical denticle, aseptate, verrucose, brown, 3.2-4 µm in diam.

Specimen examined: On *Anopheles* sp. 2nd instar larva from water collection near a construction site, dry plateau, Porvorim, Goa, India, 28°C, 23.04.1999, leg. KP, direct isolation. Culture No.GU/BOT/ICMR/C19, Slide No. C19. Additional specimen examined: i) On infested scale insects on leaves of an unidentified dicot creeper, forest, Anmod, Goa, India, 28°C, 23.03.2001, leg. KP, direct isolation. Culture No. GU/BOT/Moenf/ E144, Slide No. E144.

35. *Curvularia* sp. 1.

Entomogenous hyphomycete. *Colonies* on SDA wavy circular, with concentric rings, cottony white at periphery and black and flat in the centre, margin rhizoidal, growth median to fast attaining a diam. of 4-5 cm diam, black; reverse of the colony

colourless. *Mycelium* composed of smooth, septate, freely branched, dark brown with dark septa, thick-walled, 2.4-4.8 µm wide hyphae. *Conidiophores* mononematous, erect or flexuous, smooth, septate, thick-walled, dark brown, 60-180 x 3-9 µm. *Conidiogenous cells* polytretic, integrated, terminal, later becoming intercalary, with cicatrized scars, cylindrical, variedly curved, dark brown, paler above,6-22 x 3.2-6.4 µm. *Conidia* solitary, pyriform, distinctly curved at the third cell from the base, sigmoid, dark brown, end cells paler, 15-28 x 9-15 µm.

Specimen examined: On a *Culex* sp. 2nd instar larvae, from a drain, Vasco, Goa, India, 27°C, 02.06.1999, leg. KP, moist chamber incubation. Culture No.GU/BOT/ICMR/C25, and Slide No. C25.

36. *Curvularia* sp. 2.

Entomogenous hyphomycete. *Colonies* on SDA wavy circular, with concentric rings, cottony white at periphery and black and flat in the centre, margin rhizoidal, growth median to fast attaining a diam. of 4-5 cm in 7 days, black; reverse of the colony colourless. *Mycelium* composed of smooth, septate, freely branched, thin-walled, hyaline, 3.2-7.2 µm wide hyphae. *Conidiophores* mononematous, erect or flexuous, smooth, septate, pale brown above, hyaline below, 1.6-3.2 µm. *Conidiogenous cells* polytretic, integrated, terminal, later becoming intercalary, cicatrized, cylindrical, variously curved, pale brown, 6-26 x 2.4-4 µm. *Conidia* solitary, spindle-shaped, verrucose, 3-6 septate, sometimes curved at the third cell from the base, olive brown, 30-45 x 8-14 µm. Brown triradiate conidia of 30-38 x 8-14 µm are also produced along with normal conidia.

Specimen examined: On infested immature stages of homopteran insects on leaves of *Terminalia paniculata,* forest, Molem, Goa, India, 28°C, 29.09.2000, leg. KP, direct isolation. Culture No.GU/BOT/Moenf/E79, Slide No. E79. Additional specimen examined: i) On infested homopteran larva on leaves of an unidentified dicot plant, undershrub, scrub jungle, Chandreshwar, Goa, India, 28°C, 15.01.2001, leg. KP, direct isolation. Culture No.GU/BOT/Moenf/E141, Slide No. E141.

37. *Cylindrocladium* sp. **(Fig. 4.1.27)**

Entomogenous hyphomycete: *Colonies* on CMA circular, flat, colourless, with hyaline masses, smooth margin, fast growing, attaining a diam. of 3 cm in 7 days; reverse colourless, cottony, wet with slimy droplets. *Mycelium* smooth, septate, freely branched, thin-walled, hyaline, 2-3 µm wide hyphae. *Stipe* vesiculate, septate, hyaline, in the middle of the conidiophore, unbranched, thin-walled, 80-130 x 1.6-2.4 µm, with narrow ellipsoidal vesicles. *Conidiophores* mononematous, penicillate, smooth, septate, branched (up to 6 times) hyaline, thick-walled, bearing a terminal penicillium, 140-300 x 2-4 µm wide. *Conidiogenous cells* phialidic, discrete, without a distinct neck, occurring singly or in the groups of up to 6 at the end of branches, hyaline, 12-20 x 2.4-4 µm. *Conidia* slimy, solitary, cylindrical with truncate ends, 0-1-septate, smooth, hyaline, 10-18 x 2-3 µm. *Chlamydospores* thick-walled, olive brown, 12-16 µm in diam.

Specimen examined: On infected water insects collected from foam in a forest stream, Chorlem, Goa, India, 27°C, 30.09.2000, leg. KP, direct isolation. Culture No.GU/BOT/Moenf/E87, Slide No. E87. Additional specimen examined: i) On infested *Anopheles* sp. 2nd instar larva, collected water near the roadside, Porvorim, Goa, India, 27°C, 05.05.1999, leg. KP, direct isolation. Culture No. GU/BOT/ICMR/C21, Slide No. C21. ii) On baits used in the water samples from a puddle, scrub jungle, Cotigao, Goa, India, 27°C, 16.08.1999, leg. KP, isolation using baits. Culture No.GU/BOT/ICMR/W1, Slide No. W1.

38. *Fusarium* sp. 1.

Entomogenous hyphomycete. *Colonies* on SDA circular, cottony, floccose, with wavy elevations, attaining a diam. of 6 cm in 7 days, cottony, with water droplets on surface, creamy white to pale pink, with rhizoidal margin, pale yellow with white periphery; reverse of the colony pale yellow. *Mycelium* composed of smooth, septate, freely branched, thin-walled, hyaline, 1.6-2.4 µm wide hyphae. *Conidiophores* mononematous, branched, smooth, septate, hyaline, 1.6-3.2 µm wide. *Conidiogenous*

cells phialidic, discrete, subulate, hyaline, 20-80 x 2-3 µm. *Macroconidia* solitary, sickle-shaped, basal blunt end curved inside, straight at the apex, pointed, smooth, 3-4-septate, hyaline, 10-14 x 2.4-4.8 µm; *Microconidia* ellipsoidal to fusiform, narrow cylindrical, 1-celled, 4-16 x 2-4 µm.

Specimen examined: On a *Culex* sp. 2nd instar larva in a tank, Pernem, Goa, India, 30°C, 23.04.1999, leg. KP, direct isolation, Culture No.GU/BOT/ICMR/C16, Slide No. C16. Additional specimen examined: i) On a *Culex* sp. 4th instar larva in a well, Bicholim, Goa, India, 30°C, 05.06.1999, leg. KP, direct isolation, culture No.GU/BOT/ICMR/C28, Slide No. C28. ii) On a *Culex* sp. pupa in a well, Bicholim, Goa, India, 30°C, 16.06.1999, leg. KP, direct isolation, culture No.GU/BOT/ICMR/C30, Slide No. C30.

39. *Fusarium* sp. 2

Entomogenous hyphomycete. *Colonies* on CMA circular, cottony, floccose, cottony, creamy white, margin rhizoidal, pale yellow with white periphery, attaining a diam. of 5.5 cm in 7 days; reverse of the colony pale yellow. *Mycelium* composed of smooth, septate, freely branched, thin-walled, hyaline, 1.6-2.4 µm wide hyphae. *Conidiophores* mononematous, highly branched, smooth, septate, hyaline, 2-4 µm wide. *Conidiogenous cells* phialidic, discrete, subulate, at the apices of branches, hyaline, 15-30 x 2-4 µm. *Macroconidia* solitary, sickle-shaped, free end acute and curved, basal end blunt and rounded, smooth, 3-septate, hyaline, 14-30 x 2.4-4 µm; *Microconidia* ellipsoidal to fusiform or narrow cylindrical, 1-celled, 3-16 x 1.6-4 µm.

Specimen examined: On an infected mealy bug on the abaxial surface of the unidentified dicot plant of Solanaceae, vegetable field, Carambolim, Goa, India, 28°C, 16.08.1999, leg. KP, direct isolation, Culture No.GU/BOT/ICMR/E27, Slide No. E27. Additional specimen examined: i) On a spider cadaver on fresh leaves of *Hopea wightiana,* forest, Molem, Goa, India, 28°C, 13.09.1999, leg. KP, direct isolation, Culture No. GU/BOT/ICMR/E32, Slide No. E32. ii) On an infected dipteran adult on fresh leaves of *Dillenia indica*, forest, Molem, Goa, India, 26.09.2000, 28°C, leg. KP, direct isolation, Culture No. GU/BOT/Moenf/E70, Slide No. E70.

40. *Fusarium* sp. 3

Entomogenous hyphomycete. *Colonies* on CMA circular, cottony, floccose, fast growing, attaining a diam. of 5.5 cm in 7 days, cottony, creamy white, margin rhizoidal;

reverse of the colony colourless. *Mycelium* composed of smooth, septate, freely branched, thin-walled, hyaline, 1.6-2.4 µm wide hyphae. *Conidiophores* mononematous, highly branched, smooth, septate, hyaline, 2-4 µm wide, ends sometimes swollen to 7 µm. *Conidiogenous cells* phialidic, discrete, globose to cylindrical without a distinct neck, 10-28 x 3.2-5.6 µm. *Macroconidia* solitary, sickle-shaped, ends rounded, not curved, smooth, 3-5-septate, hyaline, 15-45 x 4-6 µm; *Microconidia* ellipsoidal to cylindrical, ends rounded, 1-celled, 4-12 x 1.6-3.2 µm.

Specimen examined: On aphids on fresh leaves of *Chromolaena odorata*, forest, Bondla, Goa, India, 28^{0}C, 18.10.1999, leg. KP, direct isolation, Culture No. GU/BOT/ICMR/E43, Slide No. E43.

41. *Fusarium* sp. 4

Entomogenous hyphomycete. *Colonies* on CMA circular, cottony, floccose, fast growing, attaining a diam. of 4-5 cm in 7 days, cottony, creamy white, margin rhizoidal, pale yellow with white periphery; reverse of the colony pale yellow. *Mycelium* composed of smooth, septate, freely branched, thin-walled, hyaline, 2-4 µm wide hyphae. *Conidiophores* mononematous, highly branched, with conidiogenous cells in whorls, smooth, septate, hyaline, 35-120 x 2-4 µm. *Conidiogenous cells* phialidic, discrete, narrow cylindrical, hyaline,15-30 x 2-4 µm. *Macroconidia* solitary, sickle-shaped, ends pointed, smooth, 3-6-septate, hyaline, 15-60 x 2.4-6 µm; *Microconidia* sickle-shaped, 1-septate, 8-15 x 2.4-3.2 µm.

Specimen examined: On a homopteran larva on fresh leaves of unidentified dicot plant, forest, Tambdi Surla, Goa, India, 28^{0}C, 25.07.2000, leg. KP, direct isolation, Culture No. GU/BOT/Moenf/E60, Slide No. E60. Additional specimen examined: i) On a homopteran larva on fresh leaves of unidentified dicot plant, forest, Tambdi Surla, Goa, India, 28^{0}C, 25.07.2000, leg. KP, direct isolation, Culture No. GU/BOT/ Moenf/E61, Slide No. E61. ii) On ants on dead barks of *Anacardium occidentale*, scrub jungle, near an iron ore mine, Cudnem, Goa, India, 28^{0}C, 12.10.2000, leg. KP, direct isolation, Culture No. GU/BOT/Moenf/E110, Slide No. E110. iii) On an infected aphids on an unidentified herb, scrub jungle, near an iron ore mine, Cudnem, Goa, India, 28^{0}C, 02.10.2000, leg. KP, direct isolation, Culture No. GU/BOT/Moenf/E111, Slide No. E111.

42. *Fusarium* sp. 5

Entomogenous hyphomycete. *Colonies* on CMA circular, cottony, floccose, fast growing attaining a diam. of 3.5 cm in 7 days, cottony, pale yellow, margin rhizoidal, reverse of the colony colourless. *Mycelium* composed of smooth, septate, freely branched, thin-walled, hyaline, 2-4 µm wide hyphae. *Conidiophores* mononematous, solitary, unbranched, 3-4-septate, smooth, hyaline, 80-130 x 2.4-4.8 µm. *Conidiogenous cells* phialidic, integrated, narrow cylindrical, 35-60 x 1.6-3.2 µm. *Macroconidia* solitary, sickle-shaped, 3-septate, ends pointed, smooth, hyaline, 30-40 x 3.2-5.6 µm. *Microconidia* Ellipsoidal to cylindrical with rounded ends, aseptate, 6-15 x 3.2-5.6 µm.

Specimen examined: On an infected scale insect on fresh leaves of unidentified dicot plant, forest, Molem, Goa, India, 28^{0}C, 30.09.2000, leg. KP, direct isolation, Culture No. GU/BOT/Moenf/E90, Slide No. E90. Additional specimen examined: i) On an infected lepidopteran larvae on fresh leaves of *Solanum melanogaster*, vegetable fields, Taleigao, Goa, India, 28^{0}C, 02.10.2000, leg. KP, direct isolation, Culture No. GU/BOT/Moenf/ E100, Slide No. E100.

Salient features of *Fusarium* spp. described above were given in a tabular form.

Isolate No	Conidiophores	Conidiogenous cells	Macroconidia	Microconidia
C16 C28 C30	Highly branched, 1.6-3.2 µm wide	Discrete, awl-shaped, 20-80 x 2-3 µm	3-4-septate, 10-14 x 2.4-4.8 µm, apex straight and pointed, base blunt and curved	Ellipsoidal to fusiform, Narrow, cylindrical, 4-16 x 2-4 µm
E27 E32 E70	Highly branched, 2-4 µm wide	Discrete, awl-shaped, 15-30 x 2-4 µm	3-septate, 14-30 x 2.4-4 µm, free end acute and curved, base rounded	Ellipsoidal to fusiform, slightly curved 3-16 x 1.6-4 µm
E43	Highly branched, ends swollen to 7 µm, 3-4 µm wide	Discrete, Globose to cylindrical, 10-28 x 3.2-5.6 µm	3-5 septate, 15-45 x 4-6 µm, ends rounded	Ellipsoidal to cylindrical, ends rounded, 4-12 x 1.6-3.2 µm
E60 E61 E110 E111	Highly branched, 3-4 µm wide	Discrete, narrow cylindrical, 5-30 x 2-4 µm	3-5 septate, 15-60 x 2.4-6 µm, ends pointed	Sickle shaped, 1-septate, 8-15 x 2.4-3.2 µm
E90 E100	Unbranched, 80-130 x 2.4-4.8 µm	Integrated, narrow cylindrical, 35-60 x 1.6-3.2 µm	3-septate, 30-40 x 3.2-5.6 µm	Ellipsoidal to cylindrical, rounded ends, 6-15 x 3.2-5.6 µm

Fusarium is an anamorphic genus affiliated with Hypocreales (Ascomycetes). The sexual stages (teleomorphs) of the species are in *Gibberella* and *Nectria*. *Fusarium* is characterized by production of slimy, hyaline, septate, canoe-shaped conidia (known as macroconidia). In addition, some species also produce distinctly different conidia in the aerial mycelium (often referred to as microconidia). According to the species and/or the ecological situation, either macroconidia or microconidia may dominate on natural substrate. Chlamydospores are also produced by some species. Modern taxonomy of *Fusarium* is based mostly on cultural characters (Nelson et al., 1983; Booth, 1971; Gerlach and Nirenberg, 1982).

F. coccophilum (Desm.) Wollenw. and Reinking group has been a confirmed and accepted entomopathogen on Diaspididae (Evans and Prior, 1990). Although there are numerous reports of entomopathogenic *Fusarium* spp. (Gabriel, 1968; Steinhaus and Marsh 1962), considering the cosmopolitan nature of the genus, Evans and Hywel-Jones (1997) hinted that these may be opportunistic nectrotrophs or saprobes.

43. *Gibellula pulchra* (Sacc.) Cavara 1894. *Att. Instit. Bot. Univ. Pavia Ser. II,* **3:** 347.

(Plate. XIII-c,d; Fig. 4.1.28)

Entomogenous hyphomycete. Infected host attached to the underside of leaf. *Mycelium* dense, mat of yellow mycelium covering the spider host, composed of smooth, septate, branched, smooth, thin-walled, hyaline, 2-3 µm wide hyphae. *Conidiophores* mononematous, septate, unbranched, thick-walled, rough and dark brown below, becoming abruptly thinner and paler towards a slender apex, 200-400 x 11-18 µm, terminating in a swollen vesicle. *Conidial heads* globose, 40-65 µm in diam. *Vesicle* globose, 12-18 µm in diam. *Conidiogenous cells* biseriate, phialidic, discrete, smooth, cylindrical, with a short neck, 6-10 x 2-2.5 µm, up to 10 borne on each metula.

Metulae broadly obovoid, hyaline, numerous borne on vesicle. *Conidia* dry, catenate, aseptate, ellipsoidal to fusoid, smooth, hyaline, 2.5-3.5 x 2-3 µm.

Specimen examined: On an infected spider on leaves of *Dillenia indica*, Bhagawan Wildlife Sanctuary, Molem, Goa, India, 17.09.2001, 27°C, leg. KP, Herb. No. GUBH 6181; Slide No. GUBH 6181.

Gibellula pulchra was originally reported from Ilan county, Taiwan by Tzean et al. (1997). Although a synnematous fungus, above mentioned isolate was mononematous on spider hosts.

44. *Gliocladiopsis* sp.

Entomogenous Hyphomycetes: *Colonies* on CMA circular, dome-shaped, white with a pale yellow tinge in the centre, with smooth margin, attaining a diam. of 3-4 cm in 7 days, cottony, with watery droplets, reverse of the colony colourless. *Mycelium* mostly submerged composed of smooth, septate, freely branched, thin-walled, hyaline, 2-3 µm wide hyphae. *Conidiophores* mononematous, smooth, septate, thin-walled, hyaline, bearing a terminal penicillium, 2-4µm wide. *Conidiogenous cells* phialidic, discrete, 5-10 x 3-3.5 µm. *Conidia* slimy, cylindrical with obtuse ends in yellowish slimy masses, smooth, hyaline, 3-5 x 2.4-3 µm size.

Specimen examined: On a scale insect on fresh leaves of *Calotropis gigantea,* scrub jungle, GU campus, Taleigao plateau, Goa, India, 27°C, 18.09.2000, leg. KP, direct isolation, Culture No.GU/BOT/Moenf/C66, Slide No. C66. Additional specimen examined: i) On aphids on fresh leaves of *Crotalaria* sp., scrub jungle, iron ore mines, Cudnem, Goa, India, 27°C, 12.10.2000, leg. KP, direct isolation, Culture No.GU/BOT/Moenf/E108, Slide No. E108.

The fungus with its penicillia, cylindrical slimy conidia is morphologically similar to members of *Cylindrocladium*. However, absence of sterile stipe, 0-1 septate, cylindrical conidia with obtuse ends in pale yellow slimy masses keeps it away from *Cylindrocladium* spp. in *Gliocladiopsis*.

45. *Gliocladium* sp.1 **(Fig. 4.1.29)**

Entomogenous hyphomycete. *Colonies* on CMA circular, pale creamy white, with smooth hairy margin, growth median, attaining a diam. of 3-4 cm in 7 days, slimy, concentric rings of wet globules of conidia after initial 1-2 cm floccose growth with thick mycelial strands bearing short conidiophores and wet heads of conidia; reverse of the colony colourless. *Mycelium* composed of smooth, septate, branched, thin-walled, hyaline, 2-3 µm wide hyphae. *Conidiophores* two types: primary conidiophores of *Verticillium*-type, and secondary conidiophores of penicillate type, mononematous, smooth, sometimes pitted, septate, branched, thin-walled, hyaline, mostly 100-150 µm in length, 60-200 µm long, 2-3 µm at the base, 3-4 µm at the middle and 2-3 µm at the base of penicillia, the base of the conidiophore narrows. *Conidiogenous cells* phialidic, discrete, 10-25 x 2-3 µm tapering towards the tip. *Conidia* slimy, solitary, asymmetrically navicular, hyaline, 4-7 x 3-4 µm.

Specimen examined: On aphids on the fresh leaves of *Chromolaena odorata*, paddy field bunds, Pernem, Goa, India, 28°C, 29.06.1999, leg. KP, direct isolation, Culture No.GU/BOT/ICMR/E16, Slide No. E16. Additional specimen examined: i) on immature homopteran insects on the fresh leaves of *Ixora coccinea,* mixed farms, Kasaragod, Kerala, India, 33°C, 18.05.1999, leg. KP, direct isolation, Culture No. GU/BOT/ICMR/ E6, Slide No. E6. ii) On aphids on fresh leaves of *Chromolaena odorata,* scrub jungle, Pernem, Goa, India, 29°C, 29.06.1999, leg. KP, direct isolation, Culture No. GU/ BOT/ICMR/E13, Slide No. E13 iii) On aphids on fresh leaves of *Cassia tora,* scrub jungle, Pernem, Goa, India, 28°C, 29.06.1999, KP, direct isolation, Culture No. GU/BOT/ ICMR/E15, Slide No. E15. iv) On aphids on fresh leaves of *Calotropis gigantea*, scrub jungle, Pernem, Goa, India, 29°C, 29.06.1999, leg. KP, direct isolation, Culture No. GU/ BOT/ICMR/E19, Slide No. E19. v) On aphids on fresh leaves of on *Ixora coccinea,* scrub jungle mixed with paddy fields and plantation, Curchorem, Goa, India, 28°C, 22.07.1999, leg. KP, direct isolation, Culture No.GU/BOT/ICMR/E23, Slide No. E23. vi) On *Drosophila* sp. on fresh leaves of on *Strobilanthus ixiocephalus,* scrub jungle, GU campus, Goa, India, 28°C, 30.06.2000, leg. KP, direct isolation, Culture No. GU/BOT/ Moenf/E48, Slide No. E48.

Typified by *Gliocladium penicilloides* Corda, the genus *Gliocladium* Corda can be seen as a counterpart of *Penicillium* with slimy conidia. Characteristic features of the species are densely penicillate conidiophores with slimy heads of 1-celled hyaline or brightly pigmented conidia. Along with penicillate conidiophores, there are also

predominantly verticillate conidiophores. However, typically asymmetrical conidia of such members help in distinguishing them from *Verticillium* spp. Taxonomy of *Gliocladium* has been considered to be less satisfactory (Domsch et al., 1980). In a wider sense, presence of verticillate or penicillate conidiophores, with divergent conidiogenous cells, hyaline asymmetrically navicular conidia of 4-7 x 3-4 µm size of the above mentioned species brings it very close to *G. roseum*. In *G. roseum* conidia are with pink shades but any such colour was not found in the conidia of species described above.

46. *Gliocladium* sp. 2

Entomogenous hyphomycete. *Colonies* on CMA circular, creamy white, flat, smooth hairy margin, growth median attaining a diam. of 3-4cm in 7 days, slimy, with concentric rings of wet globules of conidia, reverse of the colony colourless; *Mycelium* composed of smooth, septate, branched, thin-walled, hyaline, 2-3 µm wide hyphae. *Conidiophores* mononematous, smooth, sometimes pitted, septate, branched, thin-walled, hyaline, mostly 100-150 (60-200) µm long, 2-3µm wide at the base, 3-4µm at the middle, 2-3µm at the base of penicillia, with base of the conidiophore abruptly narrow. *Conidiogenous cells* phialidic, discrete, 16-25 x 2-3 µm tapering towards the tip. *Conidia* slimy, solitary oval with twisted proximal tip, 4-6 x 2-3µm.

Specimen examined: On aphids on fresh leaves of *Chromolaena odorata,* scrub jungle mixed with paddy fields and plantation, Curchorem, Goa, India, 28°C, 22.07.1999, leg. KP, direct isolation, Culture No.GU/BOT/ICMR/E20, Slide No. E20.

47. *Gliocladium* sp. 3

Entomogenous hyphomycete. *Colonies* On CMA circular, creamy white, flat, smooth hairy margin, attaining a diam. of 5-6cm in 7 days, wet, with concentric rings of wet globules of conidia, reverse of the colony colourless. *Mycelium* composed of

smooth, septate, branched, thin-walled, hyaline, 2-3 μm wide hyphae. *Conidiophores* mononematous, penicillate, smooth, septate, branched, thin-walled, hyaline, 60-150 x 3-4 μm. *Conidiogenous cells* phialidic, discrete, 8-25 x 2.4-3.2 μm, sometimes up to 40 x 4.8μm, tapering towards the tip. *Conidia* slimy, solitary oval with twisted proximal tip, 3-7 x 2.4-4 μm.

Specimen examined: On ants on dead log of wood, scrub jungle, GU Campus, Taleigao Plateau, Goa, India, 28°C, 30.06.2000, leg. KP, direct isolation, Culture No. GU/BOT/ ICMR/E49, Slide No. E49.

The base of the conidiophore is not constricted as in *Gliocladium* sp. 1 & 2. Size of the conidium is bigger than that of former.

48. *Hirsutella* sp.1 **(Plate. XIII-a,b; Fig. 4.1.30)**

Entomogenous hyphomycete. Infected host attached to the underside of leaf, *Colonies* creamy white and spreading. *Mycelium* dense mat, creamy white to creamy gray, covering the unidentified insect host, composed of septate, branched, smooth, thin-walled, hyaline, highly interwoven, prostrate, 3-4 μm wide hyphae. *Conidiophores* arising from highly interwoven prostrate hyphal mat, mononematous, septate, unbranched, thin-walled, 25-30 x 2.5-3.5 μm. *Conidiogenous cells* mono- to polyphialidic, erect, hyaline, smooth, solitary, ampulliform to sub-cylindrical 12-18 x 2-3 μm with inflated base and abruptly tapering apex, forming a narrow and smooth to verrucose neck, up to 12 μm long and less than 1 μm wide. *Conidia* dry, catenate, aseptate, globose to obovoid, smooth, hyaline, 1.5-2 x 1-1.5 μm.

Specimen examined: On immature stages of homopteran insect on fresh leaves of *Hopea ponga*, SBWS, Molem, Goa, India, 16.12.2001, 31°C, leg. KP, Herb. No. GUBH 6155. Slide No. 6146. Additional specimen examined: i) On immature stages of homopteran insect on fresh leaves of *Hopea ponga*, SBWS, Molem, Goa, India, 21.07.2001, leg. KP, Herb. No. GUBH 6107. ii) On immature stages of homopteran insect on fresh leaves of *Hopea ponga*, SBWS, Molem, Goa, India, 05.08.2001, 28°C, leg. KP, Herb. No. GUBH 6111. iii) On immature stages of homopteran insect on fresh leaves of *Hopea ponga*, SBWS, Molem, Goa, India, 07.10.2001, 29°C, leg. KP, Herb. No. GUBH 6125. iv) On immature stages of homopteran insect on fresh leaves of *Hopea ponga*, SBWS, Molem, Goa, India, 18.11.2001, 28°C, leg. KP, Herb. No. GUBH 6146. v) On immature stages of homopteran insect on fresh leaves of

Hopea ponga, SBWS, Molem, Goa, India, 13.01.2002, 31°C, leg. KP, Herb. No. GUBH 6161. vi) On immature stages of homopteran insect on fresh leaves of *Hopea ponga*, SBWS, Molem, Goa, India, 24.03.2002, 33°C, leg. KP, Herb. No. GUBH 6175. vii) On immature stages of homopteran insect on fresh leaves of *Hopea ponga*, SBWS, Molem, Goa, India, 16.06.2002, leg. KP, Herb. No. GUBH 6179.

Hirsutella sp. was found on unidentified insects throughout the year on lower sides of leaves of *Hopea ponga* in the riparian habitats of Western Ghats, though the colonies were found infested and degraded by mites and mycoparasites during summer season. Interestingly, it did not produce any synnema during the year-long *in situ* observation (discussed in the later part of the thesis). It produced typical *Hirsutella* conidiogenous cells and conidia from prostrate hyphae on all parts of the colony.

49. *Hirsutella* sp. 2

Entomogenous hyphomycete. *Colonies* on CMA circular, cottony, dry, pale pink, margin hairy, attaining a diam. of 5-6 cm in 7days, with concentric rings, velvety, with water droplets, reverse of the colony colourless. *Mycelium* partly immersed, partly superficial composed of smooth, septate, branched, hyaline, thin-walled and thick-walled, 2-4 µm wide hyphae. *Conidiophores* erect, septate, smooth, thick-walled, hyaline, 4-6 µm wide with many whorls of conidiogenous cells with or without metulae attached to it terminally and sub-terminally; *Metulae* cylindrical, smooth, wavy, hyaline, 6-12 x 3-4 µm. *Conidiogenous cells* either on metulae, or solitary on hyphae, phialidic, discrete, bottle- shaped with highly wavy wall, with a long narrow tip (up to 25 µm), smooth, hyaline, 8-40 x 2.5-5 µm, sometimes with a septa below the narrow tip. *Conidia* solitary, smooth, 1-celled, ellipsoidal to narrowly cylindrical, sometimes slightly curved, with truncate base and rounded apex, hyaline, 4-15 x 2-4 µm.

Specimen examined: On infested homopteran larvae on leaves of a *Tridax procumbens*, forest, Tambdi Surla, Goa, India, 26^{0}C, 02.12.2000, leg. KP, direct isolation, Culture No.GU/BOT/Moenf/E128, Slide No. E128.

50. *Paecilomyces javanicus* (Friederichs & Bally) Brown & Smith, 1957. *Trans. Br. mycol. Soc.* **40**, 65. **(Plate. IV-d, Fig. 4.1.31)**

Entomogenous hyphomycete. *Colonies* on CMA circular, snow white, dry, powdery, slow growing, attaining a diam. of 0.8-1 cm in 7 days, margin rhizoidal, reverse of the colony colourless. *Mycelium* partly immersed and partly superficial, composed of smooth, septate, branched, thin-walled, hyaline, 1.5-2.5 µm wide hyphae. *Conidiophores* erect, smooth, septate, branched, thin-walled, hyaline, 1.5-2.5 µm with many whorls of conidiogenous cells; *metulae* cylindrical, clavate, smooth, hyaline, 6-12 x 2-3.5 µm. *Conidiogenous cells* in verticels of 2-10, divergent, rarely solitary, phialidic, discrete, flask-shaped with ovoid base, tapering to form distinct necks, smooth, hyaline, 5-14 x 2-4 µm. *Conidia* dry, catenate, 1-celled, ellipsoidal, smooth, hyaline, 4-7 x 1.5-2 µm.

Specimen examined: On infested lepidopteran larva on leaves of an unidentified riparian dicot plant, forest, Anmod, Goa, India, 30^{0}C, 23.03.2001, leg. KP, direct isolation, culture No. GU/BOT/MOENF/ E145, Slide No. E145.

The fungus is similar to the one reported from Taiwan and Japan (Wa tanabe, 2002; Tzean et al., 1997).

51. *Paecilomyces* sp. 1. **(Fig. 4.1.32)**

Entomogenous hyphomycete. *Colonies* on CMA circular, white, cottony in the centre, slow growing, attaining a diam. of 0.5-0.8 cm in 7 days, with concentric rings, margin rhizoidal, reverse of the colony colourless. *Mycelium* immersed and superficial composed of smooth, septate, branched, thin-walled, hyaline, 1.5-2.5 µm wide hyphae. *Conidiophores* erect, smooth, septate, branched, thin-walled, hyaline, 80-150 x 1.5-2 µm. *Conidiogenous cells* solitary or in verticels of 3-4, phialidic, discrete, narrow, subulate, smooth, hyaline, 25-60 x 1.5-2 µm. *Conidia* catenate, smooth, 1-celled, ellipsoidal to fusiform, hyaline, 2.5-3 x 1.5-2 µm.

Specimen examined: On infested undetermined insect on grass leaves, riparian forest, Ambe, Goa, India, 29°C, 31.07.2001, leg. KP, direct isolation, culture No. GU/BOT/MOENF/ E186, Slide No. E186.

In its verticellate nature of conidiogenous cells, the fungus resembles *P. puntoni* (Vuill.) Nannizzi (Samson, 1974), but conidia of *Paecilomyces* sp.1 is much smaller when compared to that of *P. puntoni,* i.e., 4.5-6.3 x 2.5-2.8 µm (Watanabe, 2002).

52. *Paecilomyces* sp. 2

Entomogenous hyphomycete. *Colonies* on CMA circular, pale pink, margin smooth to hairy, fast growing, attaining a diam. of 3-3.5 cm in 7days, in concentric rings, velvety, with water droplets on surface, reverse of the colony colourless. *Mycelium* partly immersed and partly superficial composed of smooth, septate, branched, thin-walled, hyaline, 1.5-2.5 µm wide hyphae. *Conidiophores* erect, verrucose, septate, thin-walled, hyaline, 80-200 x 1.5-3 µm with many whorls of conidiogenous cells with or without metulae attached terminally and sub-terminally; *metulae* cylindrical, smooth to minutely verruculose, hyaline, 6-12 x 3-5 µm. *Conidiogenous cells* phialidic, discrete, bottle- shaped with a narrow tip 2.5-4 µm, smooth to minutely verruculose, hyaline, 8-12 x 2.5-3 µm. *Conidia* catenate, smooth, 1-celled, ovoid to fusiform, hyaline, 3-4 x 1.5-2.5 µm.

Specimen examined: On infested aphids on leaves of *Chromolaena odorata*, scrub jungle, Pernem, Goa, India, 26^{0}C, 29.06.1999, leg. KP, direct isolation, culture No. GU/BOT/ ICMR/E12, Slide No. E12. Additional specimen examined: i) On infested aphids on leaves of *Anacardiun occidentale*, forest, Alorna, Goa, India, 26^{0}C, 22.10.2000, leg. KP, direct isolation, culture No. GU/BOT/ICMR/E116, Slide No. E116.

53. *Paecilomyces* sp. 3 **(Fig. 4.1.33)**

Entomogenous hyphomycete. *Colonies* on CMA circular, pale pink, margin smooth to hairy, dry, velvety, fast growing, attaining a diam. of 2-2.5 cm in 7days, with concentric rings, reverse of the colony colourless. *Mycelium* immersed and partly superficial composed of smooth, septate, branched, thin-walled, hyaline, 1.5-2.5 µm

wide hyphae. *Conidiophores* two types: short erect in the periphery, long flexuose at the centre forming mycelial strands, smooth, septate, thin-walled, hyaline, 80-200 x 1.5-3 µm; *metulae* cylindrical, smooth, hyaline, 8-22 x 2-3 µm. *Conidiogenous cells* either solitary or grouped to form a terminal or intercalary penicillium, phialidic, discrete, bottle shaped with a narrow tip, smooth, hyaline, 8-26 x 2-3 µm. *Conidia* catenate, smooth, 1-celled, ovoid to ellipsoidal, hyaline, 2-3.5 x up to 2 µm.

Specimen examined: On infested insect eggs on leaves of *Hopea ponga,* riparian forest, Ambe, Goa, India, 29°C; 31.07.2001; leg. KP, direct isolation, culture No. GU/ BOT/MOENF/ E190 & E194, Slide No. E190 & E194. Additional specimen examined: i) On dead *Anopheles* sp. 2nd instar larva in containers, Vasco, Goa, India, 26°C, 24.07.1999, leg. KP, direct isolation, culture No. GU/BOT/ICMR/C40, Slide No C40. ii) On dead mites on leaves of *Chromolaena odorata,* forest, Molem, Goa, India, 28°C, 13.09.1999, leg. KP, direct isolation, culture No. GU/BOT/ICMR/E31, Slide No E31. iii) On homopteran larva on leaves of *Myristica malabarica,* forest, Bondla National Park, Goa, India, 28°C, 22.10.2000, leg. KP, direct isolation, Culture No. GU/BOT/Moenf/ E112, Slide No E112. iv) On coleopteran adult on leaves of *Strobilanthus ixiocephalus*, forest, Molem, Goa, India, 30°C, 03.03.2001, leg. KP, direct isolation, Culture No. GU/ BOT/ Moenf/E142, Slide No E142. v) On a spider on leaves of *Dillenia indica*, forest, Anmod, Goa, India, 30°C, 23.03.2001, leg. KP, direct isolation, Culture No. GU/BOT/ Moenf/E147, Slide No E147. vi) On unidentified insect on leaves of *D. indica*, forest, Dhudhsagar, Goa, India, 30°C, 25.10.2001, leg. KP, direct isolation, Culture No. GU/BOT/ Moenf/E209, Slide No E209. v) On insect exuviae on leaves of *Holarrhena antidysenterica,* riparian, forest, Ambe, Goa, India, 29°C, 31.07.2001, leg. KP, direct isolation, culture No. GU/BOT/MOENF/ E193, Slide No. E193. vi) On undetermined insect on leaves of *H. antidysenterica,* forest, Molem, Goa State, sunny, 28°C, 06.12.2001, leg. KP, direct isolation, Culture No. GU/BOT/MOENF/E219, Slide No. E219. vii) On unidentified insect on the leaves of the *Careya arborea,* forest, Molem, Goa, India, 28°C, 06.12.2001, leg. KP, direct isolation, Culture No. GU/BOT/MOENF/ E221, Slide No. E221.

Paecilomyces sp. 3 resembles *P. fumosoroseus* (Wize) Brown and Smith, in the colony characters, dimensions of conidiophores and arrangement of conidiogenous cells. But the fungus described here produces only ovoid to ellipsoidal conidia 2-3.5 x up to 2 µm where as *P. fumosoroseus* reported to produce conidia ovoid, ellipsoidal, fusiform and sometimes irregularly companulate conidia of 3-4 x 1-2 µm (Domsch et al., 1980; Tzean et al., 1997).

54. *Paecilomyces* sp. 4 **(Fig. 4.1.34)**

Entomogenous hyphomycete. *Colonies* on SDA circular, brownish yellow, superficial hyphae raised in the middle, margin rhizoidal, velvety, powdery with watery droplets, concentric rings, growth fast attaining a diam. of 5-6 cm in 7days, reverse of

the colony colourless. *Mycelium* immersed and partly superficial composed of smooth, septate, branched, hyaline, thin-walled and thick-walled, 2-7 µm wide hyphae. *Conidiophores* smooth, septate, branched, thin-walled, hyaline, 20-300 x 3-6 µm, often come together to form prostrate synnematous strands; *metulae* cylindrical, smooth, hyaline, 8-16 x 4-7 µm. *Conidiogenous cells* either solitary or groups to form a terminal or intercalary penicillium, phialidic, discrete, bottle-shaped or lageniform with a narrow tip, smooth, hyaline, 12-22 x 3-6 µm (narrow tip, 2-6 x 1-2 µm). *Conidia* catenate, smooth, 1-celled, ellipsoidal to doliiform, hyaline, 3-5 x 2-3 µm. Second type conidia-like entities are produced on smooth, septate conidiogenous cells, 5-25 x 2-4 µm, globose to oval, pigmented, olive brown, thick-walled, 4-8 µm.

Specimen examined: On Lepidopteran larva on leaves of unidentified dicot herb, paddy field, Taleigao, Goa, India, 28^{0}C, 02.10.2000, leg. KP, direct isolation, culture No.GU/BOT/Moenf/E93, Slide No. E93. Additional specimen examined: i) On *Drosophila* sp. on leaves of unidentified dicot herb, scrub jungle, Taleigao, Goa, India, 28^{0}C, 02.10.2000, leg. KP, direct isolation, Culture No.GU/BOT/Moenf/E94, Slide No. E94. ii) On homopteran larva on leaves of unidentified dicot shrub, forest, Tambdi Surla, Goa, India, 28^{0}C, 25.07.2000, leg. KP, direct isolation, Culture No.GU/BOT/Moenf/E59, Slide No. E59.

56. *Paecilomyces* sp. 5 **(Fig. 4.1.35)**

Entomogenous hyphomycete. *Colonies* on SDA circular, with ridges, pale green with white periphery, dry green spore mass under thinly spread hyaline hairy hyphae projecting outward, margin smooth, reverse colourless, growth median attaining a diam. of 2-3 cm in 7days. *Mycelium* immersed and partly superficial composed of smooth, septate, branched, thin-walled, hyaline 1.5-2.5 µm wide. *Conidiophores* erect, smooth, septate, branched, thin-walled, hyaline, 2-4 µm wide. *Metulae* cylindrical to clavate, smooth, hyaline, 8-18 x 3-5 µm. *Conidiogenous cells* solitary or in penicillia, either terminal or intercalary, phialidic, discrete, bottle-shaped, smooth, hyaline, 5-12 x 2-3 µm *Conidia* dry, catenate, smooth, 1-celled, globose to ellipsoidal, green, 3-4 x 2-3 µm.

59. *Penicillium* sp. 1 **(Fig. 4.1.37)**

Entomogenous hyphomycete. *Colonies* on CMA circular, green with white periphery, wavy or smooth, hairy margin, median growth, attaining a diam. of 2.8-3.3cm in 7 days, powdery, with watery droplets, with concentric rings, reverse of the colony colourless. *Mycelium* composed of smooth, septate, branched, hyaline, 2 µm wide hyphae. *Conidiophores* mononematous, finely verrucose, pitted, septate, rarely branched terminally, 100-300 x 2-2.5 µm; *metulae* asymmetrical, verruculose, hyaline 15-40 x 3-4 µm, swollen at the distal tip 5-8 µm. *Conidiogenous cells*, phialidic, discrete, bottle- shaped, with a distinct neck, hyaline, 8-10 x 2-2.5 µm. *Conidia* dry, catenate, globose, verrucose, 1-celled and green, 3 µm.

Specimen examined: On an *Anopheles* sp. 2nd larva, from curing water at construction site, Panaji, Goa, India, 30°C, 20.04.1999, leg. KP, direct isolation, Culture No. GU/ BOT/ICMR/C2, Slide No. C2. Additional specimen examined: i) On a *Culex* sp. 2nd instar larvae, from a well, scrub jungle mixed with plantation, Bicholim, Goa, India, 27°C, 05.06.1999, leg. KP, moist chamber incubation, culture No. GU/BOT/ICMR/C29, Slide No. C29. ii) On an *Anopheles* sp. pupa, from water stored in plastic bucket, Vasco, Goa, India, 27°C, 26.07.1999, leg. KP, direct isolation, Culture No.GU/BOT/ICMR/C44, Slide No. C44.

60. *Penicillium* sp. 2 **(Fig. 4.1.38)**

Entomogenous hyphomycete. *Colonies* on CMA circular, pale yellow to green, with smooth hairy margin, median growth, attaining a diam. of 2.5cm in 7 days, flat, with watery exudates, with green conidial chains. *Mycelium* composed of smooth, septate, branched, thin-walled, hyaline, 2-2.5 µm wide hyphae. *Conidiophores* mononematous, smooth to finely verruculose, septate, unbranched, thin-walled, hyaline, 250-300 x 2-2.5 µm with a terminal penicillia. *Conidiogenous cells* phialidic, discrete, cylindrical tapering abruptly at the distal end, 7-14 x3-5 µm. *Conidia* catenate, ellipsoidal, aseptate, finely verruculose, greenish yellow, 4-5 x 2-3µm.

Specimen examined: On an *Anopheles* sp. 3rd instar larva, from water at road-side construction site, dry plateau, Porvorim, Goa, India, 29°C, 19.04.1999, leg. KP, direct isolation, Culture No.GU/BOT/ICMR/C4, Slide No. C4.

61. *Penicillium* sp. 3 **(Fig. 4.1.39)**

Entomogenous hyphomycete. *Colonies* on CMA circular, green, median growth, attaining a diam. of 2.5-3cm in 7days, with scanty superficial hyphae, with watery droplets, reverse of the colony colourless. *Mycelium* composed of smooth, septate, branched, thin-walled, hyaline, 1.6-2.4 µm wide hyphae. *Conidiophores* mononematous, smooth to verruculose, septate, branched terminally, thin-walled, hyaline, 100-300 x 1.6-2.4 µm; *metulae* asymmetrical, hyaline, verruculose, 7-18 x 3-4 µm, swollen into a vesicle 5-6 µm diam. *Conidiogenous cells* phialidic, discrete, attached laterally onto vesicle, closely packed, 5-11 x 2.4-3.2 µm. *Conidia* dry, catenate, globose to subglobose, aseptate, verruculose, 2-3µm in diam.

Specimen examined: On a *Culex* sp. 3rd instar larvae, from a drain, Vasco, Goa, India, 29°C, 05.05.1999, leg. KP, direct isolation, Culture No.GU/BOT/ICMR/C20, Slide No. C20. Additional specimen examined: i) On exuviae of unidentified mosquito larvae in transient rainwater accumulated on terrace, Althino, Panaji, Goa, India, 27°C, 26.07.1999, leg. KP, direct isolation, Culture No.GU/BOT/ICMR/C42, Slide No. C42. ii) On 12 immature stages of homopteran insects on *Ixora coccinea*, mixed farm, Kasaragod, Kerala, India, 29°C, 24.05.1999, leg. KP, moist chamber incubation, Culture No. GU/ BOT/ICMR/E9, Slide No. E9. iii) On scale insects on anthurium plants, GU glass house, Taleigao Plateau, Goa, India, 29°C, 16.08.1999, leg. KP, direct isolation, Culture No.GU/BOT/ICMR/E26, Slide No. E26.

62. *Penicillium* sp. 4 **(Fig. 4.1.40)**

Entomogenous hyphomycete. *Colonies* on CMA wavy circular, green with a white periphery, flat with a smooth margin, median growth, attaining a diam. of 2.5-4.3 cm in 7 days, powdery, with watery exudates, reverse of the colony colourless. *Mycelium* submerged and partly superficial, composed of smooth, septate, branched, hyaline, 2 µm wide hyphae. *Conidiophores* mononematous, smooth to finely verruculose, septate, branched only near apical region, 100-200 x 2-3 µm; *metulae* symmetrical, finely verrucose, hyaline, 7-15 x 2-3 µm, swollen to 5-8 µm at the tip.

Conidiogenous cells phialidic, discrete, bottle-shaped, with a distinct neck, hyaline, 7-13 x 2-3 µm. *Conidia* catenate, globose, verrucose, one celled and green, 2-3 µm.

Specimen examined: On an *Anopheles* sp. 3rd instar larvae, from curing water at construction sites, Panaji, Goa, India, 29°C, 20.04.1999, leg. KP, direct isolation, culture No.GU/BOT/ICMR/C5, Slide No. C5. Additional specimen examined: i) On a *Culex* sp. 2nd instar larvae, from a drain, Vasco, Goa, India, 27°C, 03.06.1999, leg. KP, moist chamber incubation, Culture No.GU/BOT/ICMR/C26, and Slide No. C26. ii) On an *Anopheles* sp. 3rd instar larva, pond in a paddy field, Taleigao, Goa, India, 26°C, 22.09.1999, leg. KP, direct isolation, Culture No.GU/BOT/ICMR/C51, Slide No. C51. iii) On moribund homopteran larvae on *Chromolaena odorata,* mixed farm, Kasaragod, Kerala, India, 31°C, 07.05.1999, leg. KP, Culture No.GU/BOT/ICMR/E2, Slide No. E2. iv) On moribund immature stages of homopteran insects on *C. odorata,* plantation, Pernem, Goa, India, 29°C, 25.06.1999, leg. KP, direct Isolation, culture No.GU/BOT/ICMR/E11, and Slide No. E11.

The identity of isolates of *Penicillium* could not be established down to species level, though distinctive features were recognized in their morphology. These are tabulated below.

Isolat-e	Host	Colony (cm)	Conidiophore (µm)	Metulae (µm)	Conidiogeno-us cells (µm)	Conidia (µm)
C2	*Anopheles* sp. 2nd instar larva	Wavy circular 2.8-3.3	Verruculose 100-300 x 2-2.5	Asymmetrical 15-40 x 3-4	8-10 x 2-2.5	Verrucose 3-4
C20	*Culex* sp. 3rd instar larva	Circular 2.5-3	Smooth 200-300 x 2-2.5	Asymmetrical 7-11x 3	9-11 x 3	Verrucose 2.5-3
C5	*Anopheles* sp. 3rd instar larva	Wavy circular 3-3.5	Verruculose 100-150 x 3	Symmetrical 7-8 x 3	7-8 x 3	Verrucose 2-3
C4	*Anopheles* sp. 3rd instar larva	Circular 2.5	Verruculose 250-300 x 2-2.5	Asymmetrical 15-25 x 3.2-4	7-14 x 3-5	Verruculose 4-5 x 2-3

63. *Pleurothecium* sp. **(Fig. 4.1.41)**

Entomogenous hyphomycete. *Colonies* on SDA circular, light brown, margin smooth, fast growing, attaining a diam. of 5 cm in 7 days, cottony, with water droplets, creamy white to pale pink, with margin rhizoidal, wavy elevations, reverse of the colony pale yellow. *Mycelium* composed of smooth, septate, freely branched, thin-walled, hyaline, 1.6-3.2 µm wide hyphae. *Conidiophores* mononematous, variedly branched, smooth, septate, hyaline, 1.6-2.4 µm wide. *Conidiogenous cells* polyblastic, integrated,

hyaline, denticulate, 10-60 x 1.6-2.4 µm. *Conidia* solitary, ellipsoidal to obovoid, smooth, 0-septate, hyaline, 4-14 x 3-5 µm.

Specimen examined: On immature stages of homopteran insects on abaxial surface of unidentified dicot plant, undershrub forest, Tambdi Surla, Goa, India, 28^{0}C, 25.07.2000, leg. KP, direct isolation, Culture No.GU/BOT/Moenf/E55, Slide No. E55.

64. *Trichoderma* sp. 1 **(Fig. 4.1.42)**

Entomogenous hyphomycete. *Colonies* on CMA circular, hyaline submerged hyphae with green patches of conidial mass and rhizoidal margin, fast growing, attaining a diam. of 9cm in 7 days, slimy, fruit-bodies in bush like patches, initially white later changing to green, with watery exudates, reverse of the colony colourless. *Mycelium* composed of smooth, septate, freely branched, thin-walled, hyaline, 4-7 µm wide hyphae. *conidiophores* mononematous, coarse with tubercles, septate, profusely branched, thin-walled, hyaline, 40-70 x 2-3µm. *Conidiogenous cells* phialidic, discrete, rough, hyaline, 5-7x 3-3.5µm with a very narrow beak like distal tip. *Conidia* globose, smooth, sticky forming balls of 10-15 conidia over each conidiogenous cell, green, 3µm.

Specimen examined: On an *Anopheles* sp. 3rd instar larvae, curing water at construction sites, Porvorim, Goa, India, 30°C, 23.04.1999, leg. KP, direct isolation, culture No.GU/BOT/ICMR/C13, Slide No. C13.

65. *Trichoderma* sp. 2

Entomogenous hyphomycete. *Colonies* on CMA circular, hyaline hyphae with green patches of conidial mass, fast growing, attaining a diam. of more than 9cm in 7 days, wavy rhizoidal margin, slimy, with watery exudates, conidial mass in bush like patches of green, reverse of the colony colourless. *Mycelium* composed of smooth, septate, freely branched, thin-walled, hyaline, 3-7 µm wide hyphae. *Conidiophores*

mononematous, smooth, septate, branched, thin-walled, hyaline, 30-40 x 2-5 μm. *Conidiogenous cells* phialidic, discrete, smooth, 5-7 x 3-3.5 μm, with a very narrow beak like distal tip. *Conidia* globose, smooth, forming sticky balls of 10-15, green, 3μm diam.

Specimen examined: On a *Culex* sp. 3rd instar larvae, from curing water at construction sites, Cuncolim, Goa, India, 28°C, 24.07.1999, leg. KP, direct isolation, culture No.GU/BOT/ICMR/C36, Slide No. C36.

66. *Trichoderma* sp. 3 **(Fig. 4.1.43)**

Entomogenous hyphomycete. *Colonies* on CMA circular, hyaline submerged hyphae with, superficial patches of green conidial mass and rhizoidal margin, growth fast attaining a diam. more than 9cm in 7 days, slimy, fruit-bodies in patches, initially white later changing to green, with watery exudates, reverse of the colony colourless. *Mycelium* composed of smooth, septate, freely branched, thin-walled, hyaline, 4-7 μm wide hyphae. *Conidiophores* mononematous, coarse with tubercles, septate, profusely branched, thin-walled, hyaline, 30-40 x 2-3 μm. *Conidiogenous cells* phialidic, discrete, rough, 5-7x 3-3.5 μm with a very narrow beak like distal tip. *Conidia* globose, smooth, forming sticky balls of 10-15 on each conidiogenous cell, green, 2-3 μm diam.

Specimen examined: On an *Anopheles* sp. 3rd instar larva, curing water at construction sites, Cuncolim, Goa, India, 26°C, 24.09.1999, leg. KP, direct isolation, culture No.GU/BOT/ICMR/C37, Slide No. C37. Additional specimen examined: i) From a *Culex* sp. 3rd instar larva, from water stored in plastic bucket, Vasco, Goa, India, 27°C, 26.07.1999, leg. KP, direct isolation, Culture No.GU/BOT/ICMR/C48, Slide No. C48.

67. *Trichoderma* sp. 4

Entomogenous hyphomycete. *Colonies* on CMA circular, flat, hyaline with smooth margin, fast growing, attaining a diam. more than 9cm in 7 days, slimy, fruit-bodies in patches, initially white later changing to green, reverse of the colony colourless. *Mycelium* slightly superficial composed of smooth, septate, freely branched,

thin-walled, hyaline, 4-7 µm wide hyphae. *Conidiophores* mononematous, smooth, septate, profusely branched, thin-walled, hyaline, 25-30 x 2-3µm. *Conidiogenous cells* phialidic, discrete, 5-7x 3-3.5 µm. *Conidia* wet, sub-globose to elliptical, smooth, sticky forming masses, yellowish green, 3-5 x 2.4-3µm size.

Specimen examined: On a *Culex* sp. 3rd instar larva, from a water tank, Pernem, Goa, India, 27°C, 22.09.1999, leg. KP, direct isolation, culture No.GU/BOT/ICMR/C52, Slide No. C52. Additional specimen examined: i) On an *Anopheles* sp. 3rd instar larva, from rain water accumulated in abandoned mineral water bottles, Pernem, Goa, India, 27°C, 15.10.1999, leg. KP, direct isolation, culture No.GU/BOT/ICMR/C53, Slide No. C53. ii) On a *Culex* sp. 3rd instar larva, from curing water at construction sites, Cuncolim, Goa, India, 26°C, 18.11.1999, leg. KP, direct isolation, culture No.GU/BOT/ICMR/C54, Slide No. C54. iii) On a spider cadaver on leaf of *Dillenia indica*, forest, SBWS, Molem, Goa, India, 26°C, 13.09.1999, leg. KP, direct isolation, Culture No.GU/BOT/ICMR/E37, Slide No. E37. iv) On numerous moribund mites on leaves of *Terminalia paniculata*, forest, SBWS, Molem, Goa, India, 26°C, 15.10.1999, Culture No.GU/BOT/ICMR/E39, Slide No E39. v) On aphids on *Chromolaena odorata*, forest, Bondla national Park, Goa, India, 26°C, 18.10.1999, leg. KP, direct isolation, Culture No.GU/BOT/ICMR/E42, Slide No. E42. vii) On a *Culex quinquefasciatus* 3rd instar larvae, forest, Bondla National Park, Goa, India, 27°C, 15.10.1999, leg. KP, Isolation using baits, Culture No. GU/BOT/ ICMR/W6, Slide No. W6.

68. *Trichoderma* sp. 5 **(Fig. 4.1.44)**

Entomogenous hyphomycete. *Colonies* on CMA circular, wet, slimy, cottony, watery droplets margin rhizoidal, superficial hyphae abundant, 0.6-0.7 cm height, inoculation point flat, slimy green conidial mass thinly dispersed throughout, fast growing, attaining a diam. of 9cm in 7 days, reverse colourless on CMA, orange on SDA. *Mycelium* composed of smooth, septate, freely branched, thin-walled, hyaline, 1.6-5.6 µm wide hyphae. *Conidiophores* mononematous, smooth, septate, profusely branched, thin-walled, hyaline, up to 140 x 2.4-3.2 µm. *Conidiogenous cells* phialidic, discrete, with inflated base, narrow beak like apex, 4.6-12 x 2.4-3.2 µm. *Conidia* sub-globose to ellipsoidal, smooth, sticky, forming masses, green, 1.6-3.2 µm diam. *Chlamydospores* present, ellipsoidal, pyriform to rectangular, thick-walled, hyaline, 7-14 x 3.2-5.6 µm.

Specimen examined: On infested scale insects on fresh leaves of unidentified grass in paddy fields, Talcigao, Goa, India, 27°C, 02.10.2000, leg. KP, isolation using baits, Culture No.GU/BOT/Moenf/E106, Slide No. E106.

The identity of isolates of *Trichoderma* could not be established down to species level, though distinctive features recognized in their morphology are tabulated below.

Isolate	Conidiophore (µm)	Conidiogenous cells (µm)	Conidia (µm)
C13	Coarse, 40-70 x 2-3	Rough, 5-7 x 3-3.5	3
C36	Smooth, 30-40 x 2-5	Smooth, 5-7 x 3-3.5	3
C37	Coarse, 30-40 x 2-3	Rough, 5-7 x 3-3.5	2-3
C52	Smooth, 25-30 x 2-3	Smooth, 5-7 x 3-3.5	3-5 x 2.4-3
E106	Smooth, 140 x 2.4-3.2	Smooth, 4.6-12 x 2.4-3.2	1.6-3.2

69. Undetermined taxon 1 (C7)

Entomogenous hyphomycete. *Colonies* on CMA circular, flat with hairy rhizoidal margin, white with radial pattern, growth fast attaining a diam. more than 9cm in 7 days, with watery droplets, with mycelium becoming superficial on the edges of plates, reverse of the colony colourless. *Mycelium* smooth, septate, freely branched, thin-walled, hyaline, 2-3µm wide hyphae. *Hyphal bodies* thick-walled with 5-15 cells, 3-4µm wide; numerous rhomboidal to tetrahedral crystals encasing the hypha seen. Not sporulating on CMA and SDA.

Specimen examined: On an *Anopheles* sp. 2nd instar larva, from curing water at construction sites, Porvorim, Goa, India, 30°C, 20.04.1999, leg. KP, direct isolation, Culture No.GU/BOT/ICMR/C7, Slide No. C7. Additional specimen examined: i) On an *Anopheles* sp. 2nd instar larva, collected from transient water collection at construction site, Porvorim, Goa, India, 29°C, 23.04.1999, leg. KP, direct isolation, culture No.GU/BOT/ICMR/C17, Slide No. C17. ii) On a *Culex* sp. 2nd instar larva, from well, Pernem, Goa, India, 28°C, 04.06.1999, leg. KP, direct isolation, Culture No. GU/BOT/ ICMR/C27, Slide No. C27.

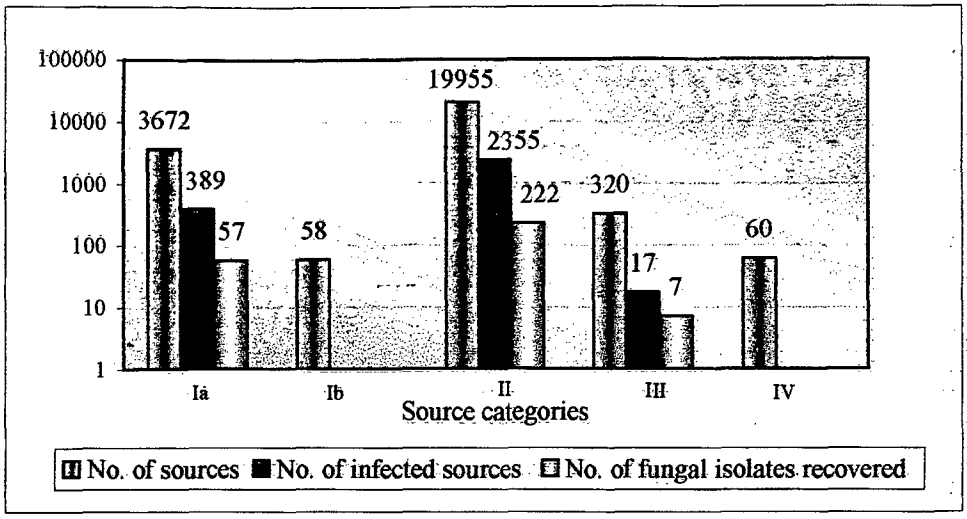
70. Undetermined Taxon 2 **(Fig. 4.1.45)**

Entomogenous hyphomycete. *Colonies* on CMA circular, cottony, floccose, moderately fast growing, attaining a diam. of 5.5 cm in 7 days, cottony, margin rhizoidal, reverse of the colony colourless. *Mycelium* composed of smooth, septate,

freely branched, hyaline, thin- and thick-walled, 2.4-4 μm wide hyphae. *Conidiogenesis* thallic, formed by the breaking up of thick-walled hyphal cells. *Conidia* rectangular to subglobose, thick-walled, single celled, hyaline, 4-6.4 x 4-4.8 μm; numerous rhomboidal and tetrahedral crystals are seen. Sometimes these crystals encased the hypha.

Specimen examined: On infected unidentified insect eggs on fresh leaves of unidentified dicot plant, forest, Cotigao, Goa, India, 28^{0}C, 22.07.2001, leg. KP, direct isolation, Culture No. GU/BOT/Moenf/E182, Slide No. E182.

Fig. 4.1.1. Sourcing of entomogenous fungi



Note: Ia - mosquito larvae; Ib - mosquito adults; II - other insects and arachnids; III - larval baits in water samples; IV - larval baits in simulation float chambers

Fig. 4.1.2. Recovery of fungi from mosquito larvae

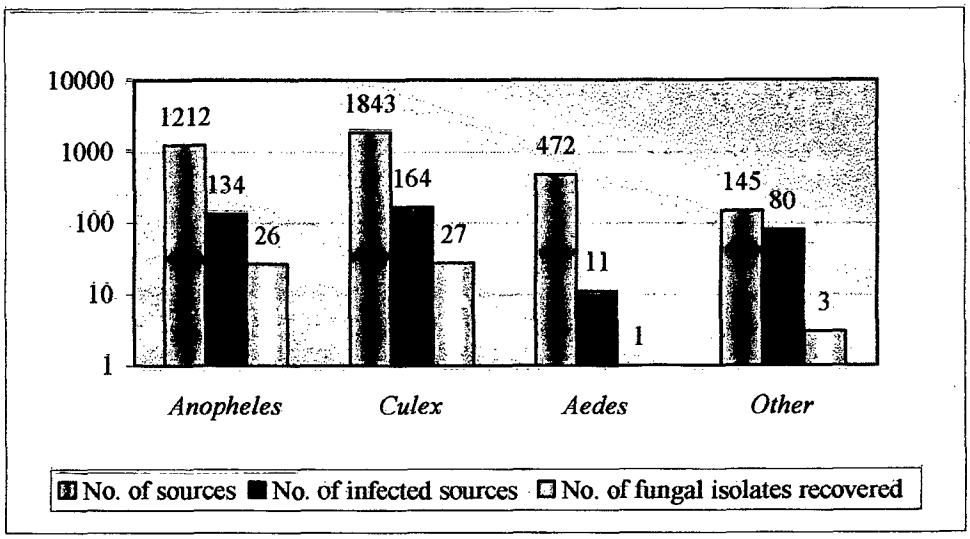


Fig. 4.1.3. Recovery of fungi from non-mosquito insects and arachnids

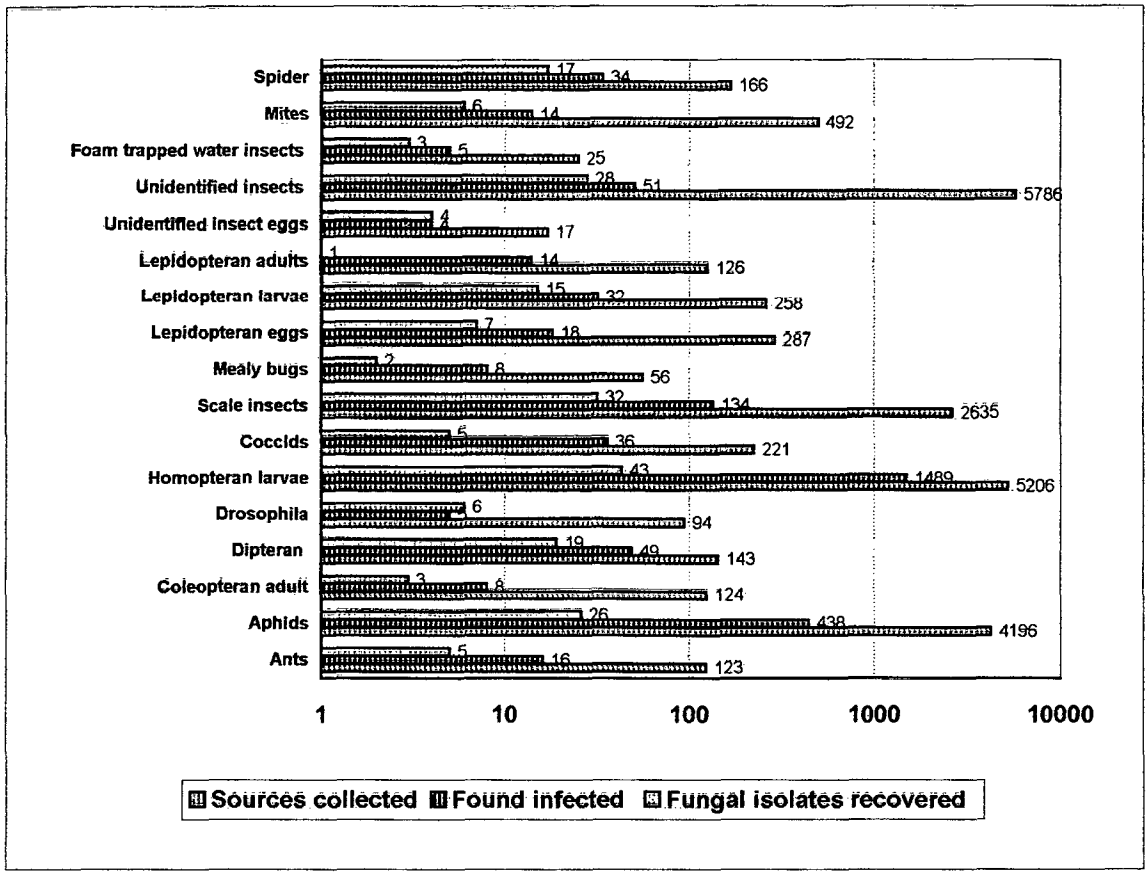
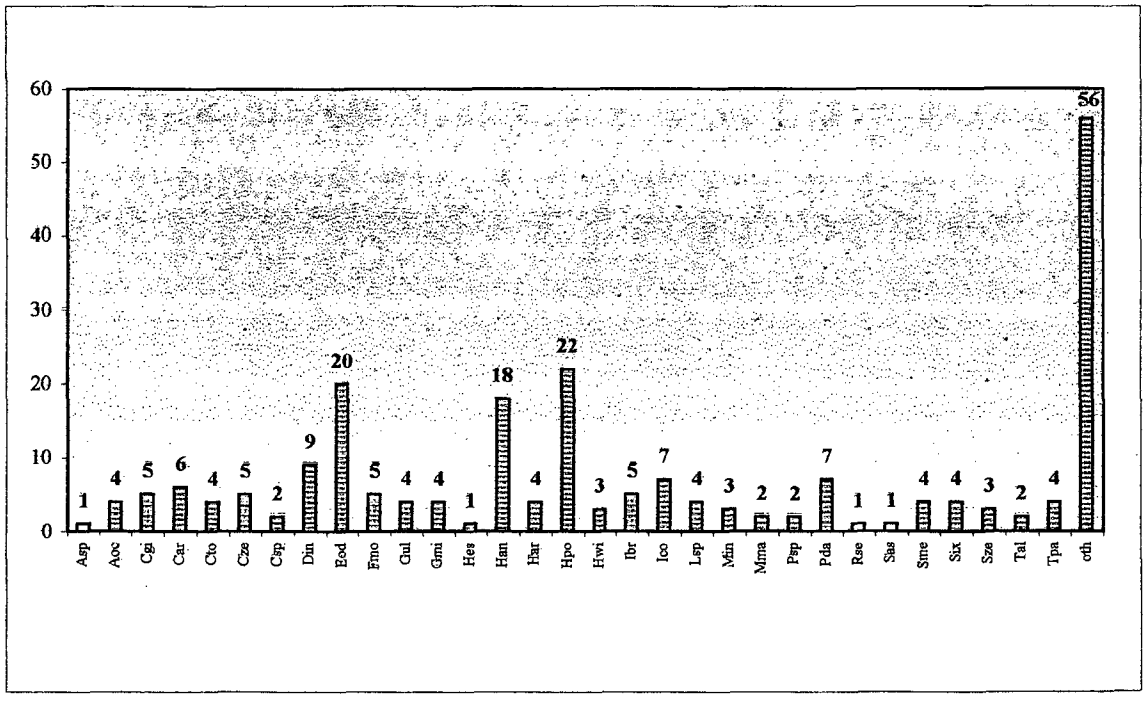
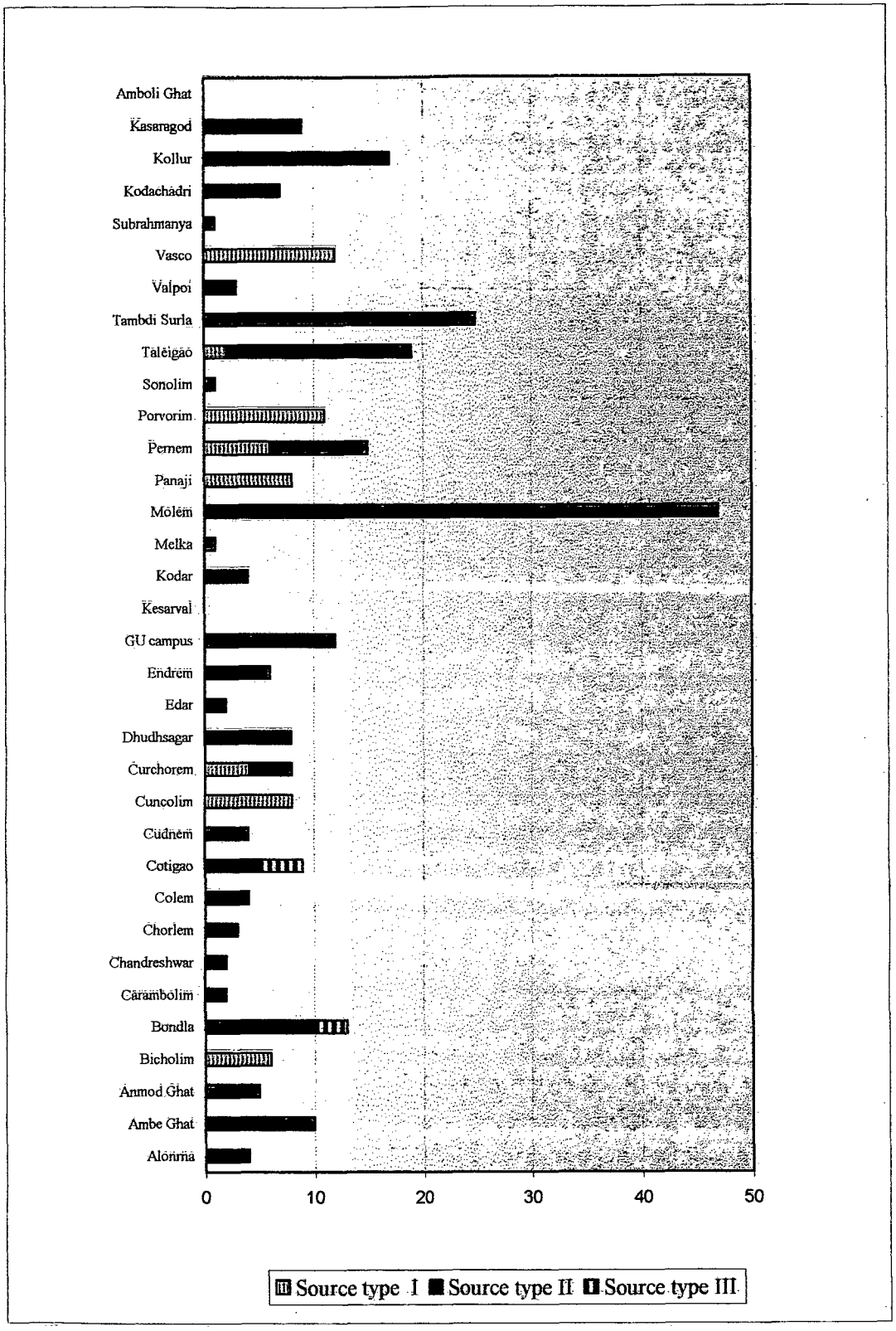


Fig. 4.1.4. Recovery of fungal isolates from insects and arachnids infesting different plant hosts.



Note: Legends are given in Table.

Fig. 4.1.5. Localitywise recovery of fungal isolates different type of sources



Note: source type I - mosquito larvae/pupae; II - other insects and arachnids; III - water sample

LEGENDS FOR FIG. 4.1.6. TO 4.1.45.

Fig. 4.1.6. ***Conidiobolus obscurus.*** Conidiophores and conidia.

Fig. 4.1.7. ***Hypocrella*** **sp.** (a) Vertical section through the stroma showing pycnidium and perithecium; (b) Asci; (c) Portion of asci showing apical cap and ascospores; (d) Ascospores.

Fig. 4.1.8. ***Podonectria*** **sp.** (a) Vertical section through the stroma showing perithecia; (b) Asci; (c) An ascospore.

Fig. 4.1.9. ***Aschersonia aleyrodis.*** (a) Vertical section through the stroma showing pycnidia; (b) Conidiophores with conidia; (c) Conidia.

Fig. 4.1.10. ***Aschersonia badia.*** (a) Vertical section through the stroma showing pycnidia; (b & c) A single pycnidium enlarged, (d) Conidiophores with conidia; (e) Conidia.

Fig. 4.1.11. ***Aschersonia brunnea.*** (a) Vertical section through the stroma showing pycnidium; (b) Conidiophores with conidia; (c) Conidia.

Fig. 4.1.12. ***Aschersonia indica.*** (a) Vertical section through the stroma showing pycnidia; (b) Conidiophores with conidia; (c) Conidia.

Fig. 4.1.13. ***Acremonium charticola.*** Conidiophores and conidia.

Fig. 4.1.14. ***Acremonium*** **sp. 3.** Conidiophores and conidia.

Fig. 4.1.15. ***Acremonium*** **sp. 4.** Conidiophores and conidia.

Fig. 4.1.16. ***Acremonium*** **sp. 5.** Conidiophores and conidia.

Fig. 4.1.17. ***Acremonium*** **sp. 6.** Conidiophores and conidia.

Fig. 4.1.18. ***Aspergillus clavatus*** Conidiophores and conidia.

Fig. 4.1.19. ***Aspergillus fumigatus*** Conidiophores and conidia.

Fig. 4.1.20. ***Aspergillus japonicus*** var. ***aculeatus*** Conidiophores and conidia.

Fig. 4.1.21. ***Aspergillus niger*** var. ***awamori*** Conidiophore and conidia.

Fig. 4.1.22. ***Aspergillus oryzae*** var. ***oryzae*** Conidiophores and conidia.

Fig. 4.1.23. ***Aspergillus restrictus*** Conidiophores and conidia.

Fig. 4.1.24. ***Aspergillus*** **sp. 1** Conidiophore and conidia.

Fig. 4.1.25. ***Aspergillus*** **sp. 5** Conidiophores and conidia.

Fig. 4.1.26. ***Aspergillus*** **sp. 6** Conidiophores and conidia.

Fig. 4.1.27. ***Cylindrocladium*** **sp.** Conidiophore with characteristic sterile stipe and conidia.

Fig. 4.1.28. ***Gibellula pulchra*** Conidiophores and conidia.

Fig. 4.1.29. ***Gliocladium*** **sp.1** Conidiophores and conidia.

Fig. 4.1.30. ***Hirsutella*** **sp.1** Conidiophores and conidia.

Fig. 4.1.31. ***Paecilomyces javanicus*** Conidiophores and conidia.

Fig. 4.1.32. ***Paecilomyces*** **sp. 1.** Conidiophores and conidia.

Fig. 4.1.33. ***Paecilomyces*** **sp. 3.** Conidiophores and conidia.

Fig. 4.1.34. ***Paecilomyces*** **sp. 4.** Conidiophores and conidia.

Fig. 4.1.35. ***Paecilomyces*** **sp. 5.** Conidiophores and conidia.

Fig. 4.1.36. ***Paecilomyces*** **sp. 7.** Conidiophores and conidia.

Fig. 4.1.37. ***Penicillium*** **sp. 1** Conidiophore and conidia.

Fig. 4.1.38. ***Penicillium*** **sp. 2** Conidiophore and conidia.

Fig. 4.1.39. ***Penicillium*** **sp. 3** Conidiophore and conidia.

Fig. 4.1.40. ***Penicillium*** **sp. 4** Conidiophore and conidia.

Fig. 4.1.41. ***Pleurothecium*** **sp.** Conidiophores and conidia.

Fig. 4.1.42. ***Trichoderma*** **sp. 1.** Conidiophore and conidia.

Fig. 4.1.43. ***Trichoderma*** **sp. 3.** Conidiophore and conidia.

Fig. 4.1.44. ***Trichoderma*** **sp. 5** Conidiophore and conidia.

Fig. 4.1.45. Undetermined taxon 2. Conidiophores and conidia.

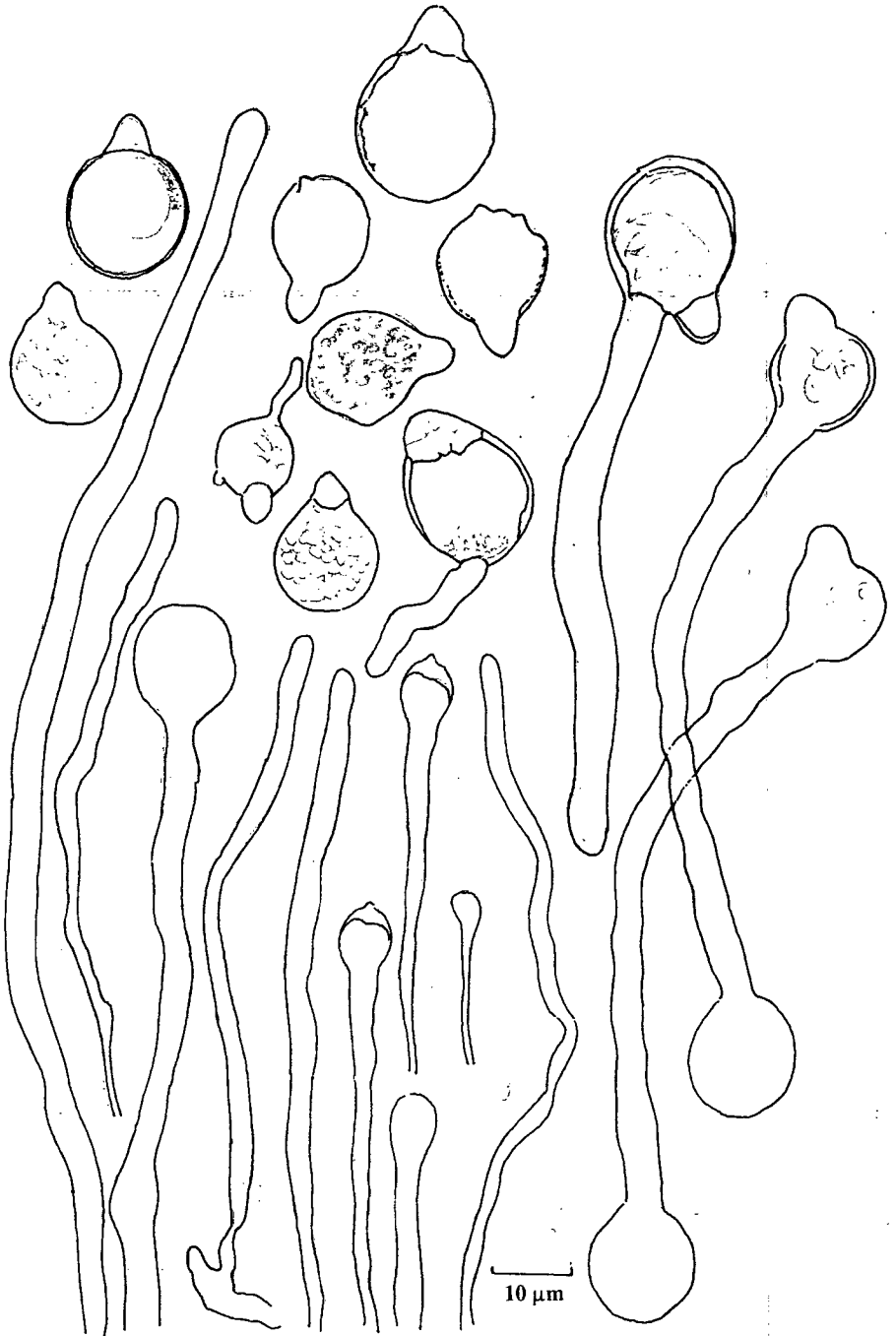


Fig. 4.1.6

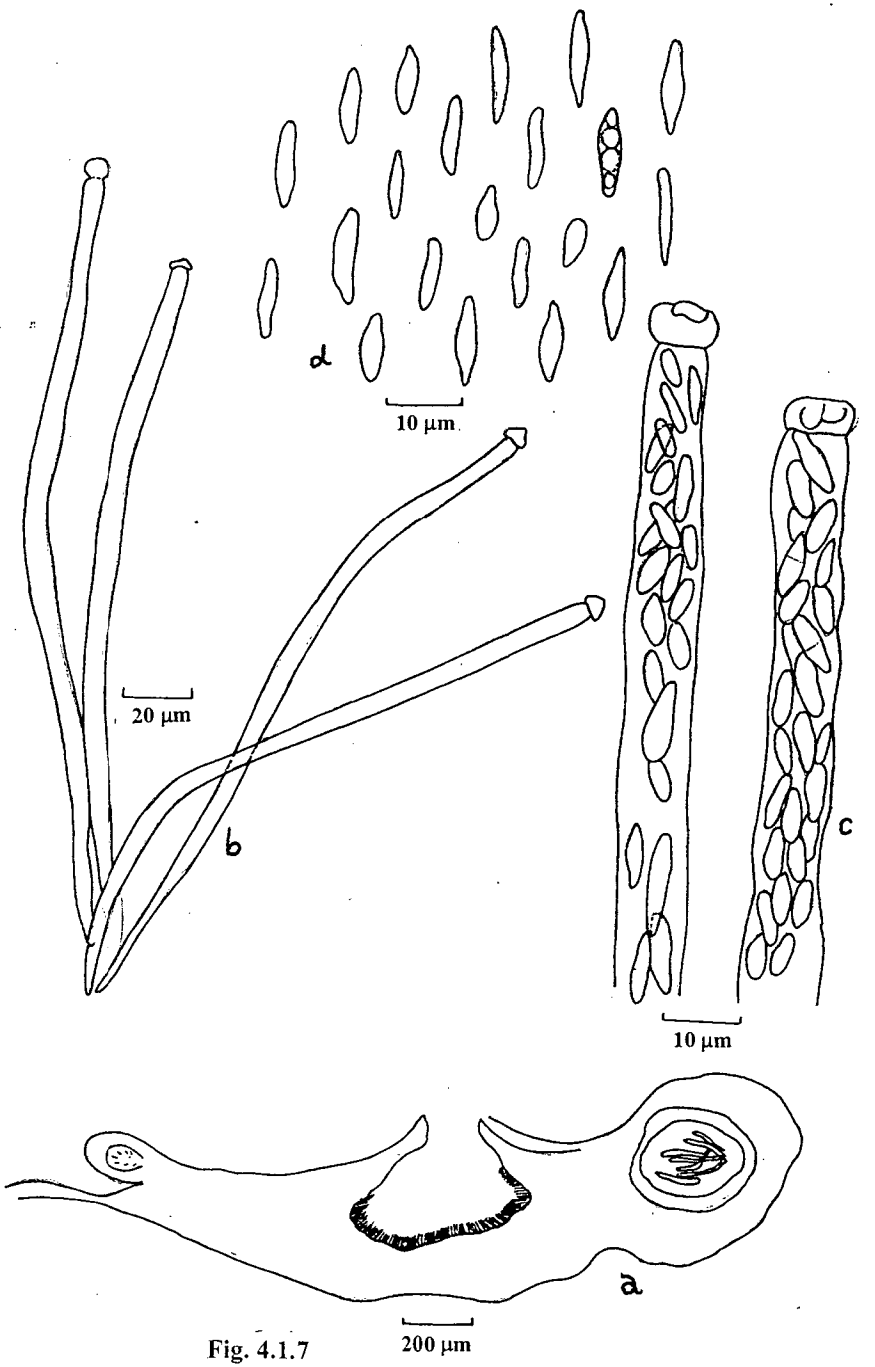


Fig. 4.1.7

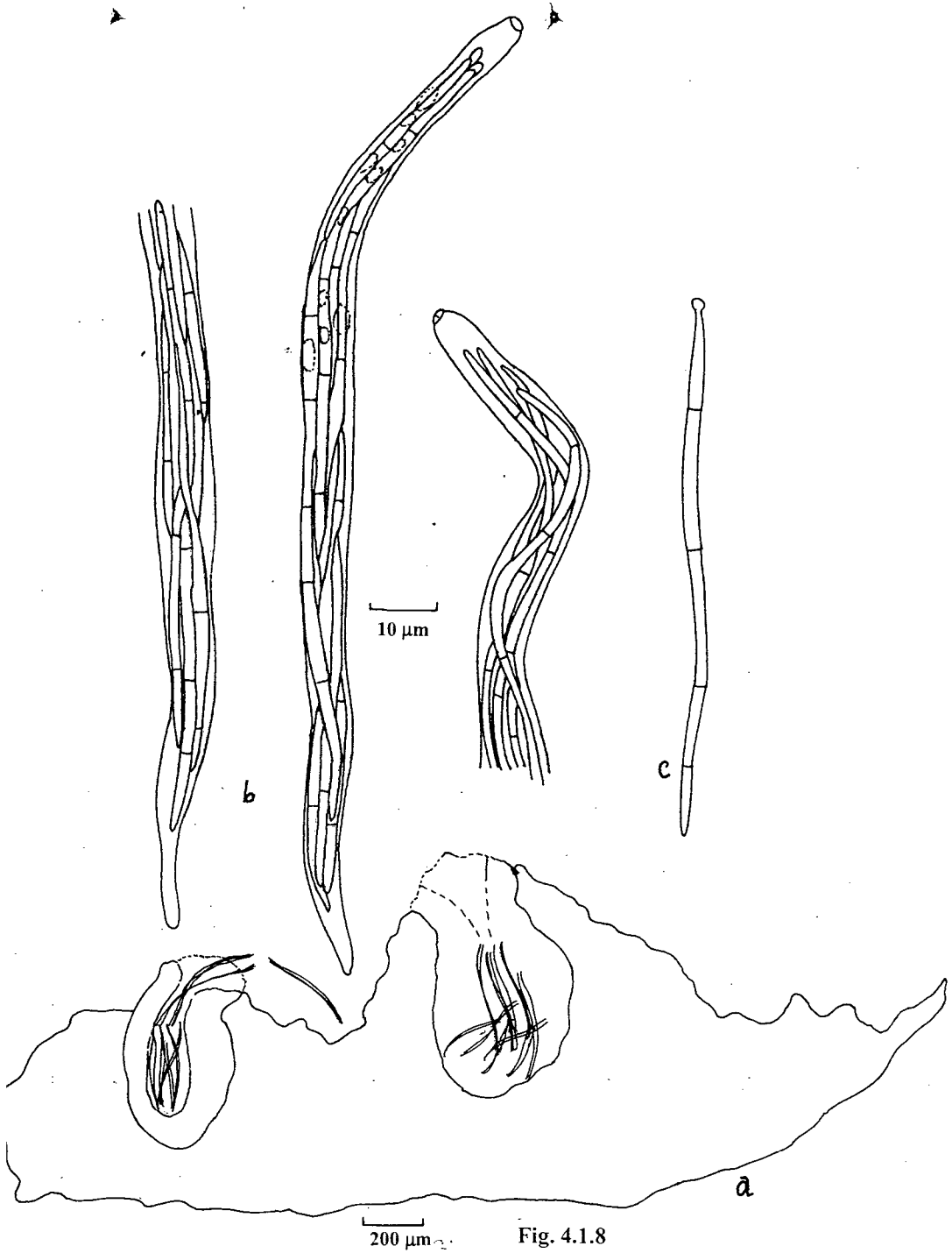


Fig. 4.1.8

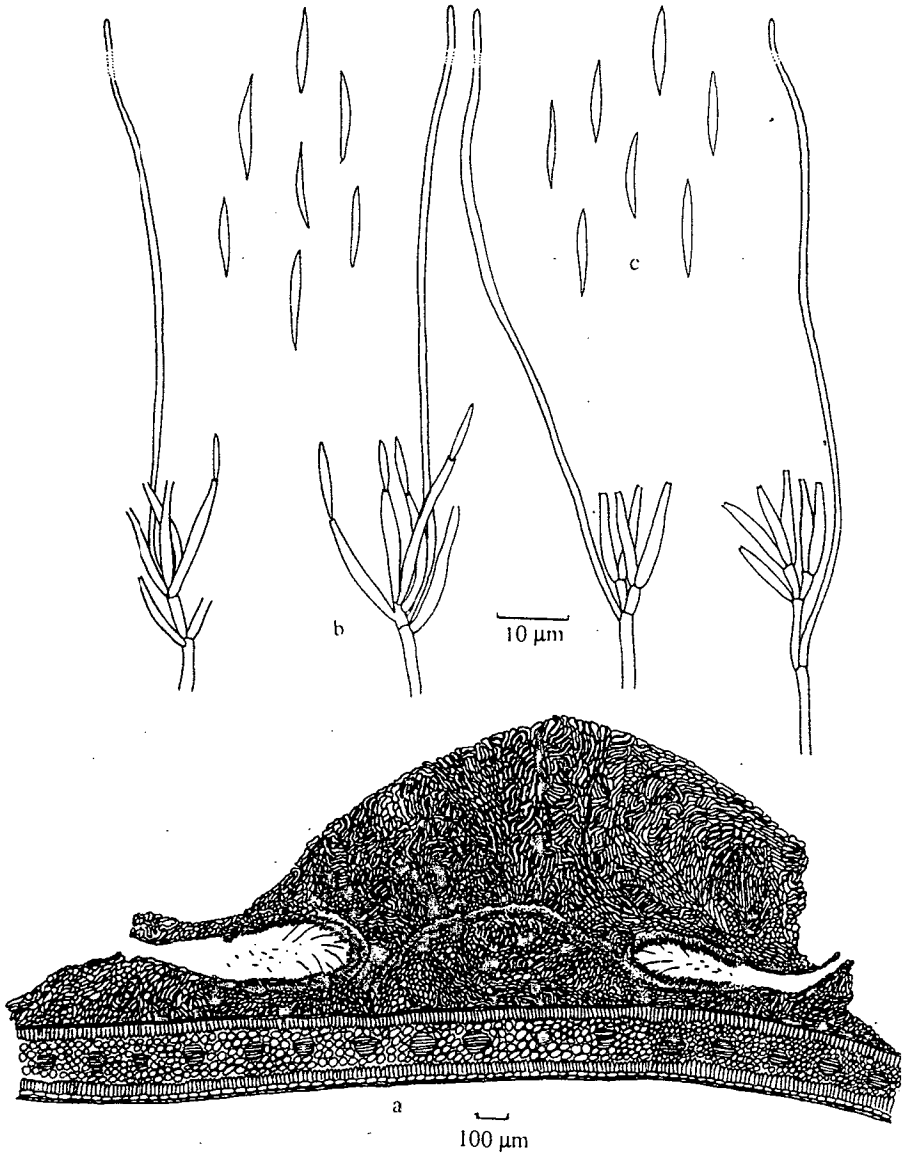


Fig. 4.1.9

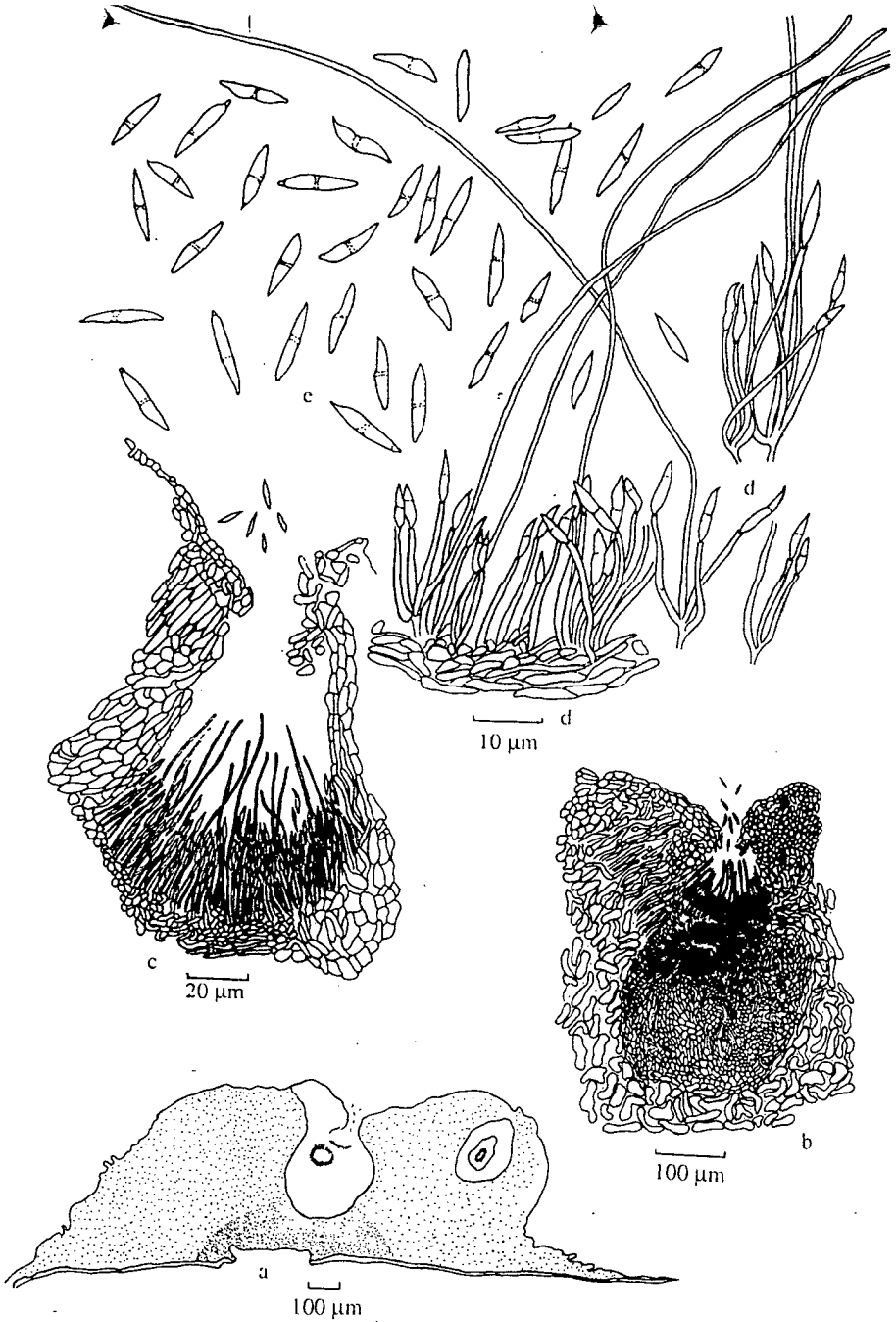


Fig. 4.1.10

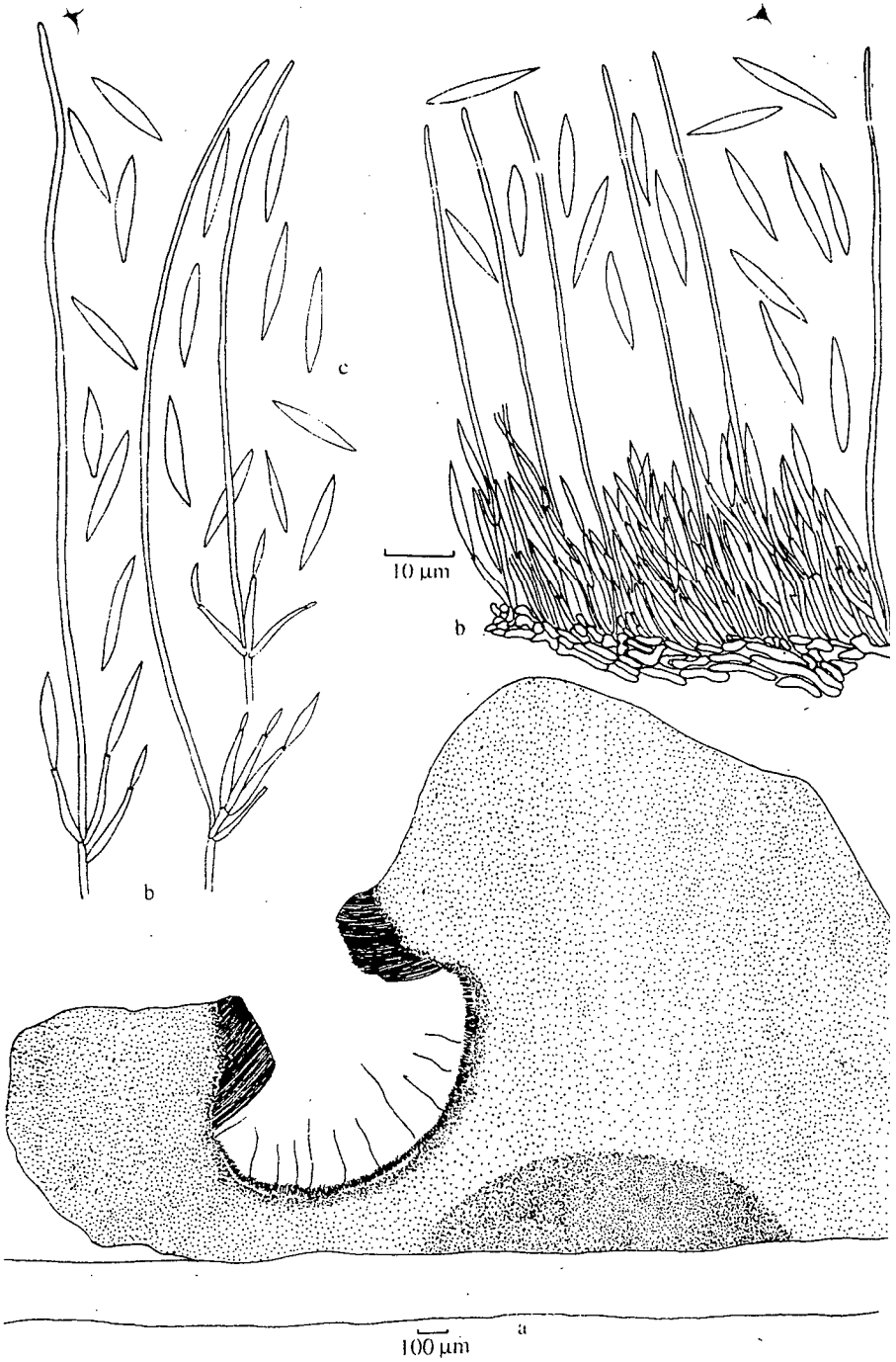


Fig. 4.1.11

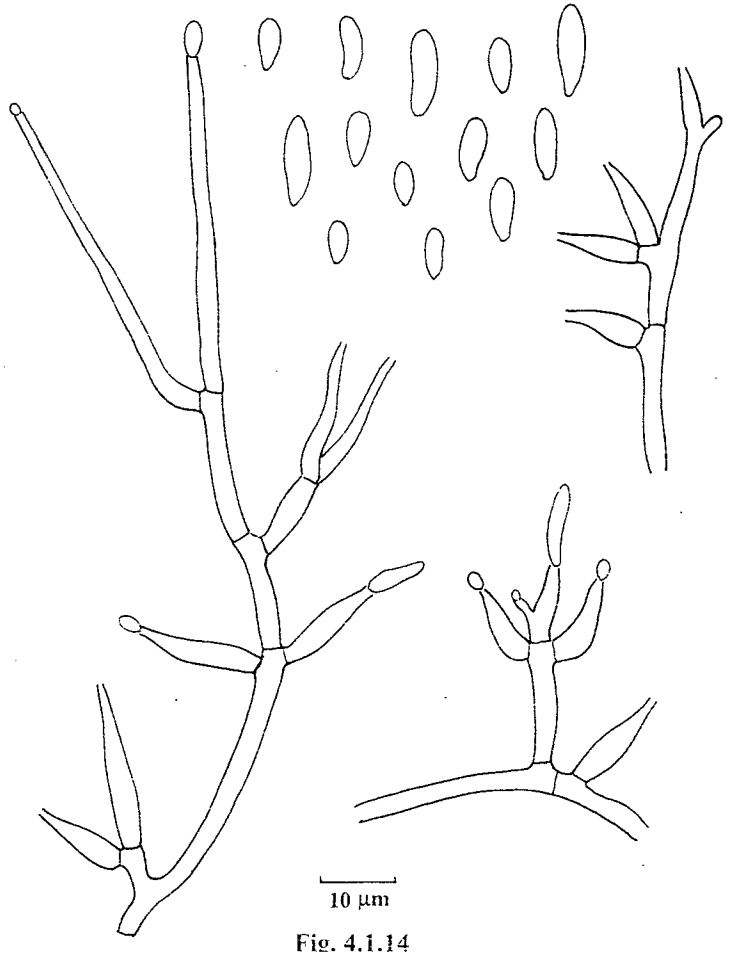


Fig. 4.1.14

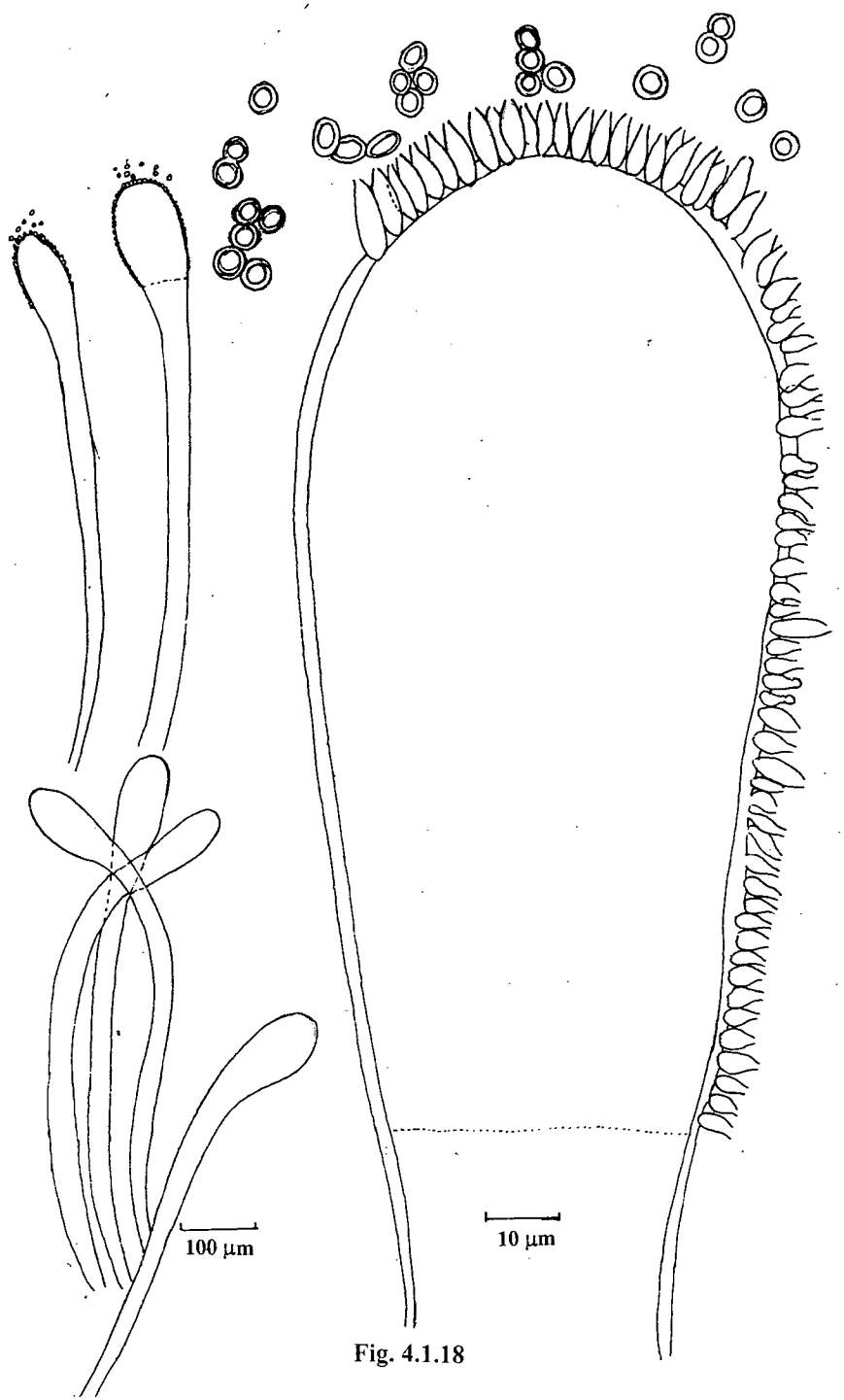
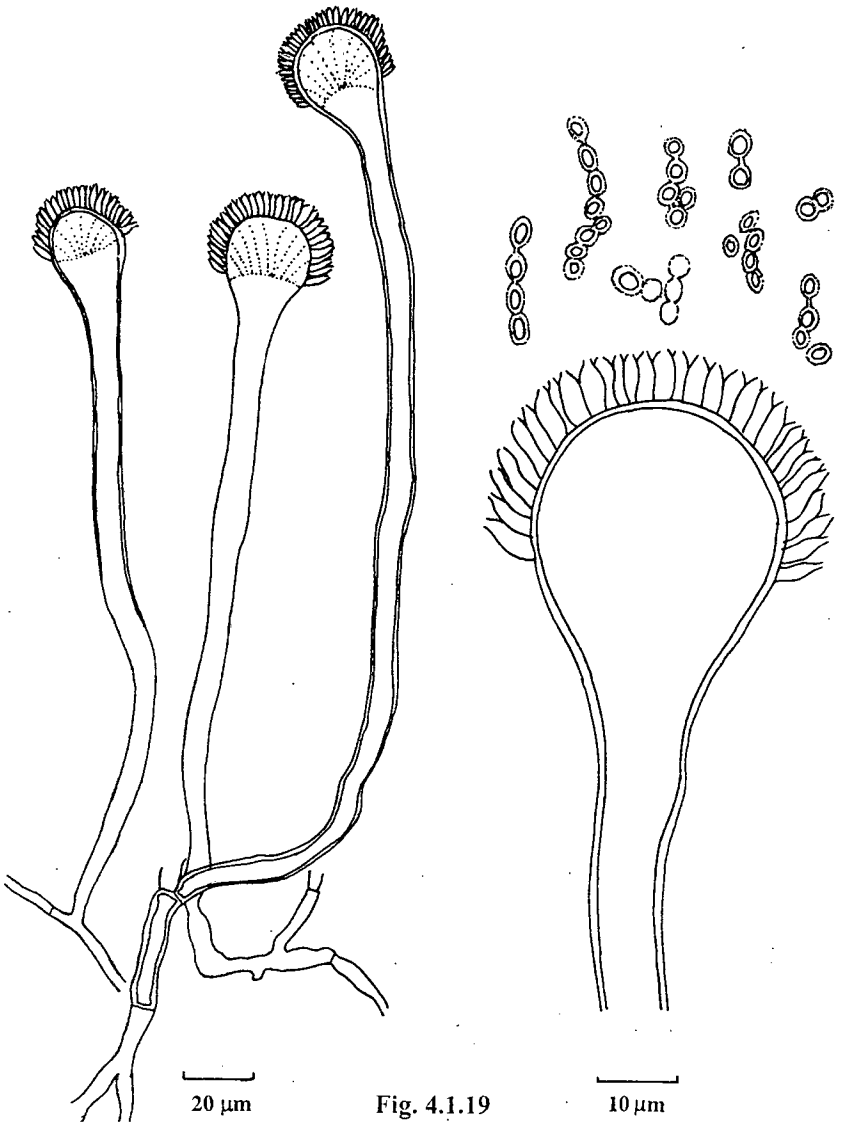


Fig. 4.1.18



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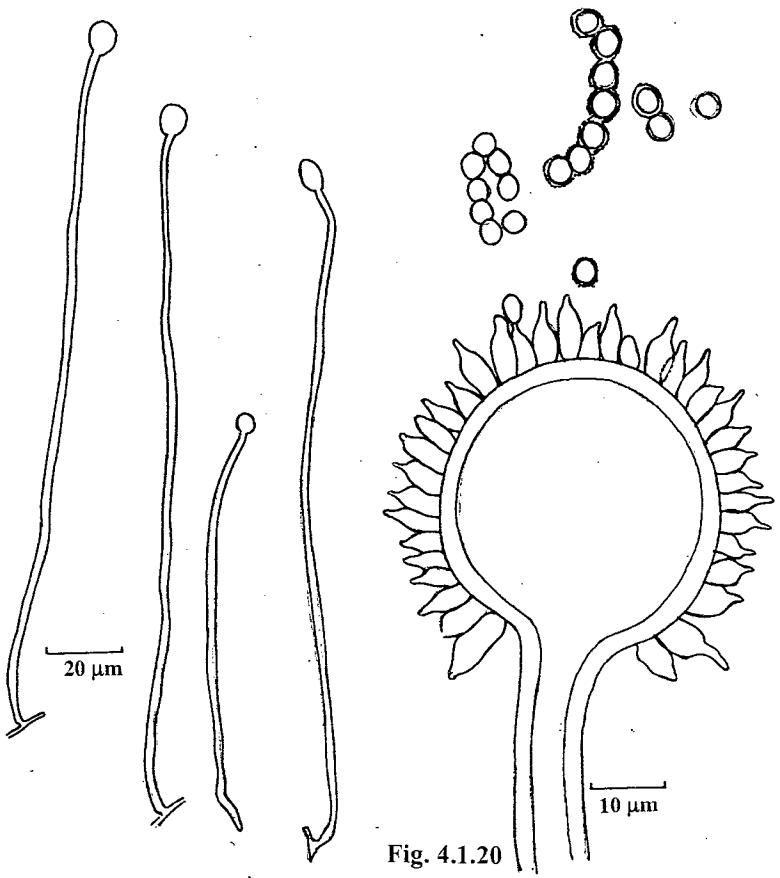


Fig. 4.1.20

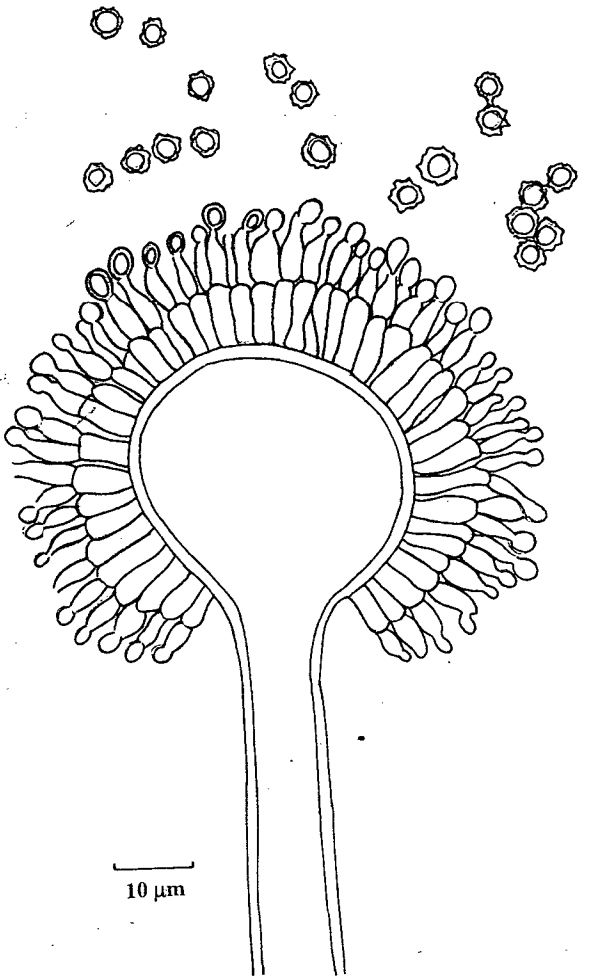
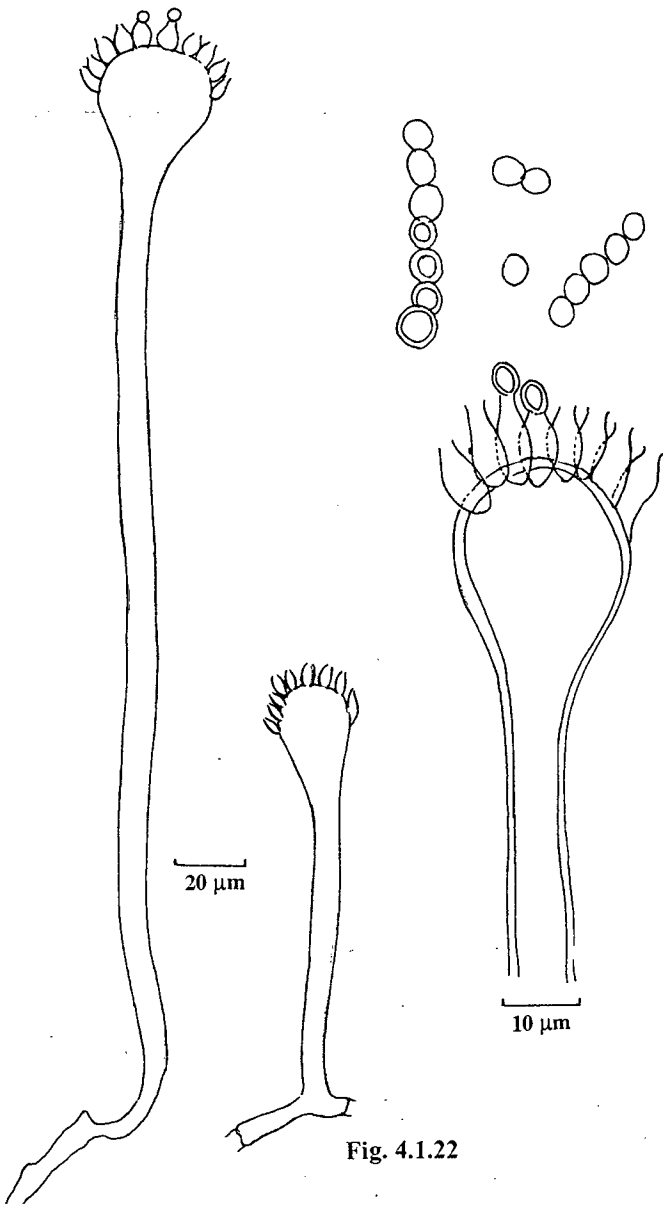


Fig. 4.1.21



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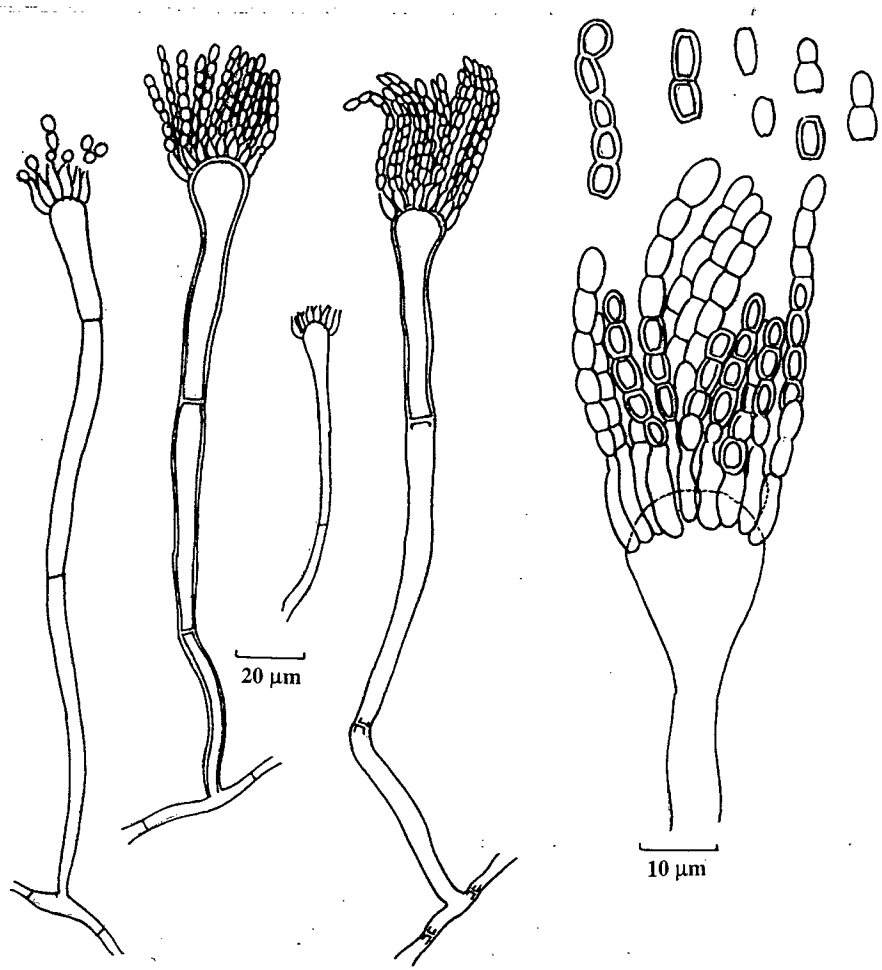
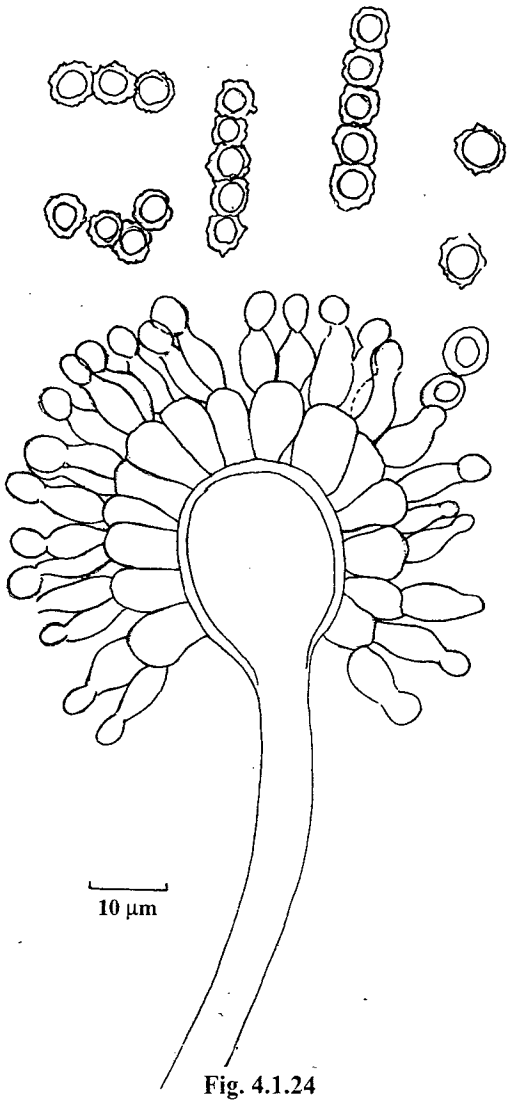


Fig. 4.1.23



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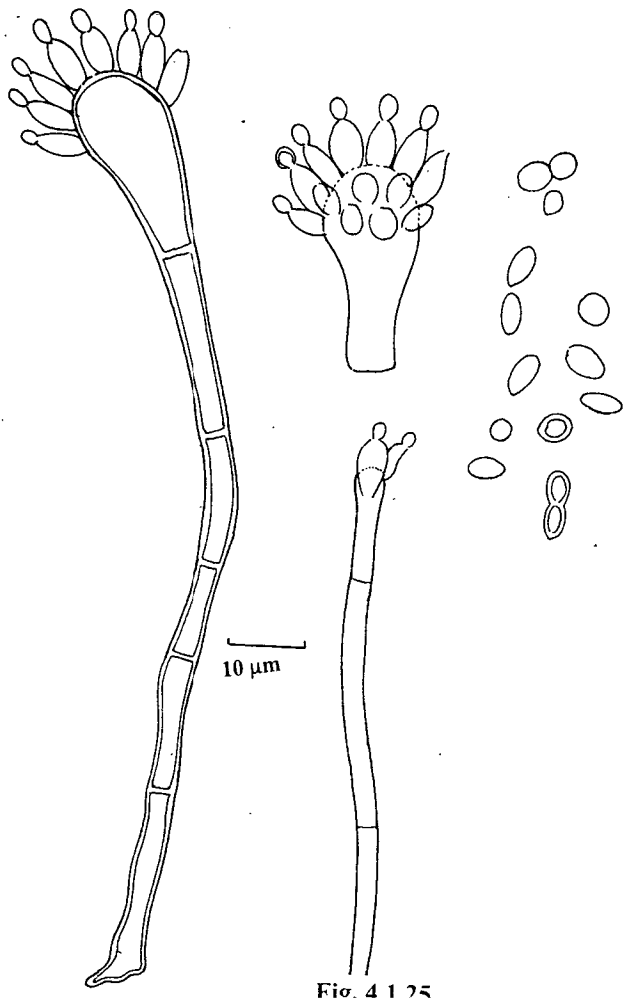

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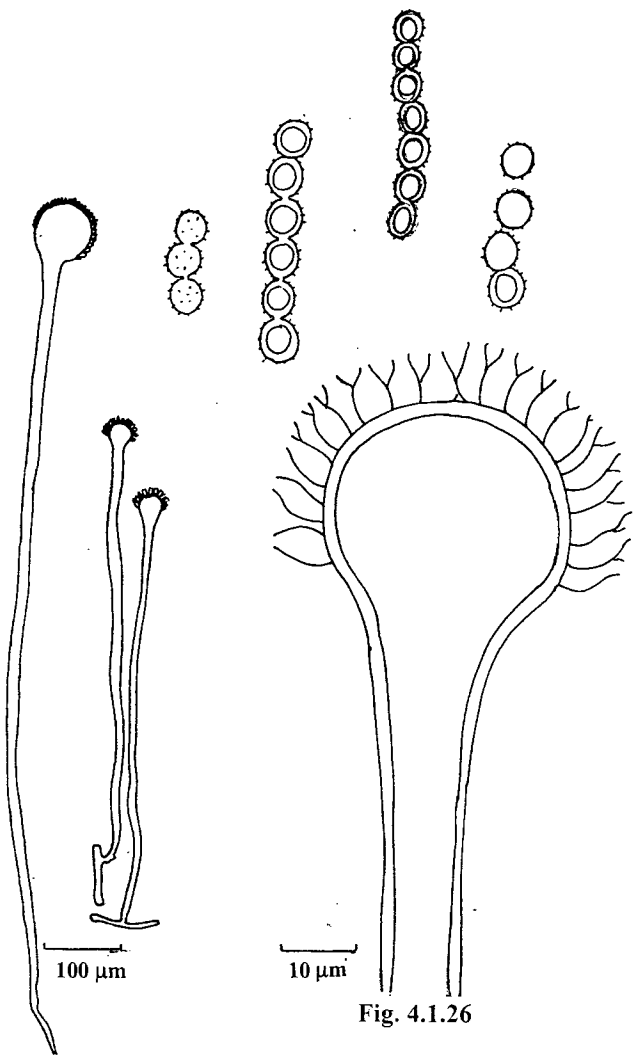

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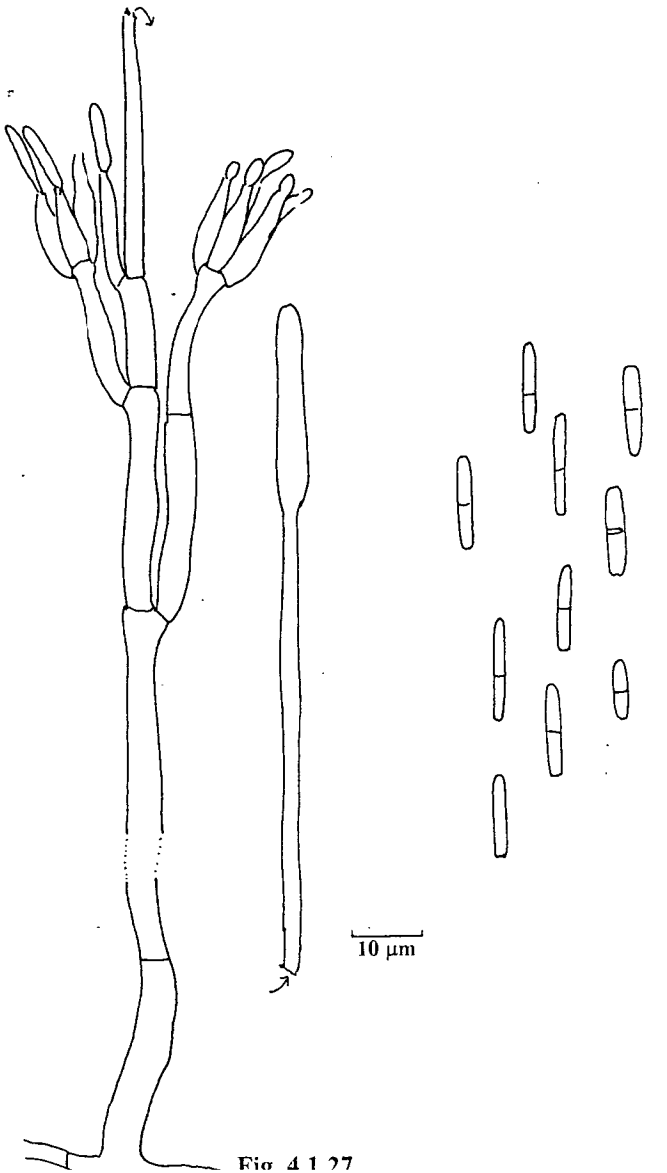

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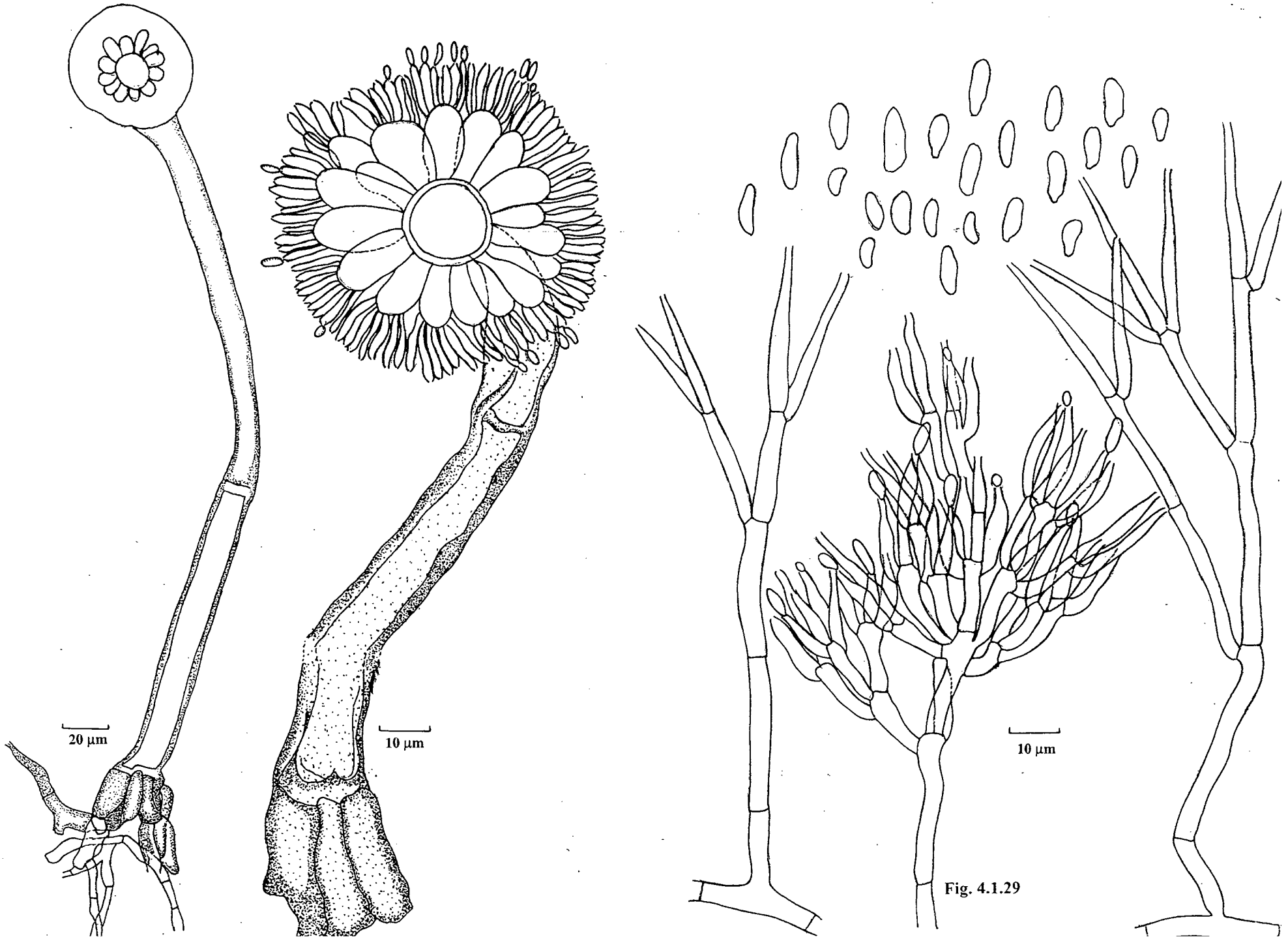

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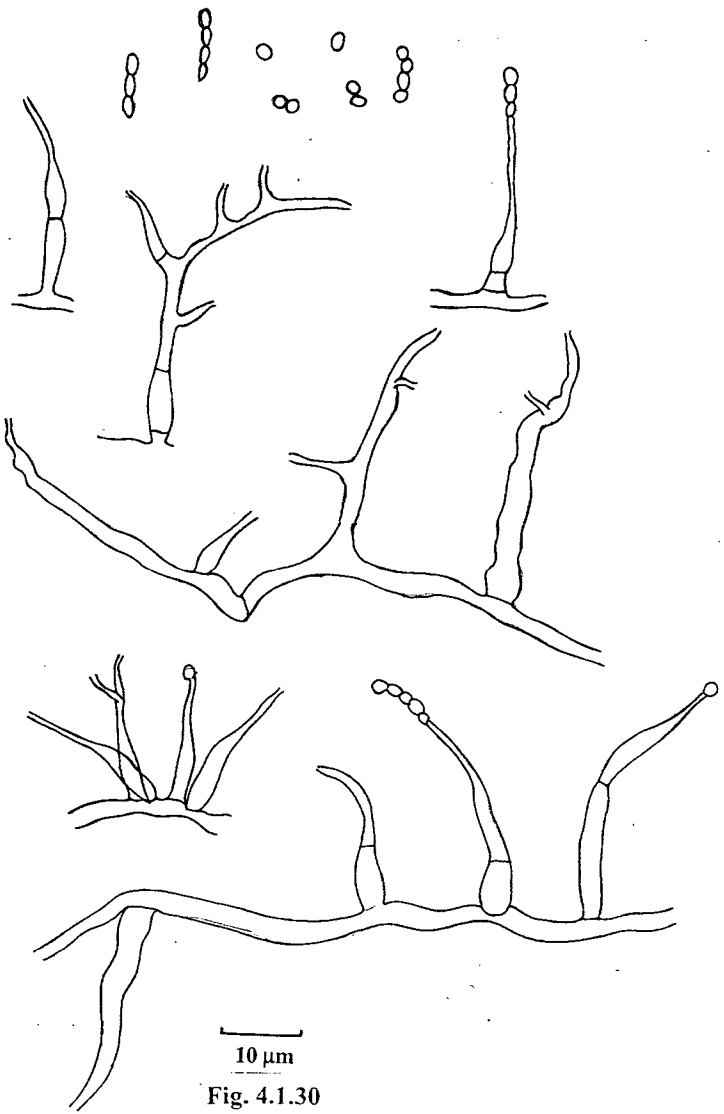
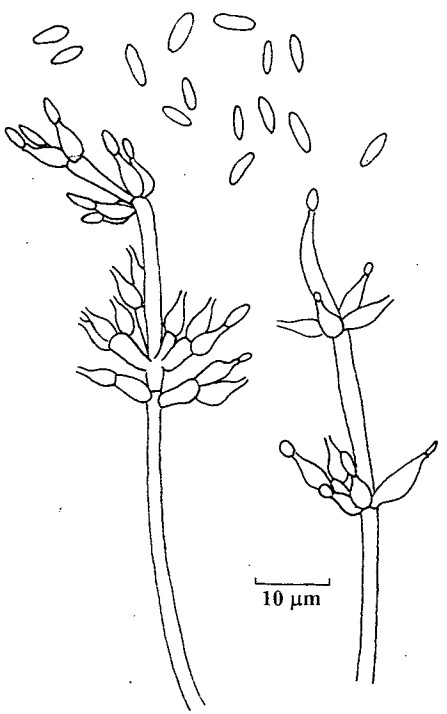


Fig. 4.1.30



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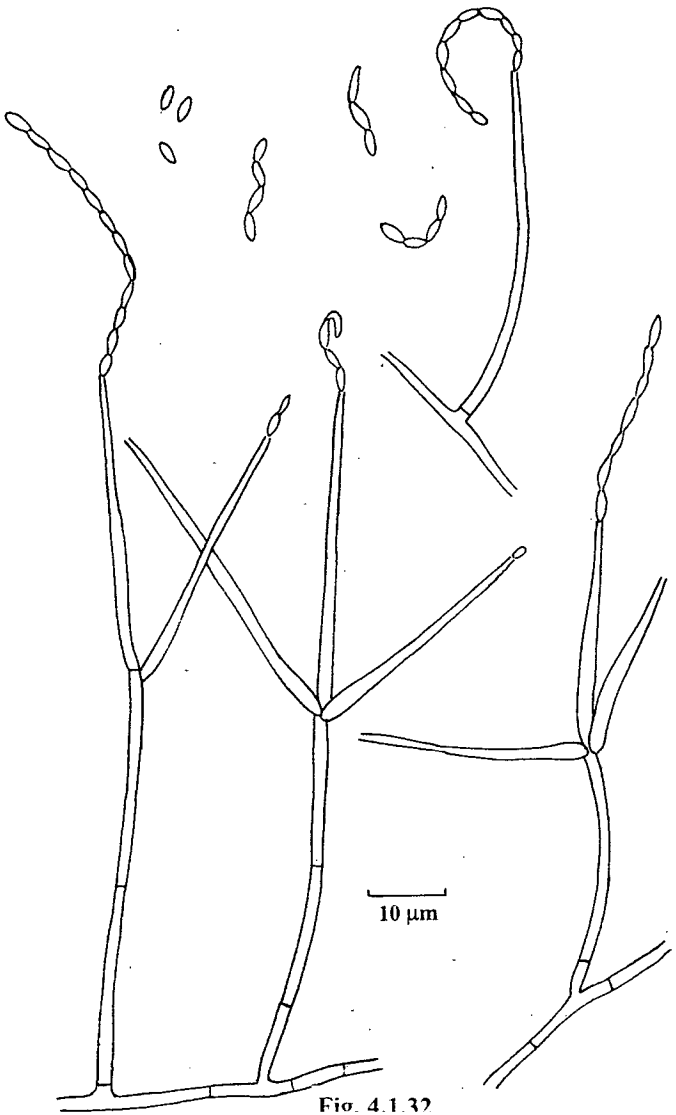

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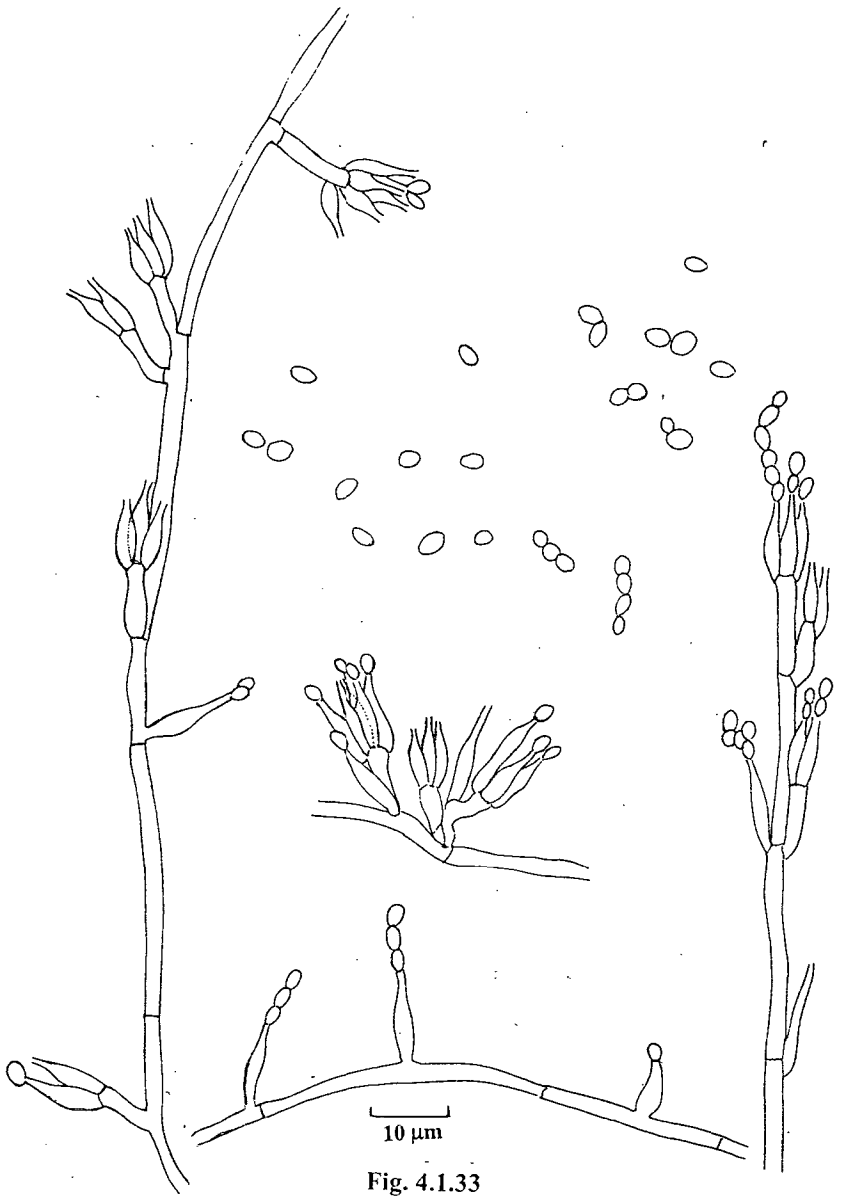

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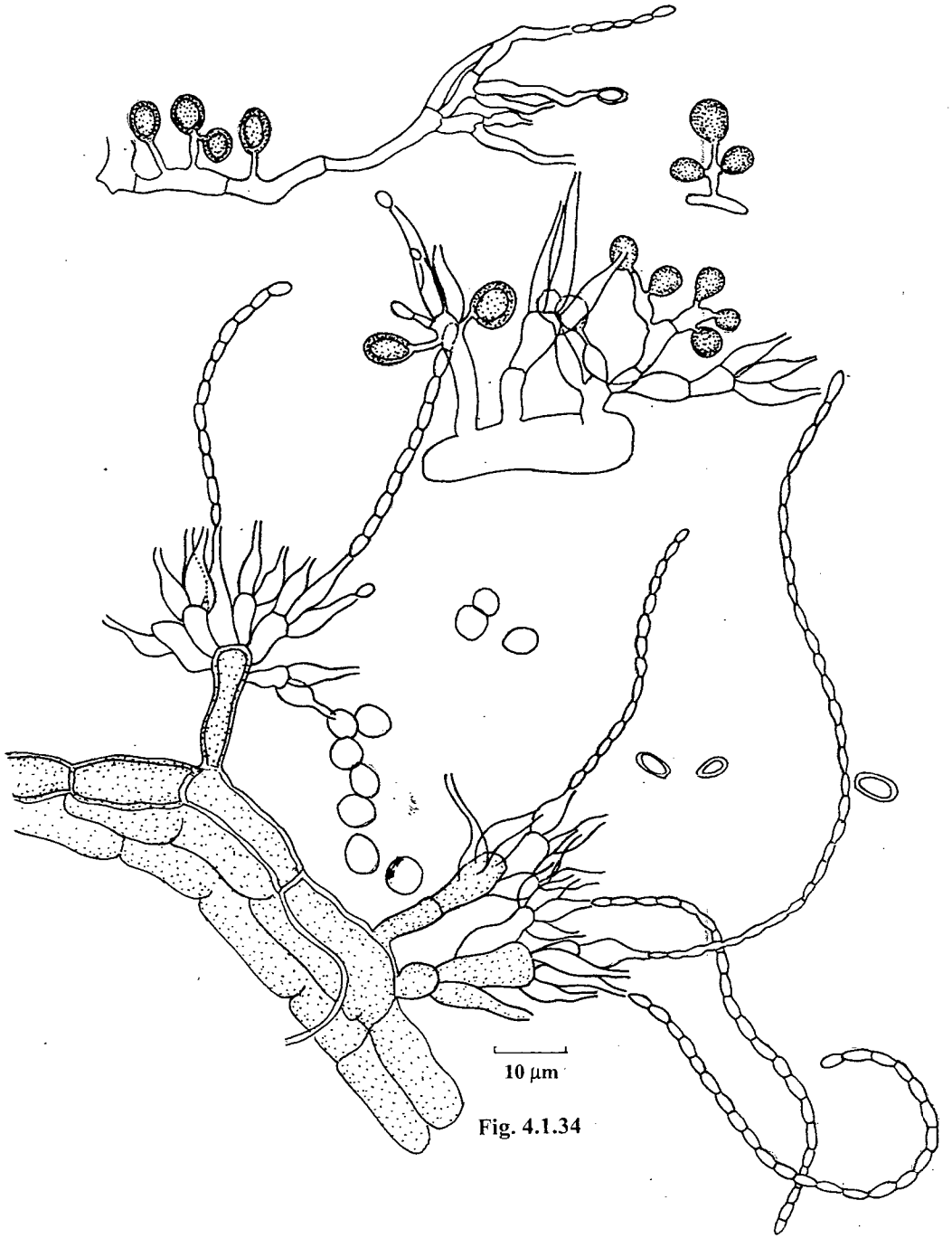

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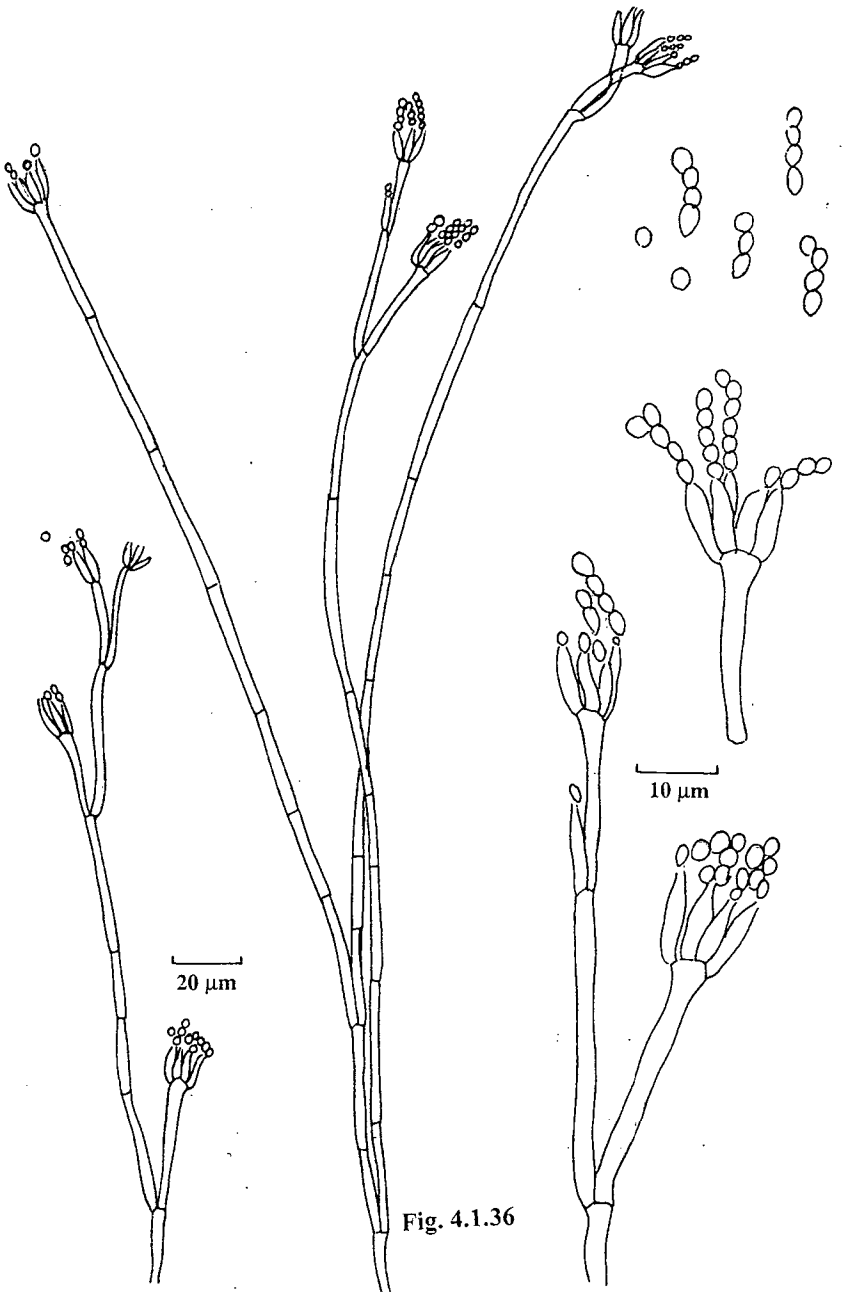

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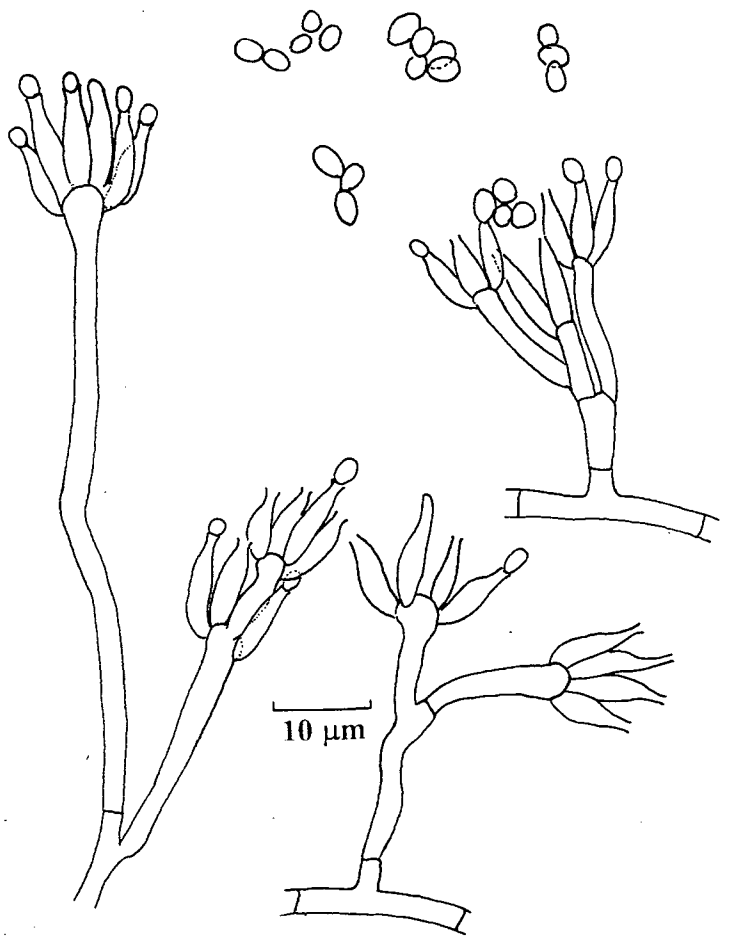

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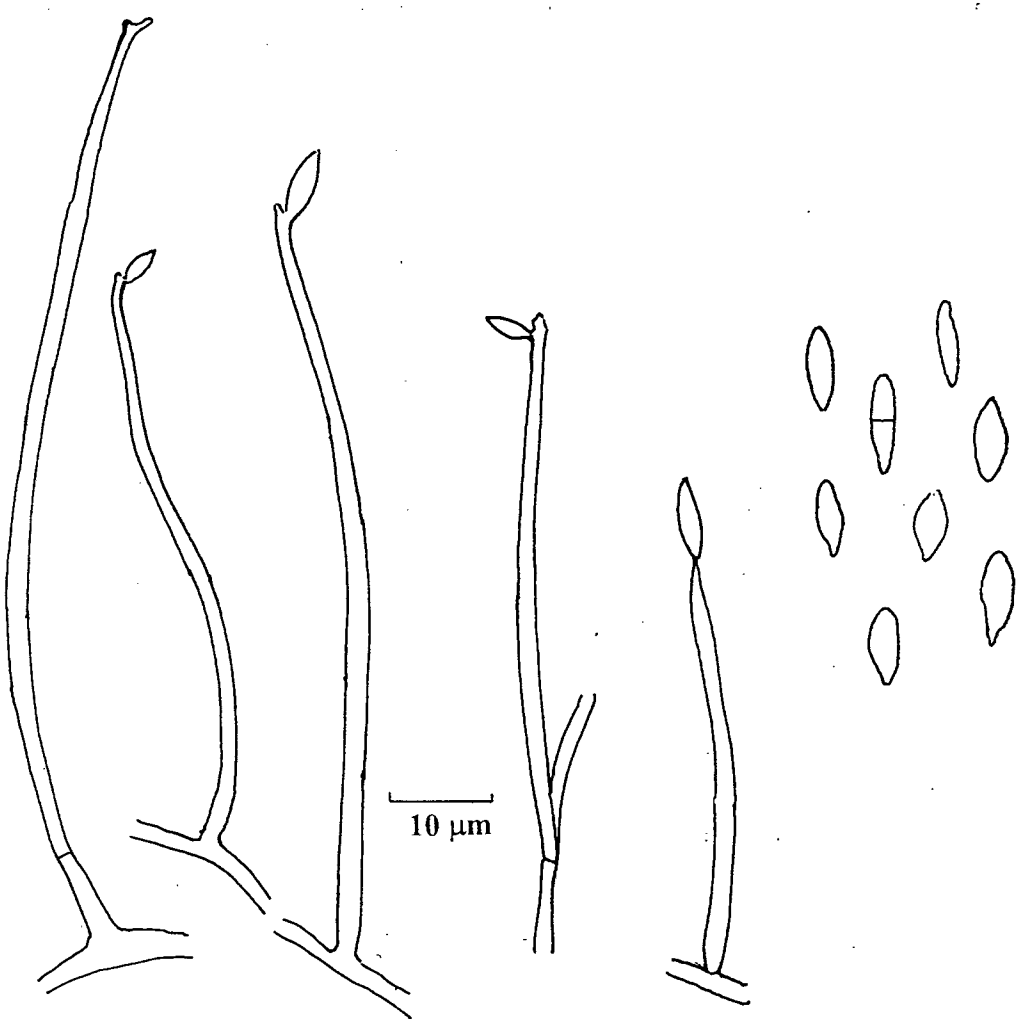

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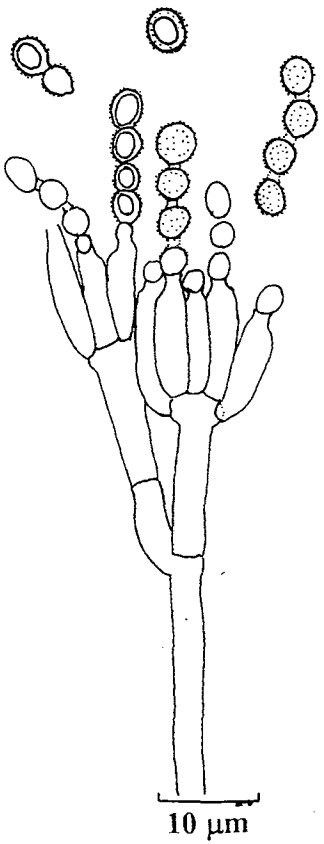

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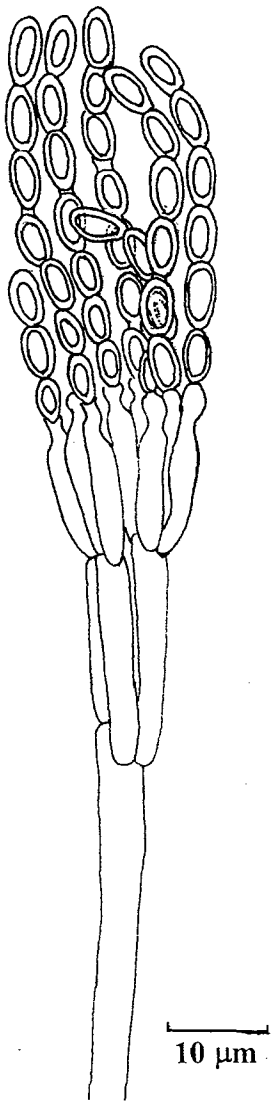

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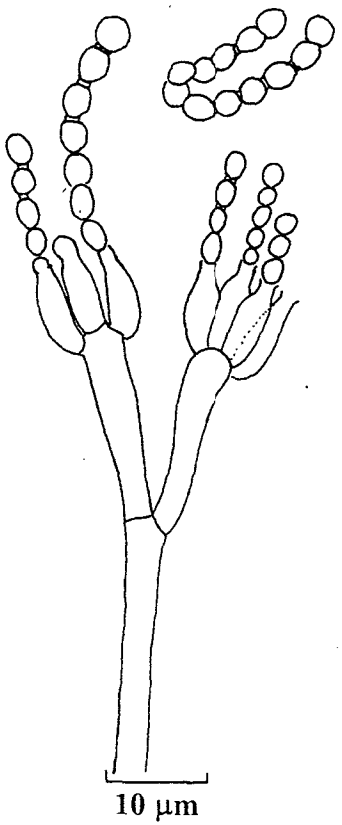

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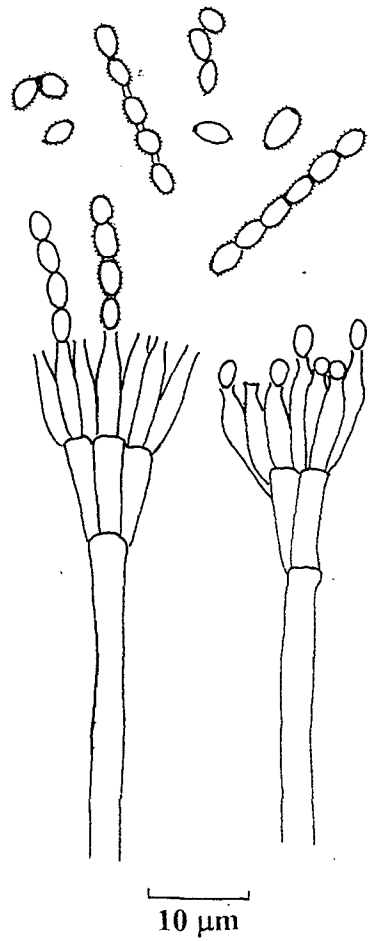
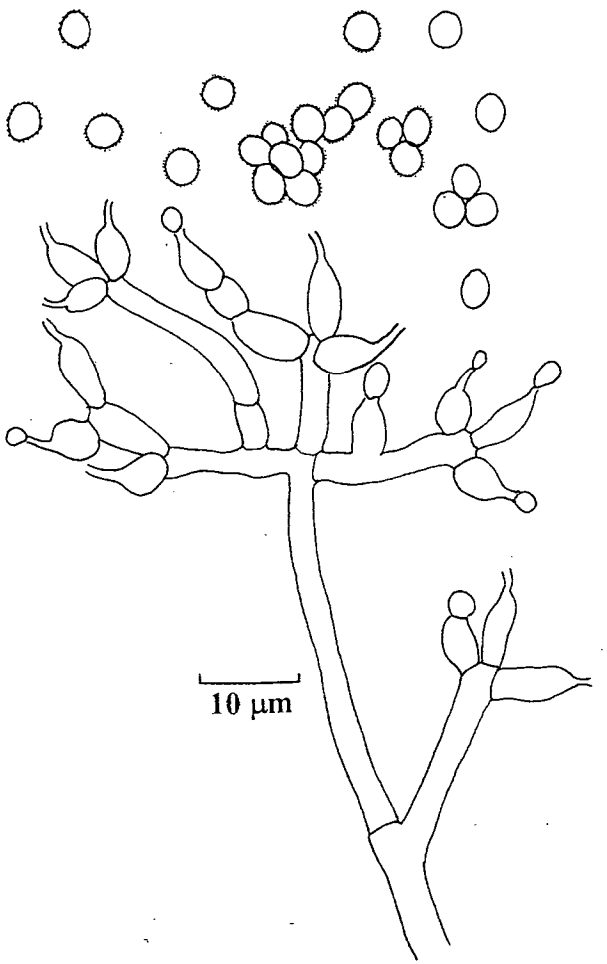


Fig. 4.1.40



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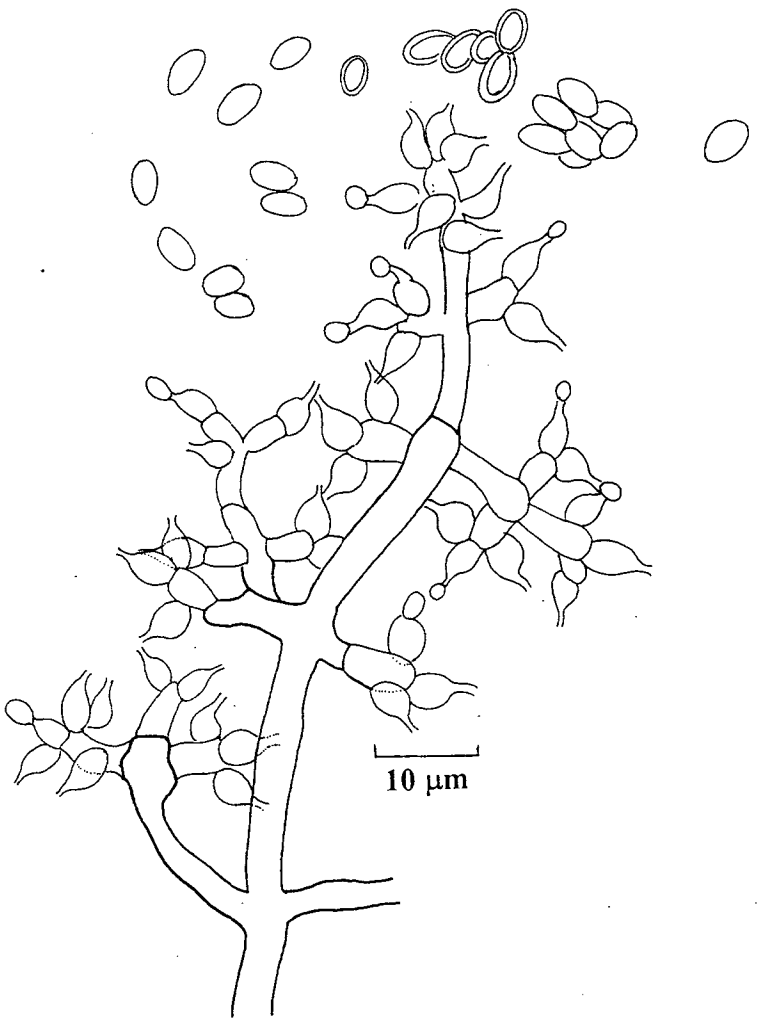
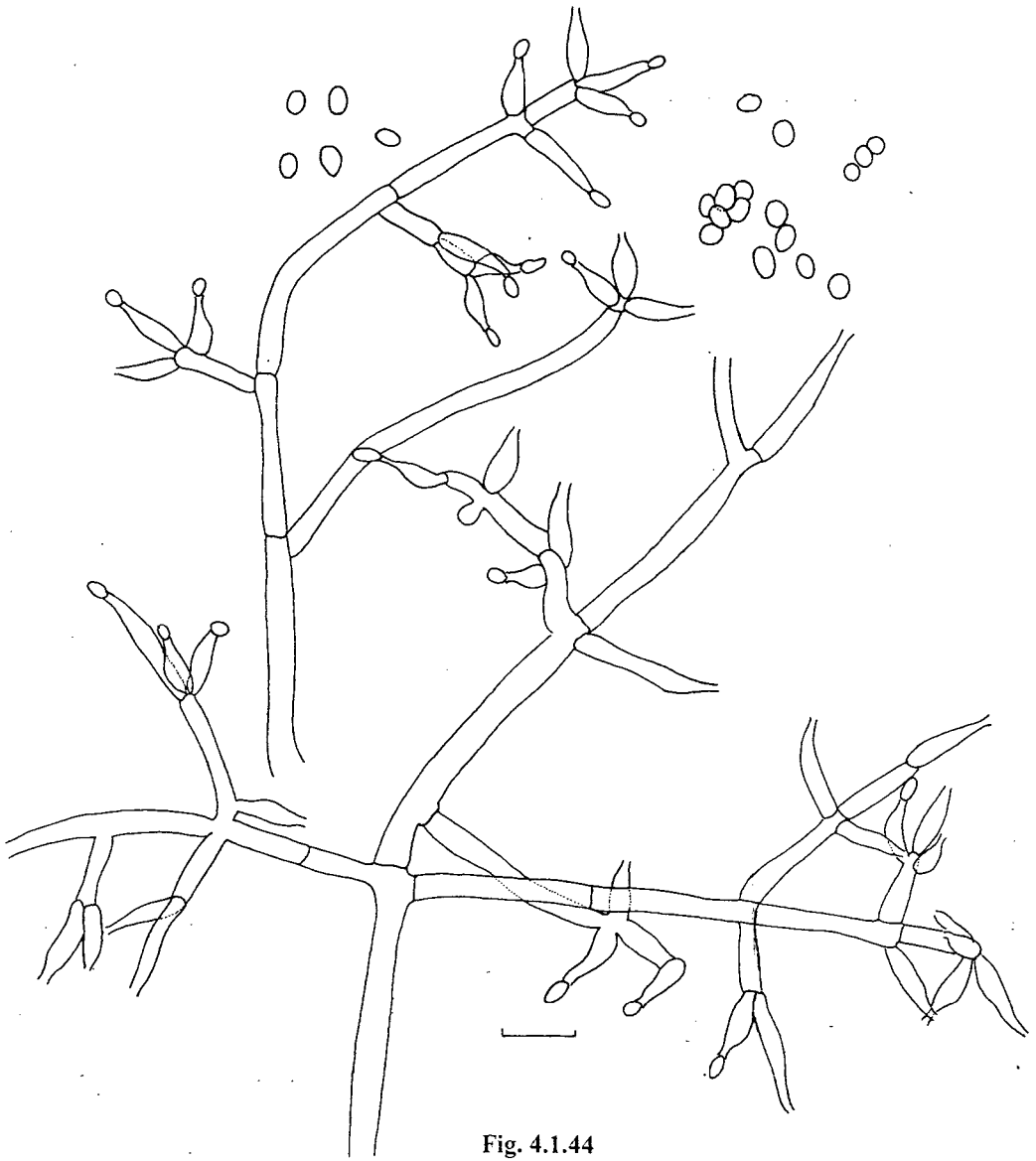


Fig. 4.1.43



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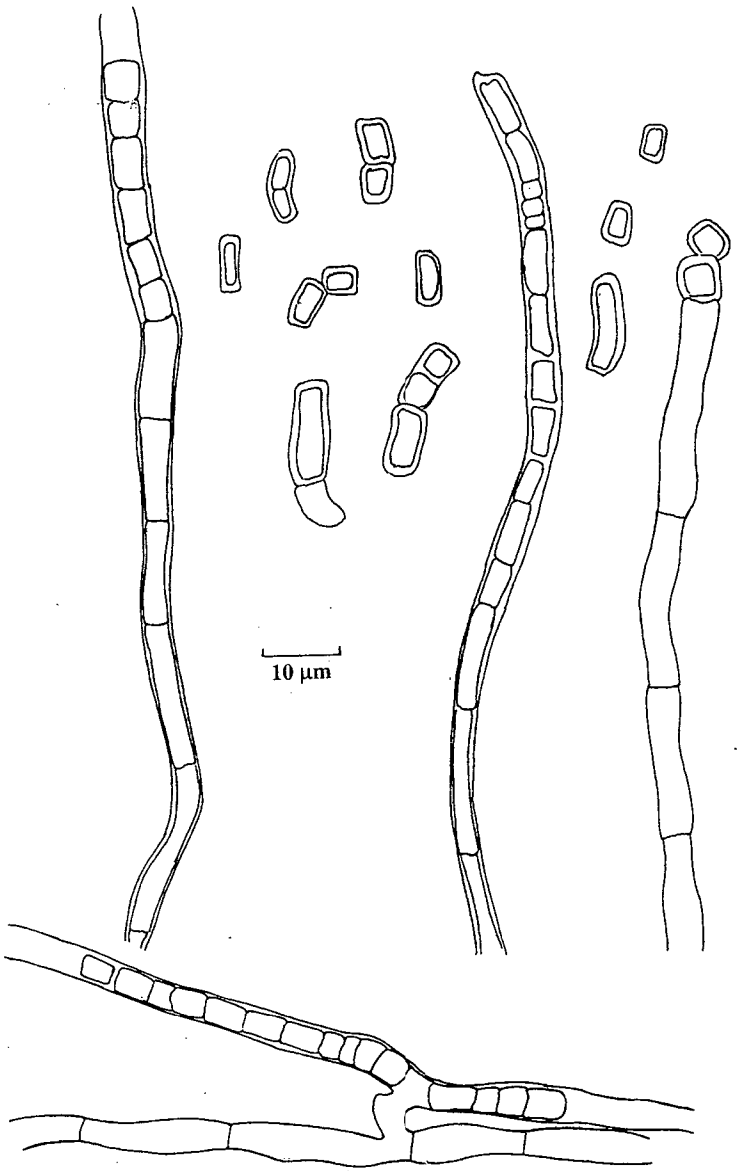


PART II:

4.2. Screening the fungi for their biological control potential against mosquito larvae

All 286 fungal isolates obtained in the present study were screened for their mosquito larvicidal potential. Out of the 57 fungi isolated from dead or inactive mosquito developmental stages, 20 (35.08 %) isolates showed more than 50% mortality, whereas of the 222 fungal isolates obtained from non-mosquito sources, 18 (8.11 %) were found active against mosquito larvae. Only 2 of the seven isolates from water samples showed more than 50% bioactivity against mosquito larvae. The promising fungal isolates belonged to the genera *Acremonium* Link (1), *Aspergillus* Link (3), *Chaetomella* Fuckel (1), *Gliocladium* Corda (8), *Penicillium* Fries (13), and *Trichoderma* Persoon (11). Three active isolates (C7, C17 and C27) could not be identified since they did not sporulate in culture. (Table 4.4).

PART III:

4.3. Assessment of mosquito-larvicidal activity of candidate species

Of the 40 promising isolates, 4 - one each belonging to genera *Acremonium* (E29), *Gliocladium* (E16), *Penicillium* (E9) and *Trichoderma* (C54) were tested by standard bioassay in 250 ml of water.

4.3.1. Mass production of conidia:

Isolates of *Acremonium* and *Trichoderma* were grown in Malt Czapek (MCz) liquid medium. Fungi inoculated in 50 ml broth grew well in 7 days forming a submerged mycelial mat. Floating culture of *Acremonium* sp. produced micro-colonies on 3rd day of incubation and covered the entire surface on day 6. From day 7, the fungus produced a diffusible pink-coloured pigment into the medium. After 20 days, a thick layer of mycelial mat with waxy appearance on the surface was seen and mean conidial yield was $\approx 2.1 \times 10^6 \pm 2.7 \times 10^5$. In case of *Trichoderma* sp. submerged hyphae covered the broth in 3 days and green masses of spores were visible after 5 days. After 20 days of incubation, mean conidial yield was $\approx 3.16 \times 10^6 \pm 3.0 \times 10^5$.

Gliocladium sp.(E16) and *Penicillium* sp. (E9) were grown in 1% corn, 4% corn, 4% corn + sugar and Czapek Malt liquid media. *Gliocladium* sp. formed submerged colonies slowly in 1% corn medium yielding very less sporulation. However, in 4% corn medium, the fungus grew fast and sporulated well. In 4% corn + sugar medium, colony growth was less as compared to 4% corn liquid media. Fast growth and sporulation was observed in Czapek Malt liquid medium, maximum being after 18 days of incubation. Order of medium on the basis of conidial yield after 20 days of incubation was Czapek Malt >4% corn > 4% corn + sugar> 1% corn with corresponding conidial yield $\approx 3.02 \times 10^6 \pm 1.33 \times 10^5 > 6.07 \times 10^5 \pm 8.20 \times 10^4 > 3.94 \times$

$10^5 \pm 3.01 \times 10^4 > 1.07 \times 10^4 \pm 9.96 \times 10^2$, per 50 ml of media used in culture bottles with 117 cm^2 surface area (Table 4.5, Fig. 4.3.1)

Penicillium sp. produced green dot-like sporulating micro colonies on the surface of broth on day 3. After colonizing the surface with a profusely sporulating mycelial mat, submerged hyphae got established in Czapek Malt liquid medium. In 1% corn, growth and sporulation was visible after 10 days. Comparatively better growth was observed in 4% corn and 4% corn + sugar liquid media. Order of media on the basis of conidial yield after 20 days of incubation was Czapek Malt >4% corn + sugar> 4% corn > 1% corn with corresponding conidial yield $\approx 1.08 \times 10^7 \pm 1.12 \times 10^6 > 1.86 \times 10^6 \pm 2.67 \times 10^5 > 5.71 \times 10^5 \pm 9.44 \times 10^4 > 9.22 \times 10^4 \pm 3.09 \times 10^4$, per 50 ml of media used in culture bottles with 117 cm^2 surface area (Table 4.6, Fig. 4.3.2).

4.3.2. Standard Bioassay in 250 ml of water

Results of bioassays for spore dose range studies of the four isolates are given in the Table. 4.7. Dose range of *Acremonium* sp. giving 0-100% mortality for *Culex quinquefasciatus* and *Anopheles stephensi* was 1×10^3- 5×10^4 spores/ml and for *Aedes aegypti* 1×10^4-1×10^5 spores/ml. Conidia of *Gliocladium* sp. yielded the range of 1×10^2-1×10^4 spores/ml for *Culex quinuefasciatus* and *Anopheles stephensi* and 1×10^4-5×10^4 spores/ml. In case of *Penicillium* sp., dose range was found to be 1×10^3-5×10^4 spores/ml for *Culex quinuefasciatus* and *Culex quinuefasciatus,* and 1×10^4-1×10^5 spores/ml for *Aedes aegypti. Trichoderma* sp. caused 0-100% mortality on all three species of mosquito larvae in the dose range of 1×10^4-1×10^5 spores/ml.

When bioassays were performed for *Acremonium* sp. in 250 ml of water, a high average percentage mortality of 85.6 ± 4.56 (24 h), 88 ± 2.83(48 h.) and 88.8±1.79 (72 h.) was obtained in 3rd instar *Culex quinuefasciatus* larvae (n=25) against a dose of

42000 spores/ml. In case of *Anopheles stephensi,* against a dose of 48000 spores/ml, average percentage mortality was 76.8 ± 5.22, 80±4 and 84.8±5.22 at the end of day 1, 2 and 3 of the treatment respectively. Against a dose of 120000 spores/ml, average percentage mortality of 56.8 ± 12.46, 57.6 ± 11.52 and 59.2 ± 11.1 were recorded among *Aedes aegypti* tested, in 24, 48 and 72 h exposure. The results of dose response relationship in different concentrations among different species of mosquito larvae tested are given in Fig 4.3.3.

In bioassays of *Acremonium* sp. against three species of mosquito larvae, increase in the duration of treatment had no significant effect at dose values. Interaction accounted for only less than 0.15% of the total variance in all cases. However, variation in the dose had significant effect accounting for 98.86%, 98.87% and 94.79% of the total variance in case of *Culex quinuefasciatus, Anopheles stephensi* and *Aedes aegypti* respectively. The F and P values of ANOVA are shown in Table 4.8.

In case of *Gliocladium* sp., 100% mortality was recorded within 24 hrs of treatment in all 3 species of larvae subjected to bioassay, i.e. *Culex quinuefasciatus, Anopheles stephensi* and *Aedes aegypti* against corresponding concentrations of 9800, 14200 and 49500 spores/ml. The dose response relationship is shown in Fig. 4.3.4. However, increase in the duration of treatment of *Gliocladium* sp. did not have any significant effect on *Culex quinuefasciatus* and *Aedes aegypti* and accounted for only less than 0.1% of the total variance. However, in case of *Anopheles stephensi,* interaction was significant. 'Bonferroni post t' tests showed that increase in treatment duration had extremely significant effect on results at dose of 7100 spores/ml. Increase in treatment duration however did not affect results in cases of *Culex quinuefasciatus* and *Aedes aegypti.* Variation in the dose had extremely significant effect accounting for

99.38%, 99.43% and 97.95% of the total variance in case of *Culex quinuefasciatus, Anopheles stephensi* and *Aedes aegypti* respectively. The F and P values of ANOVA have been depicted in Table 4.8.

Penicillium sp. impacted absolute mortality in 24 h against a dose of 12850 spores/ml for *Culex quinuefasciatus,* 17887 spores/ml for *Anopheles stephensi* and 98444 spores/ml for *Aedes aegypti.* Dose response relationship of *Penicillium* sp. versus mosquito larvae of three species tested has been shown in Fig. 4.3.5.

In bioassay of *Penicillium* sp. against three species of mosquito larvae, increase in treatment duration did not yield significant effect at all values of dose. Interaction accounted for only 1.52% in case of *Culex quinuefasciatus* and less than 0.01% of total variance in other two cases. Increase in treatment duration also did not affect results significantly. Variation in the dose had extremely significant effect accounting for 88.55%, 98.34% and 95.66% of the total variance in case of *Culex quinuefasciatus, Anopheles stephensi* and *Aedes aegypti* respectively. The F and P values of ANOVA test are shown in Table 4.8.

Trichoderma sp., though found slow in growth, effected a highest average percentage mortality of 64.8 ± 5.2 at the end of 120 h treatment, among *Culex quinuefasciatus* larvae at a dose of 120000 spores/ml. Similarly, average percentage mortality of 41.6 ± 9.6 and 16.8 ± 10.35 were recorded against dose of 120000 spores/ml in cases of *Anopheles stephensi* and *Aedes aegypti* respectively. Graphs were plotted to visualize relation between dose and mortality caused by *Trichoderma* sp. conidia among mosquito larvae tested (Fig. 4.3.6).

In bioassay of *Trichoderma* sp. with *Culex quinuefasciatus* increase in treatment duration yielded significant results. Interaction accounted for only 8.42% of the total

variance. Against *Anopheles stephensi,* increase in treatment duration had extremely significant effect on results with *Trichoderma* sp. Interaction accounted for only 27.63% of the total variance and considered extremely significant. In case of bioassays against *Aedes aegypti*, interaction and increase in treatment duration had no significant effect on results, accounting for only 2.40% and 3.26% of the total variance respectively. Bonferroni post t-tests showed that in case of *Culex quinuefasciatus,* increase in treatment duration from 24 to 48 h had extremely significant effect on results at doses of 60000 and 120000 spores/ml, but from 48 to 72 h no significant effect was seen at all values of dose. Increase from 72 to 96 h had very significant effect at the dose of 40000 spores/ml, and extremely significant effect at the doses of 60000 and 120000 spores/ml. Increase from 96 to 120 h also had extremely significant effect at the doses of 40000 and 120000 spores/ml (Table 4.9).

In case of *Anopheles stephensi*, Bonferroni post t-tests showed that there was no significant effect of increase in treatment duration from 24 to 48 h on mortality at all doses. Increase from 48 to 72 h had extremely significant % mortality at doses of 60000 and 120000 spores/ml. Increase from 72 to 96 h had a very significant effect on mortality at a dose of 60000 and extremely significant effect at 120000 spores/ml. An increase in treatment duration from 96 to 120 h resulted in very significant effect on mortality at 60000 spores/ml whereas extreme significance was seen at a dose of 120000 spores/ml (Table 4.9).

Variation in the dose had extremely significant effect accounting for 77.25%, 36.03% and 33.25% of the total variance in case of *Culex quinuefasciatus, Anopheles stephensi* and *Aedes aegypti* respectively. The F and P values of ANOVA have been depicted in the tabular form. (Table 4.10).

It was observed that 3rd instar *Culex quinuefasciatus* larvae were most susceptible (LD_{50}^{24h} = 19712; LD_{50}^{48h} = 18992; LD_{50}^{72h} = 18885 spores/ml) than *Anopheles stephensi* (LD_{50}^{24h} = 26385; LD_{50}^{48h} = 25341; LD_{50}^{72h} = 24561 spores/ml) and *Aedes aegypti* were least susceptible (LD_{50}^{24h} = 108638; LD_{50}^{48h} = 107026; LD_{50}^{72h} = 104974 spores/ml) to *Acremonium*. (Table 4.10). When exposure time for *Acremonium* was increased to 48 h and 72 h it was observed that LD_{50} value was 3.65% and 4.2% less in case of *Culex quinuefasciatus,* 3.96% and 6.01% less in case of *Anopheles stephensi* and 1.48% and 3.37% less in case of *Aedes aegypti* (Table 4.10).

On the other hand in case of *Gliocladium* sp., larvae of *Culex quinuefasciatus* were more susceptible (LD_{50}^{24h} = 4373; LD_{50}^{48h} = 4250; LD_{50}^{72h} : 4147 spores/ml) followed by *Anopheles stephensi* (LD_{50}^{24h} = 5324; LD_{50}^{48h} = 5039; LD_{50}^{72h} = 5039spores/ml) and *Aedes aegypti* were the least susceptible (LD_{50}^{24h} = 27406; LD_{50}^{48h} = 27653; LD_{50}^{72hrs} = 27738 spores/ml) (Table 4.10).

When the exposure time of *Gliocladium* spores was increased to 48 h and 72 h from 24 h it was observed that LD_{50} value was 2.81% and 5.17% less in case of *Culex quinuefasciatus,* 5.35% and 5.35% less in case of *Anopheles stephensi* and 0.9% and 1.21% more in case of *Aedes aegypti* (Table 4.8).

The 3rd instar *Culex quinuefasciatus* larvae were the most susceptible (LD_{50}^{24h} = 1828; LD_{50}^{48h} = 1536; LD_{50}^{72h} = 1288 spores/ml) than *Anopheles stephensi* (LD_{50}^{24h} =1822; LD_{50}^{48h} =1723; LD_{50}^{72h} =1637 spores/ml) and *Aedes aegypti* were the least susceptible (LD_{50}^{24h} =31733; LD_{50}^{48h} =30804; LD_{50}^{72h} =30503 spores/ml) to *Penicillium* sp. (E9) spores (Table 4.10).

When the exposure time of *Penicillium* spores was increased to 48 h and 72 h from 24 h, it was observed that LD_{50} value was 15.97% and 29.54% less in case of

Culex quinuefasciatus, 5.43% and 10.15% less in case of *Anopheles stephensi* and 2.93% and 3.82% less in case of *Aedes aegypti.*

Trichoderma sp. (C54) was slow in its activity. LD_{50} values calculated only for *Culex quinuefasciatus* for 96 and 120 h of treatment (LD_{50}^{96h} =106936; LD_{50}^{120h} =84332 spores/ml) and *Anopheles stephensi* for 120 h of treatment as only in these cases there was appreciable response in the bioassay (Table 4.10).

The 'goodness of fit test' showed that there was good co-relation between dose and mortality relationship as revealed by R^2 values (being close to 1).

Other observations

Conidia of *Acremonium* sp. in suspension were becoming sticky while vortexing. Spores accumulated in the gut due to nonselective filter-feeding habit of larvae. No faecal pellets were seen in higher doses, though at lower doses spores were successfully ejected from the hind-gut. Septecaemia was observed in dead larvae within 24 h of exposure. The fungus was recovered on plating thoroughly washed gut of dead and live mosquito larvae.

In case of *Gliocladium* sp., the thoracic and foregut region of mosquito larvae was semi-transparent when observed within first 4 h of exposure under a dissecting microscope, indicating involvement of a process of cell lysis in gut epithelium. Test larvae were in disorder and swirling their head continuously whereas those in control were moving orderly. Most of the larvae were melanised at the end of 24 h of incubation. Faecal pellets were observed in the initial 4-6 h of exposure and subsequently larvae stopped feeding and became sluggish. The test fungus was recovered in culture by plating thoroughly washed gut of dead and live mosquito larvae exposed to the fungus.

In case of *Penicillium* sp., conidia packed the gut of mosquito larvae within one h of exposure, which could be seen as a green internal tube against transluscent light. Larvae fed continuously for 3-4 h. During this period, larvae could not come up to surface of water and remained in the bottom of the bowl with their posterior position down. At the end of 24 h, fungal filaments were observed emerging out through anal papilla and inter-segmental areas. Though the larvae did not rise up to the surface of the bowl, slight movements were observed in head region. No defecation was observed even after 24 h. When exposed to autoclaved conidia of the fungus, larvae survived without any sign of sluggishness, successfully defecating the fungal spores. Faecal pellets did not contain any intact fungal spores.

Conidia of *Trichoderma* sp. showed a bleached appearance (or colourless) in infected larvae. The gut tube was green. Haemocoel became opaque, septicemia and melanisation. Fungus was recovered by plating thoroughly washed dissected out gut.

Bioassays against larvae of strains of *An. stephensi, Cx. quinquefasciatus* and *Ae. aegypti* from Pune were conducted and the larvae were found to be susceptible to conidia of *Penicillium* sp. At dose of 10000 spores/ml, 95% and 100% mortality were recorded for *Culex quinuefasciatus* 3rd instar larvae at 24 and 48 hrs of treatment. Absolute mortality for *Anopheles stephensi* was recorded at 5000 spores/ml dose in 24 hrs. A maximum of 41.67 ± 2.89 percentage mortality was obtained for *Aedes aegypti* larvae against a dose of 150000 spores/ml after 48 hrs treatment.

When a comparison was made between mosquito larval strains of Goa and Pune based on dose response data, it was observed that Pune strain of *Anopheles stephensi* larvae was much more susceptible (100% mortality at a dose of 5000 spores/ml in 24 hrs) as compared to Goa strain at a similar dose (100% at 17887 spores/ml in 24 hrs).

However, *Aedes aegypti* Pune strain was found to have less susceptible (41.67 ± 2.89 % mortality at a dose of 150000 spores/ml in 48 hrs) than the Goa strain (100% mortality at a dose of 98444 spores/ml in 24 hrs) to *Penicillium* spores. There was not much difference in the susceptibility of *Culex quinuefasciatus* strains of the two regions (Pune strain: dose range was 5000-10000 spores/ml for 83.3 ± 2.89% to 100% mortality in 48 hrs; Goa strain: dose range was 6425-12850 spores/ml for 92.8 ± 6.57 to 100%). Dose response relationship is given in Table 4.11.

Regression analysis showed that there is no significant difference in the susceptibility of Goa and Pune strains of 3rd instar larvae of *Culex quinuefasciatus* and *Anopheles stephensi* as there was no significant difference between the slopes and Y-intercepts of best fit regression lines in cases of 24 and 48 h treatment. Although goodness of fit values were on the lower side, test of significance of nonlinearity was not significant.

In case of *Aedes aegypti,* susceptibility of Goa and Pune strains were markedly different. Regression line comparison showed extremely significant difference among the slopes. Goa strain of *Aedes aegypti* was much more susceptible than the Pune strain for *Penicillium* (E9) spores. Values of best fit, goodness of fit, runs test have been listed in tabular form (Table 4.12). Comparison between slope and intercepts have been given in Table 4.13. Regression lines were depicted graphically (Fig. 4.3.7)

4.3.3. Standard Bioassay in 1500 ml of water

Only *Gliocladium* sp. (E16) and *Penicillium* sp. (E9) were bioassayed further in 1500 ml of water against all three species of mosquito larvae. When the bioassay were performed for *Gliocladium* sp. in 1500 ml of water, mortality of 20% (24 h) and 32% (48 h) were obtained in 3rd instar *Culex quinuefasciatus* larvae against a dose of

20000 spores/ml. In case of *Anopheles stephensi,* against a dose of 20000 spores/ml, mortality of 28% and 100% were obtained at the end of treatment day 1 and 2 respectively. Against a dose of 60000 spores/ml, a 100% mortality was recorded after 24 h among *Aedes aegypti* tested. Dose response relationship among different species of mosquito larvae tested were plotted (Fig. 4.3.8).

In bioassays of *Gliocladium* sp. against three species of mosquito larvae in 1500 ml of water, increase in treatment duration had significant effect on results. Interaction accounted for 4.16%, 17.1% and 0.19% of the total variance in cases of *Culex quinuefasciatus. Anopheles stephensi* and *Aedes aegypti* and were considered significant, extremely significant and very significant respectively. Increase in treatment duration accounted for 3.07%, 31.47% and 0.17% of the total variance in cases of *Culex quinuefasciatus Anopheles stephensi* and *Aedes aegypti* and were considered very significant in case of *Culex quinuefasciatus*, extremely significant in cases of *Anopheles stephensi* and *Aedes aegypti.* 'Bonferroni post t' tests showed that increase in treatment duration had extremely significant effect on results at most values of dose used (Table. 4.15). Variation in the dose had extremely significant effect accounting for 81.21%, 49.02% and 99.28% of the total variance in case of *Culex quinuefasciatus, Anopheles stephensi* and *Aedes aegypti* respectively. The F and P values of ANOVA have been depicted in Table 4.14.

In case of *Penicillium* sp., mortalities of 63% (24 h) and 88% (48 h) were obtained in 3rd instar *Culex quinuefasciatus* larvae against a dose of 20000 spores/ml. In case of *Anopheles stephensi,* against a dose of 20000 spores/ml, mortalities of 39% and 99% were obtained at the end of treatment day 1 and 2 respectively. Against a dose of 120000 spores/ml, 46% and 76% mortality was recorded at 24 and 48 hrs of treatment

among *Aedes aegypti*. Dose response relationship among different species of mosquito larvae tested were plotted and given in Fig. 4.3.9.

In bioassays of *Penicillium* sp., against three species of mosquito larvae in 1500 ml of water, increase in treatment duration had significant effect. Interaction accounted for 3.59%, 12.07% and 7.36% of the total variance in cases of *Culex quinuefasciatus Anopheles stephensi* and *Aedes aegypti* and were considered significant. Increase in treatment duration accounted for 14.08%, 36.67% and 10.75% of the total variance in cases of *Culex quinuefasciatus Anopheles stephensi* and *Aedes aegypti* and were considered to have significant effect on results. 'Bonferroni post t' tests showed that increase in treatment duration had extremely significant effect on results at most values of dose used (Table 4.15). Variation in the dose had extremely significant effect accounting for 76.86%, 47.88% and 79.51% of the total variance in case of *Culex quinuefasciatus, Anopheles stephensi* and *Aedes aegypti* respectively. The F and P values of ANOVA have been shown in Table 4.14.

It was observed that larvae of *Anopheles stephensi* were most susceptible (LD_{50}^{24h} = 26334, LD_{50}^{48h} = 8297 spores/ml) followed by *Aedes aegypti* (LD_{50}^{24h} = 24069, LD_{50}^{48h} = 21349 spores/ml) and *Culex quinuefasciatus* (LD_{50}^{24h} = 27992; LD_{50}^{48h} = 24439 spores/ml), were the least susceptible to *Gliocladium* sp. (E16) (Table 4.16). When the exposure time for *Gliocladium* sp. (E16) was increased to 48 h from 24 h, it was observed that LD_{50} value was 12.69% less in case of *Culex quinuefasciatus*, 68.49% less in case of *Anopheles stephensi* and 11.37% less in case *Aedes aegypti*.

On the other hand, III instar *Culex quinuefasciatus* Larvae were the most susceptible (LD_{50}^{24h} = 17347, LD_{50}^{48h} = 7631spores/ml) than *Anopheles stephensi* (LD_{50}^{24h} 25404, LD_{50}^{48h} 3070 spores/ml) and *Aedes aegypti* were the least susceptible

(LD_{50}^{24h} = 122653, LD_{50}^{48h} = 89727spores/ml) to *Penicillium* (E9) spores (Table 4.16). When the exposure time for *Penicillium* sp., was increased to 48 h from 24 h, It was observed that LD_{50} value was 56.01% less in case of *Culex quinuefasciatus*, 87.92% less in case of *Anopheles stephensi* and 26.84% less in case *Aedes aegypti.*

Increase in the treatment duration from 24 to 48 h drastically increased susceptibility of *Anopheles stephensi* as only 3.17 fold less dose was needed in case of *Gliocladium* sp. and 8.27 fold less dose in case of *Penicillium* sp. to elicit 50 % mortality.

It was observed that *Culex quinquefasciatus* and *An. stephensi* larvae were more susceptible to *Penicillium* spores than that of *Gliocladium,* but reverse was true in case of *Aedes aegypti* as 4-6 fold lower dose was needed in case of *Gliocladium* sp. (Fig. 4.3.10).

The goodness of fit test showed that there was good co-relation between dose and mortality relationship as revealed by R^2 values (being closer to 1).

Fig. 4.3.1. Mass production of conidia in different media for *Gliocladium* sp. (E16) (Incubation time 20 days)

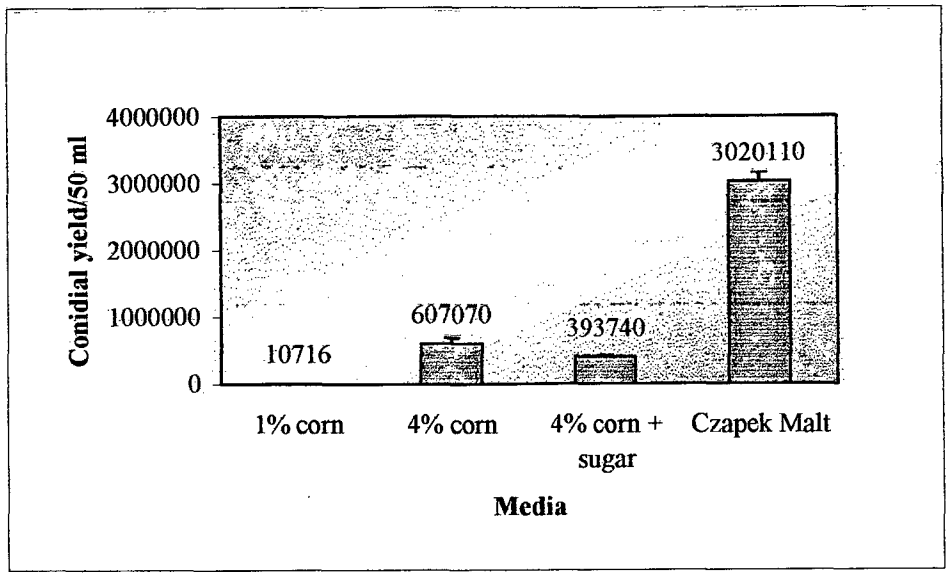


Fig. 4.3.2. Mass production of conidia in different media for *Penicillium* sp. (E9) (Incubation time 25 days)

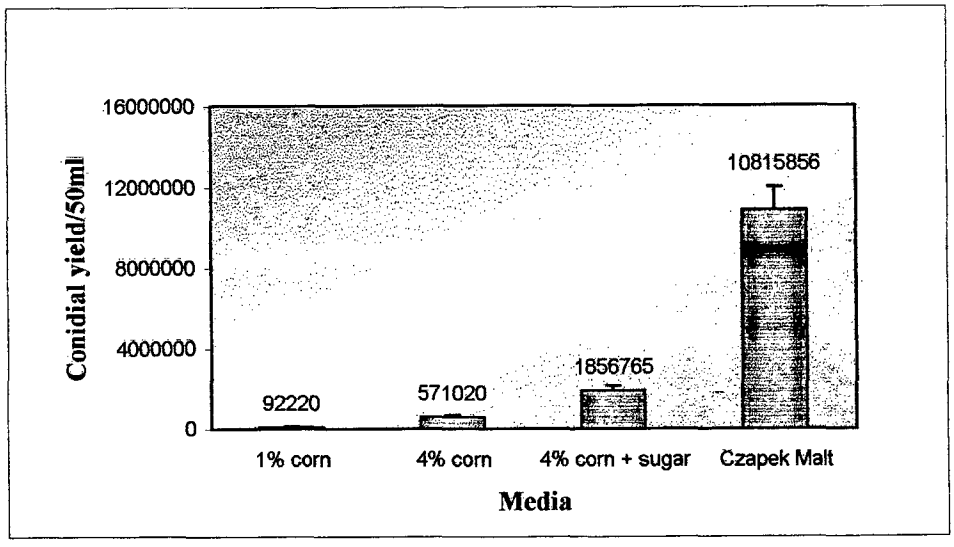


Fig. 4.3.3. Dose response relationship of *Acremonium* sp. (E29) against mosquito larvae.

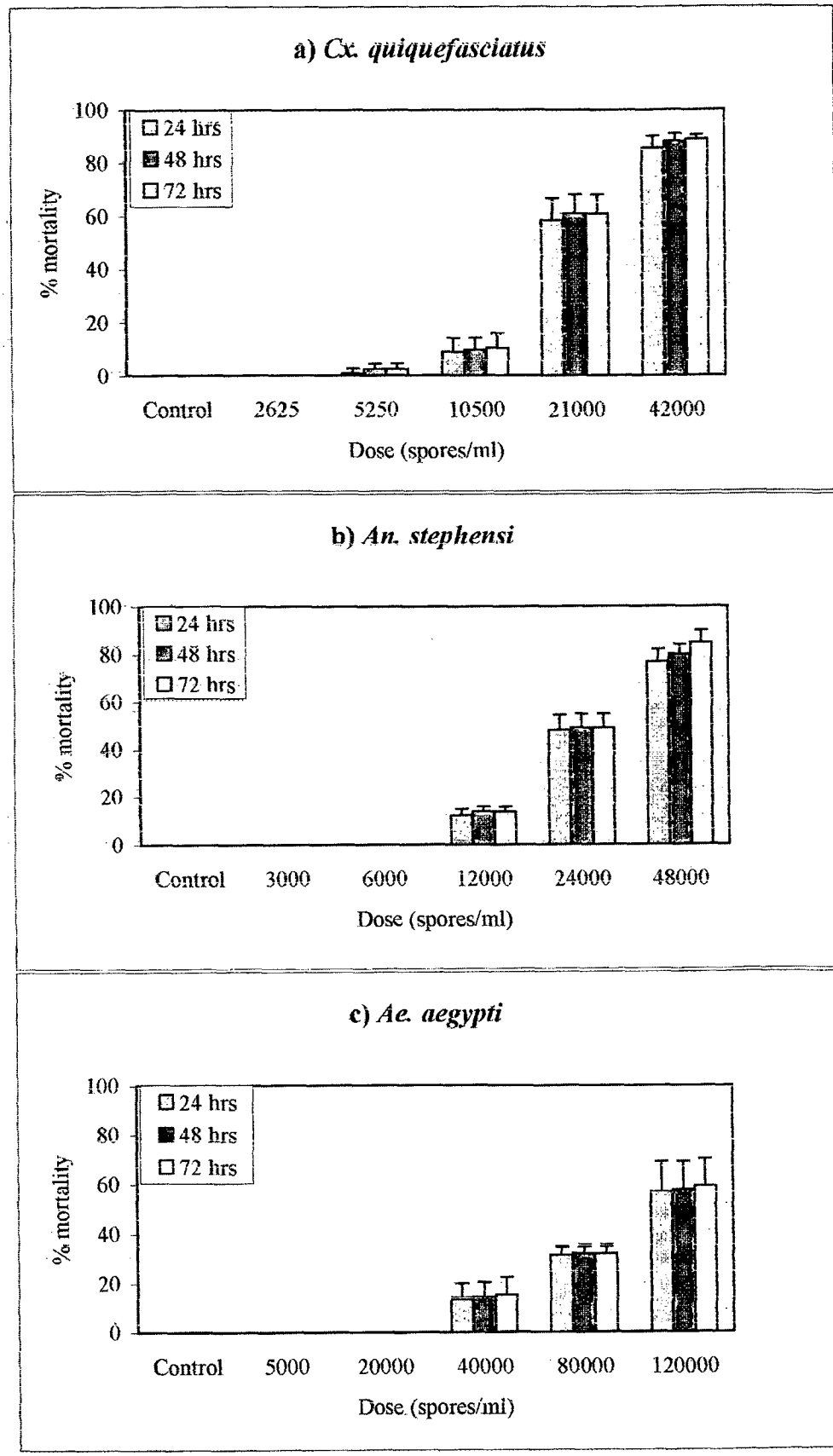


Fig. 4.3.4. Dose response relationship of *Gliocladium* sp. (E16) against mosquito larvae.

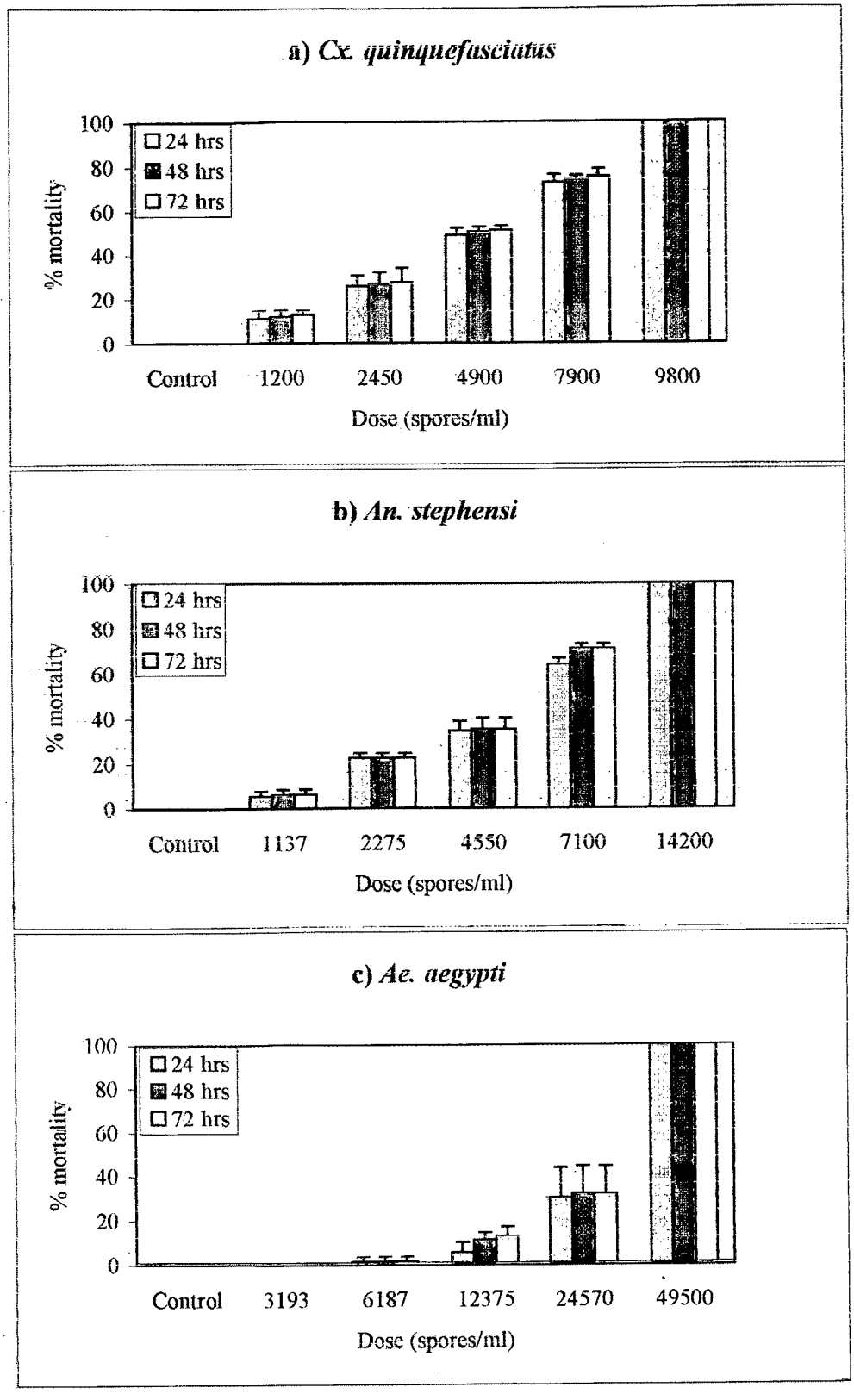


Fig. 4.3.5. Dose response relationship of *Penicillium* sp. (E9) against mosquito larvae.

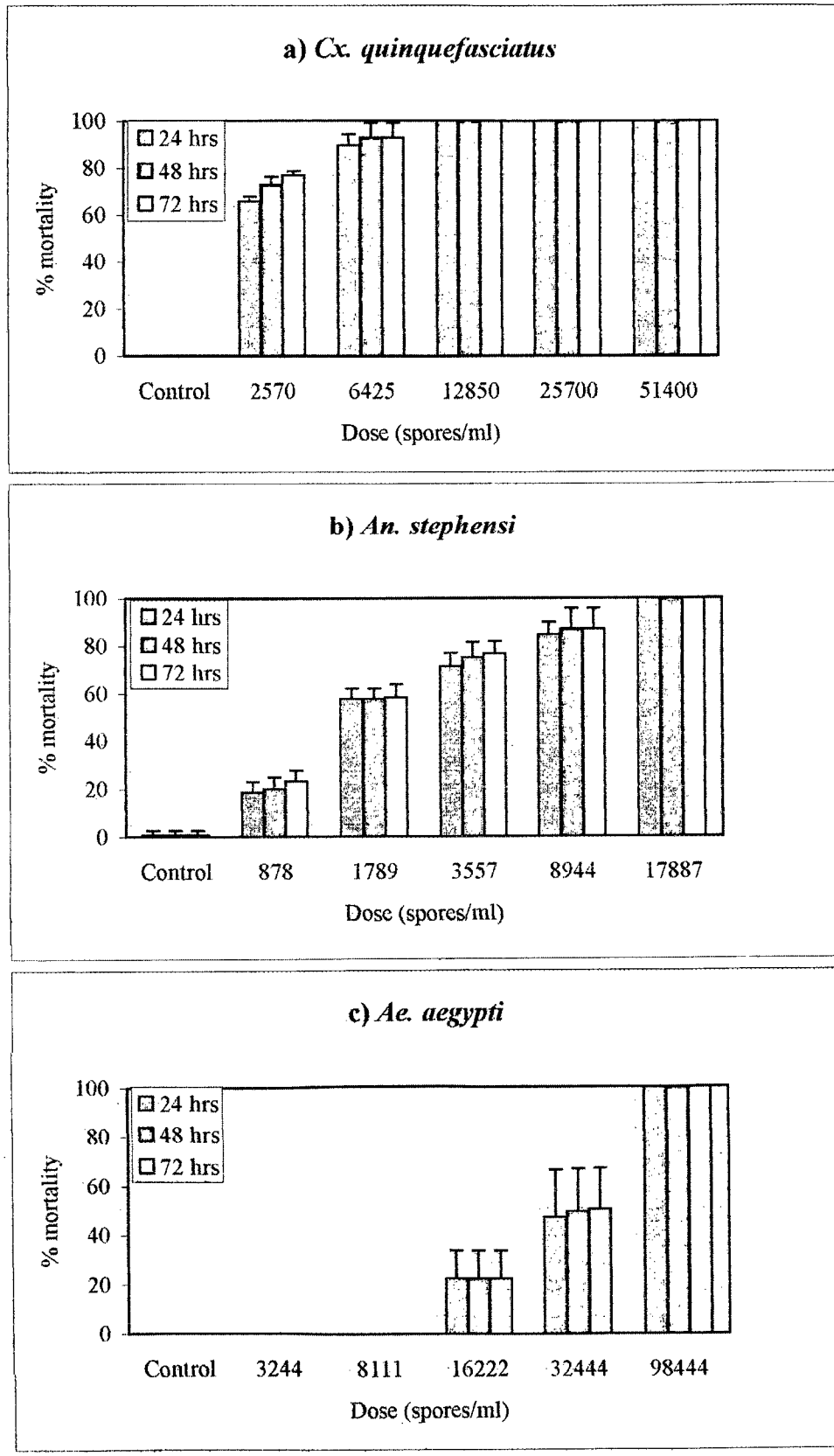


Fig. 4.3.6. Dose response relationship of *Trichoderma* sp. (C54) against mosquito larvae.

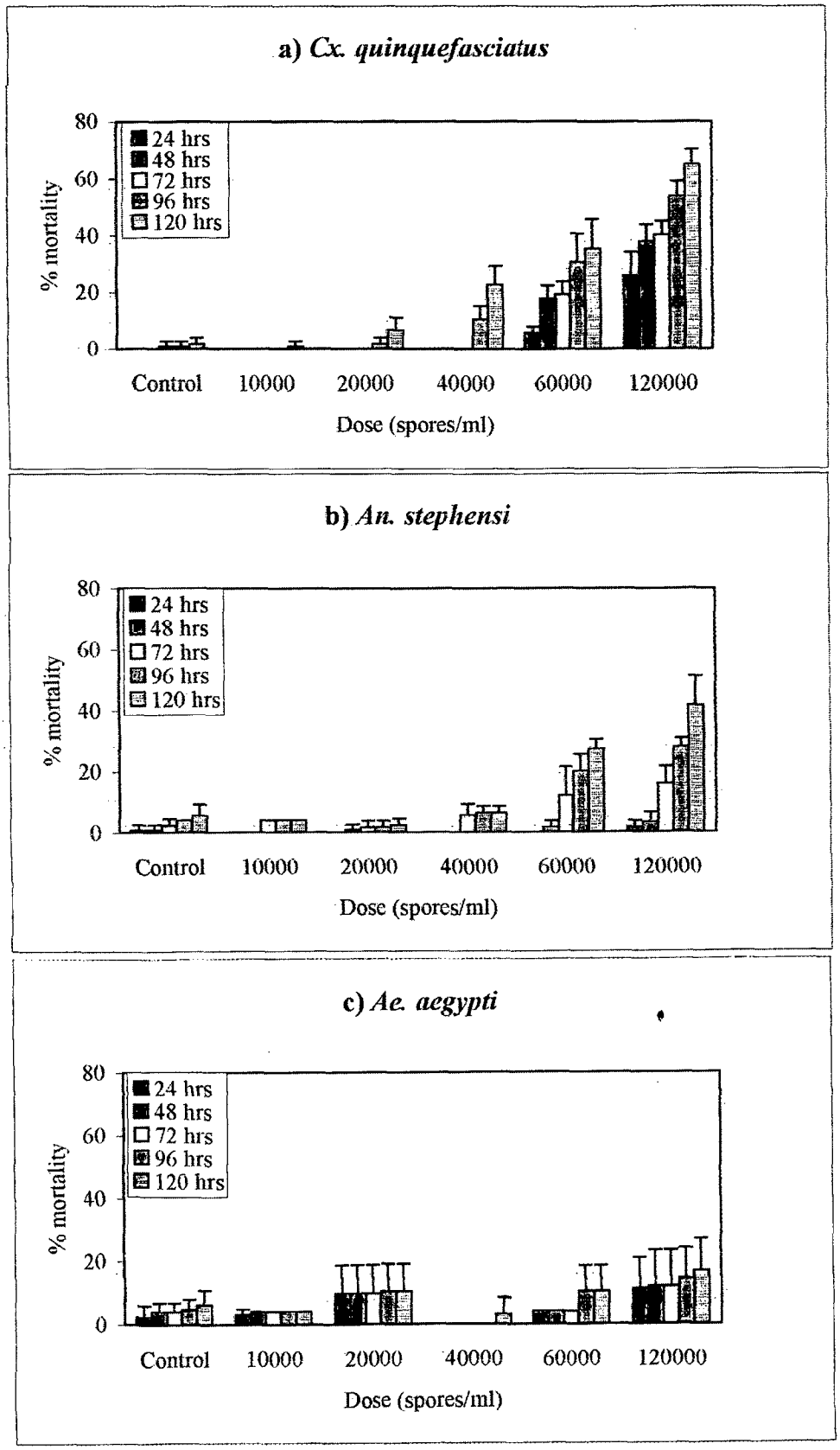
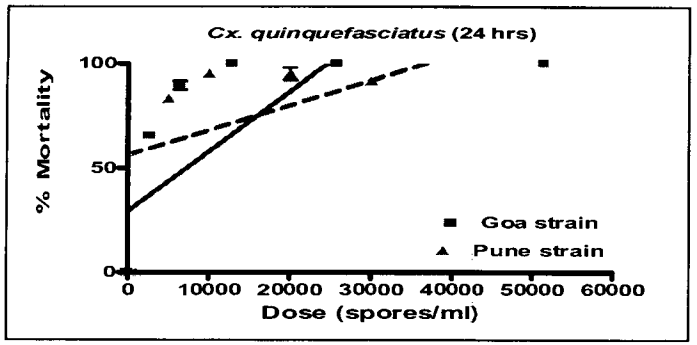
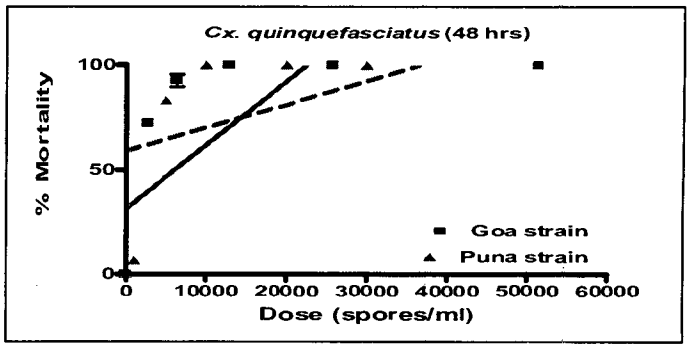
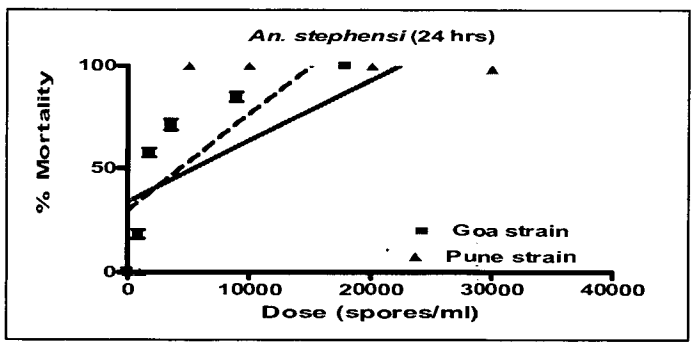
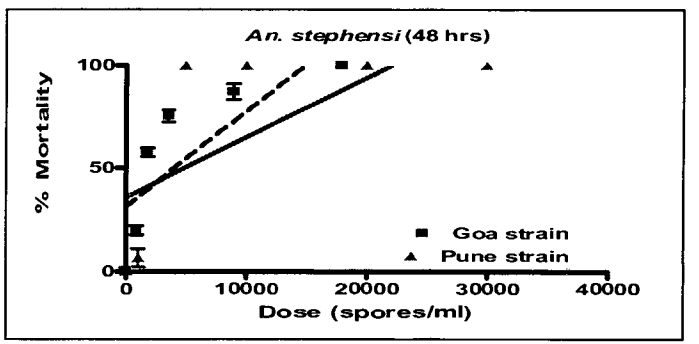


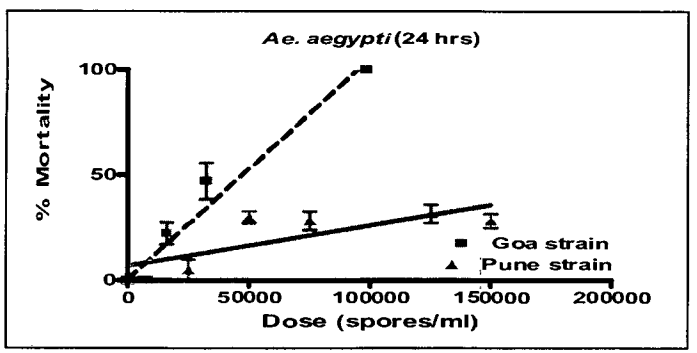
Fig. 4.3.7. Comparision of susceptibilty of Goa and Pune strains of mosquito larvae for spores of *Penicillium* sp. (E9)











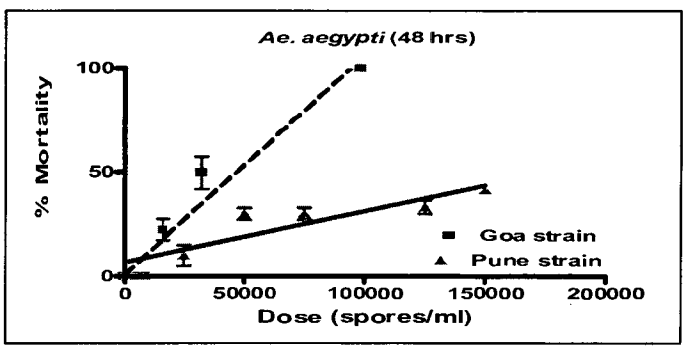


Fig. 4.3.8. Dose response relationship of *Gliocladium* sp. (E16) against mosquito larvae.

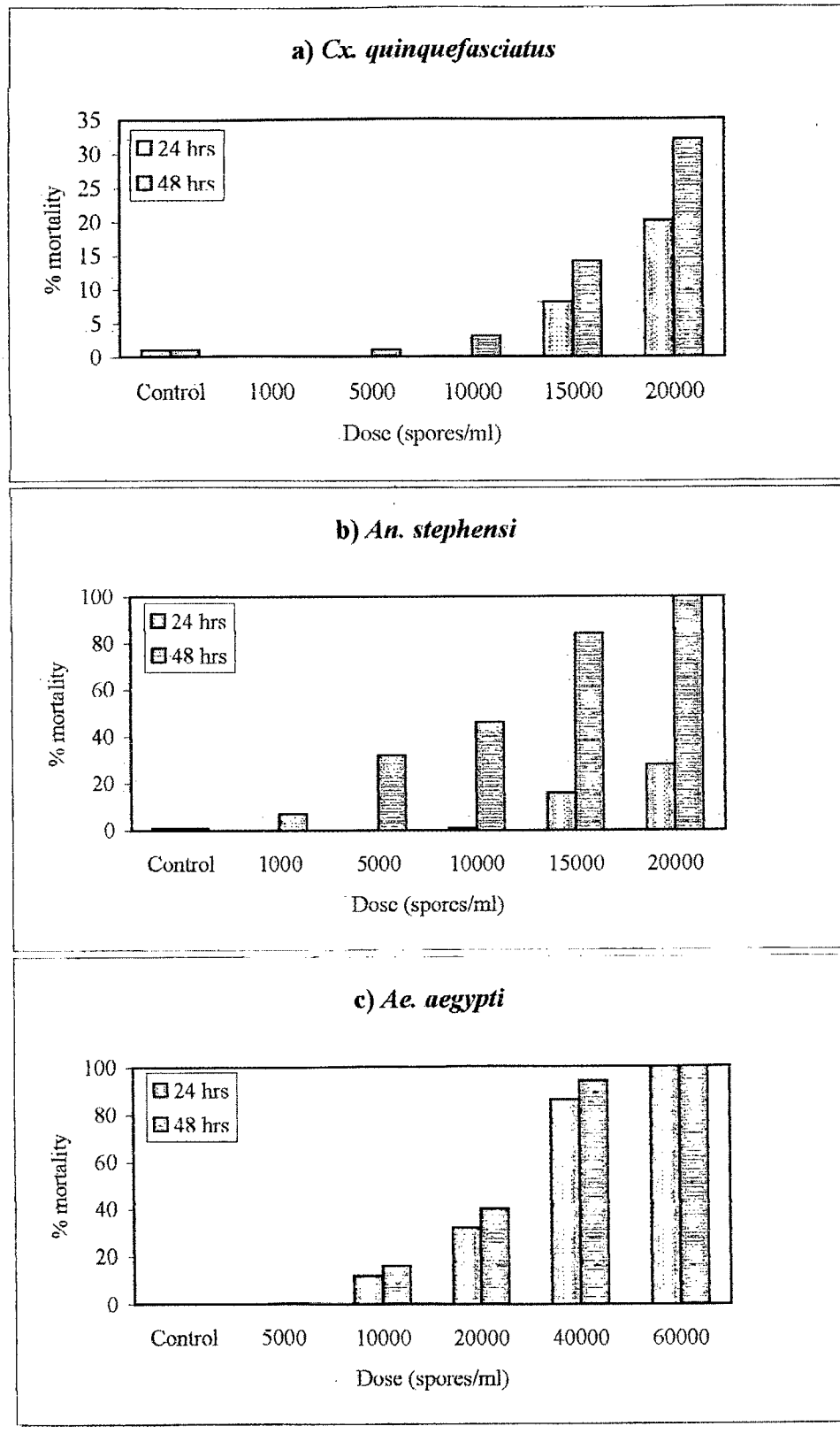


Fig. 4.3.9. Dose response relationship of *Penicillium* sp. (E9) against mosquito larvae.

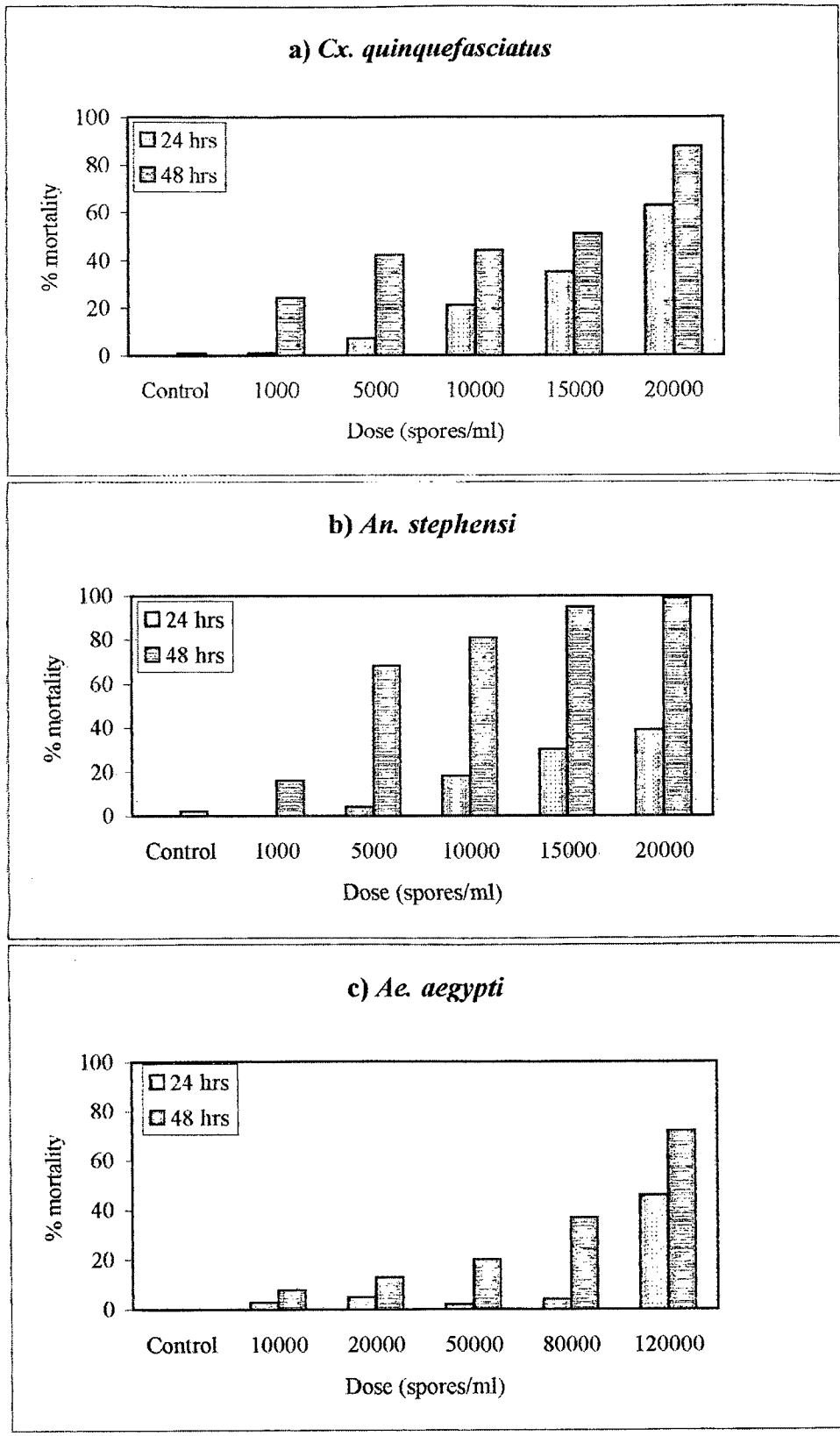
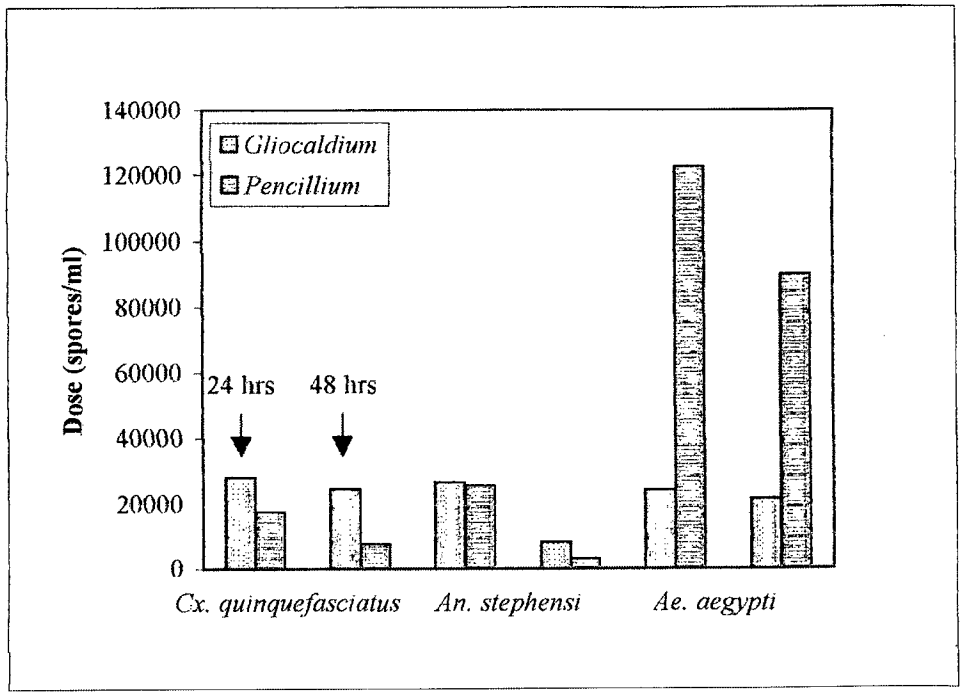


Fig. 4.3.10. LD_{50} values of *Gliocladium* sp. and *Penicillium* sp. against mosquito larvae.



PART IV:

4.4. Mosquito larvicidal activity of culture filtrate of promising isolates

Out of 40 promising isolates of entomogenous fungi tested, none of the 4-day-old-cultures showed larvicidal activity against 3rd instar larvae of *Culex quinquefasciatus*. In case of cell-free extracts of 7-day old cultures, 3 isolates, viz., *Acremonium* sp. (E29), *Penicillium* sp. (C2) and *Trichoderma* sp. (W6) resulted with 10%, 20% and 10% mortality respectively. On the other hand, cell-free extracts of 20-day old culture of 17 fungi caused mortality of 50% or more in 24 h. However, 15 isolates among the remaining produced less than 50% mortality at the end of 24 h. Extracts of last 8 promising isolates did not kill mosquito larvae tested. Results are presented in Table 4.17.

PART V:

4.5. Detection of enzymes of entomogenous fungi.

4.5.1. Fatty acid esterase (presumptive esterase) activity

Although this test is a presumptive one, effort was made to categorize positive results qualitatively into 3 categories based on intensity of activity. Isolates exhibiting colour change and formation of certain crystals in the entire plate were depicted as with +++ activity, colour change and formation of crystals beyond colony growth but not covering entire plate have been treated as those with ++ activity and , a colour change and crystal formation only within fungal colony were with + activity. Of the 286 isolates tested, 137 isolates were positive for esterase activity. Based on the grading considered above, 90 isolates were of +++ activity, 20 isolates with ++ activity and 27 isolates with + activity (Table 4.18).

4.5.2. Gelatin hydrolysis (presumptive protease) activity

According to length of gelatin in the test tube liquefied, gelatinase activity of the fungi was assigned as +1, +2, +3, +4 and +5 activity wherein +5 activity meant entire 5 cm of gelatin in the test tube liquefied by the fungus and +1 activity indicated the solubilization of only 1 cm of gelatin near the fungal colony. Only 125 amongst 286 isolates tested were able to solubilize gelatin. Of these, 96 isolates were given +5 activity status for liquefying gelatin completely, 2 isolates with +4 activity, 4 isolates with +3 activity, 9 isolates with +2 activity and 14 isolates with +1 activity (Table 4.18).

4.5.3. Chitin utilization (presumptive chitinase) activity:

Of the 286 isolates tested, only 31 showed positive to chitin utilization activity. (Table 4.18).

PART VI:

4.6. Preliminary field trials of promising isolate

Mortality rate of 53.8 ± 8.53 % and 66 ± 10.56 % were recorded at 24 and 48 h of treatment among *Anopheles stephensi* 3rd and 4th instar larvae treated with a dose of 20000 spores/ml of *Penicillium* sp. (E9) (Table 4.19). With 24 h treatment, dead larvae were at the bottom of the tray and their gut turned into a green tube. They were swollen with a contorted neck. Faecal pellets were not seen in the tray. Water contained germinating as well as intact spores. On 48 h exposure, dead larvae were covered by a green mass of fungal mycelia. Under light microscope, the fungus was found growing over the bristles and inter-segmental and caudal region of the larvae. Several short conidiophores with phialides producing conidial chains are observed. This observation clearly indicated the recycling ability of this fungus on exposure to mosquito larvae in water on exposure to certain duration. Tray did not contain any faecal pellets. Larvae, which were still alive, showed clear signs of sluggishness compared to control. Only 4% mortality was observed in control. Since it was below the acceptable 5% limit, the correction was not applied.

PART VII:

4.7. *In situ* observation of life cycle of *Aschersonia* spp.

Aschersonia aleyrodis, A. badia, A. indica and *Hirsutella* sp., found colonizing certain unidentified homopteran insects on plant foliage, were examined *in situ* at regular intervals for a duration of one year in order to understand the life cycle and distribution of these fungi. *Aschersonia badia* and *A. indica* produced their teleomorphs in *Podonectria* and *Hypocrella* respectively, during the study period. *Aschersonia aleyrodis, A. badia, A. indica* and *Hirsutella* sp. were readily distinguishable from each other by size, colour, shape and texture of exosclerotia. *Aschersonia aleyrodis* was found all over the forest, though its concentration was more in riparian habitats. *A. badia* was also found in most of the places wherever *A. aleyrodis* was present, but the former was less in number. Density of *A. badia* was more on foliage of trees around rivers. *Hirsutella* sp. and *Aschersonia indica* were found only in riparian region. Further, larger distribution of these fungi was seen only during October to January.

4.7.1. Occurrence

Collections of *A. indica, Hirsutella* sp. and *Hypocrella* sp. were made only on *H. ponga* throughout the year. *Podonectria* sp. was found on 4 plants, *A. badia* on 6 and *A. aleyrodis* on all 7 plants observed. While *Hopea ponga* harboured all 6 species of fungi *Strobilanthus ixiocephalus* hosted only *A. aleyrodis.* Although *Morinda citrifolia* harboured *A. badia,* its perfect state *Podonectria* sp. was not observed along with (Table 4.20).

4.7.2. Host insects

Insect hosts encountered were larvae/pupae of a few unidentified homopterans. These appear in large numbers on young fresh leaves during July to August. Although

several insects are seen around the host plant and since life cycle of these homopterans are not well known, affinity of the insect to fungus can not be established very well.

4.7.3. *Aschersonia aleyrodis*

During early monsoon very old colonies (exosclerotia) of *A. aleyrodis* were found on older foliages of *Calycopteris floribunda, Dillenia indica and Holarrhena antidysenterica* (Plate VII-a). These colonies were large, irregularly shaped and sometimes turned dark. These were also eaten away by mites, ants and various other unidentified insects and even colonized by fungi such as species of *Aspergillus*, *Penicillium* and *Hansfordia.* During mid-July, young colonies of *Aschersonia aleyrodis* were first observed on *Morinda citrifolia.* In August, young colonies of *A. aleyrodis* appeared *as* white mycelia around the insect pupa (Plate VII-b) on *Calycopteris floribunda, Dillenia indica, Holarrhena antidysenterica* and *Morinda citrifolia.* However, on *Strobilanthus ixiocephalus* and *Hopea ponga*, the fungus appeared in September. Colonies appeared on pupa from within and the sides, firmly fixing the pupa to host leaf. Several white, flat colonies had developed by then exhibiting pycnidia in circles at the periphery with their orifice covered by pale yellow or creamy mass of pycnidiospores (Plate VII-c,d). On contacting water, spores ooze out in large numbers in slimy droplets.

Aschersonia aleyrodis was found on tree foliage even little away from the streams during September to January. In February, almost all fruiting bodies were colonized fungi such as species of *Penicillium* and *Hanfordia.* However, young uninfected colonies with pycnidia were also seen, but in very small numbers. By end of March, as streams dry up, collection site become very humid and dry up further. During April, only mature and old colonies were seen in senescent leaves and litter of *D. indica*

and *Holarrhena antidysenterica*. Teleomorph of *A. aleyrodis* was not observed during the entire study period.

From mid-August to mid-December there was a steep increase in mean number of individuals per plant host (Fig. 4.7.1), thereafter declining steadily until end of the season.

4.7.4. *A. badia* and *Podonectria* sp.

Young colonies of *A. badia* appear on *Morinda citrifolia* and on leaves of *Calycopteris floribunda* in the month of August. The yellow orange colonies became apparent on *Dillenia indica, C. floribunda, Holarrhena antidysenterica* and *Lea* sp. in the beginning of September (Plate. IX-a,b). The fungus grew from the periphery of insect pupa covering it completely (Fig. 4.7.3-a,b,c). One to 5 small, sunken pycnidia appeared by the end of the month (Plate. IX-c; Fig. 4.7.3-f). End of September saw large pycnidia filled with conidia at deeper level in the fruiting body (Plate. IX-e,f; Fig. 4.7.3-g). *A. badia* was found distributed far away from the riparian habitat and in very less numbers when compared to *A. aleyrodis*. Between January and February, perithecia of teleomorph became apparent in some fruiting bodies of *A. badia* as many raised dark orange spots along the edges of thick stroma (Plate. XII-a; Fig. 4.7.3-h). In C.S., several tiny flask-shaped structures were found along the periphery of hemispherical stroma which contain a pycnidium at the centre and deep inside (Fig. 4.7.3-h). The spots were actually of ostioles of developing ascocarps. As the spots raised further, their adjoining areas were furrowed and each ascocarp became apparent by end of March (Plate. XII-b). As peritheia developed, the central anamorph zone shrunk making ostioles clearer. In cross section, well developed asci and ascospores were present, but centrally located pycnidium was not seen (Fig. 4.7.3-I). March onwards, most of the plants shed their

leaves and both anamorph and teleomorph stages become part of litter flora (Fig. 4.7.3-j). In subsequent dry months, the teleomorph stage could easily be detached from the main body.

Mean number of individuals of *A. badia* per host plant increased steeply, attaining a peak in December then declining until next June (Fig 4.7.2). Mean number of teleomorphs increased sharply during January-February. The mean number of individuals of *Podonectria* sp. was highest in the March, thereafter declining until next June.

Based on the year-long study, life cycle of *Aschersonia* badia was drawn and shown in Fig. 4.7.3. How the fungus gets back to insects on fresh leaves in the next year is not known.

4.7.5. *Aschersonia indica* and *Hypocrella* sp.

Very young colonies of *A. indica* appeared on minute immature stages of homopteran insects with shining golden yellow colour in mid-June and early July (Plate. XI-a; Fig. 4.7.5-a,c). Cross section of fruiting bodies showed highly interwoven hyphae. Many uninfected insect pupae were also seen on the leaves. By August, colonies of *A. indica* showed a small central darker area (pycnidial initial) (Fig. 4.7.5-d). However, no pycnidium or conidia were found in section of the stroma. During September, pycnidia appeared at the centre of stroma with a large opening, ostiole covered with orange conidia (Fig. 4.7.5-e). Pale, warty protuberances appeared at edge of the stroma (Plate. XI-b; Fig. 4.7.5-e). End of October saw no increase in number of pycnidia. Instead, peripheral area expanded with wart-like structures appearing around the periphery. Some of them elongated to form cylindrical structures with dense tissues in the center. By October, these cylindrical structures developed into flask-shaped ascocarps, cavity

of which was lined by layers of pseudoparenchymatous tissue inside which many asci were located (Plate. XI-d,e; Fig. 4.7.5-f,g). The fungus was identified as *Hypocrella* sp. Ascocarps of *Hypocrella* sp. could be detached from the anamorph very easily. By the end of the January, young colonies of *A. indica* were less in number. *Hypocrella* ascocarps were getting matured and detached (Fig. 4.7.5-h,i). Younger warts developed into ascocarps. By the end of March, though *Hypocrella* sp. were found in appreciable numbers, most of them were colonized by mycoparasites (Fig. 4.7.5-h,i).

Mean number of individuals per plant host plotted against months maintained almost a plateau from end of July till the end of October after a sharp increase from zero in June (Fig. 4.7.4). From October again there was an increase in mean number of individuals until a highest number was attained in December. The number of anamorph sharply declined between January and February. Less numbers of individuals were observed thereafter.

Though started developing in mid-October itself, a very steady increase in number of individuals was seen till mid-January. There was a sharp increase in mean number of *Hypocrella* sp. coinciding with corresponding sharp decline in the number of anamorphs. Highest peak of teleomorph reached during end of February. There was steady decline from March to July reaching zero at end of July. Life cycle of the fungus is shown in Fig. 4.7.5.

4.7.6. *Hirsutella* sp.

Young colonies of *Hirsutella* sp. appeared during July. There was not much change in its morphology except the size (Plate. XII-a,b). Mean number of individuals per plant host plotted against season reached a maximum in October, thereafter maintained a stationary phase up to November. Decline in the number until next June

along the summer was very steady, thus maintaining the inoculum for the next season (Fig. 4.7.1)

4.7.7. Colony size

When colony size was compared, mean size of *Hirsutella* sp. was the highest followed by *Hypocrella* sp., *Podonectria* sp., *A. aleyrodis*, *A. badia* and *A. indica* (Fig. 4.7.6). Highest colony size for all species was recorded during Dec. to Feb., registering a peak in plot of colony diam. against months (Fig. 4.7.7). After February, size of the stroma shrunk due to mycoparasitism and drying.

Fig. 4.7.1. *In situ* seasonal variation in mean number of individuals of the entomogenous fungi

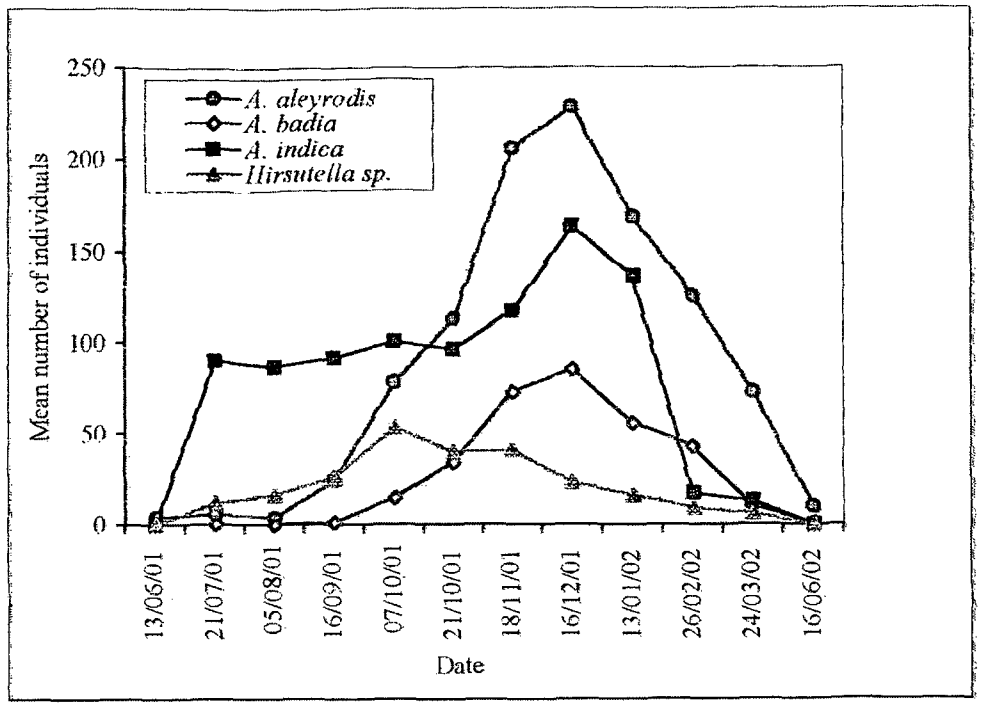


Fig. 4.7.2. *In situ* seasonal variation in mean number of colonies of *A. badia* and *Podonectria* sp. (anamorph- teleomorph).

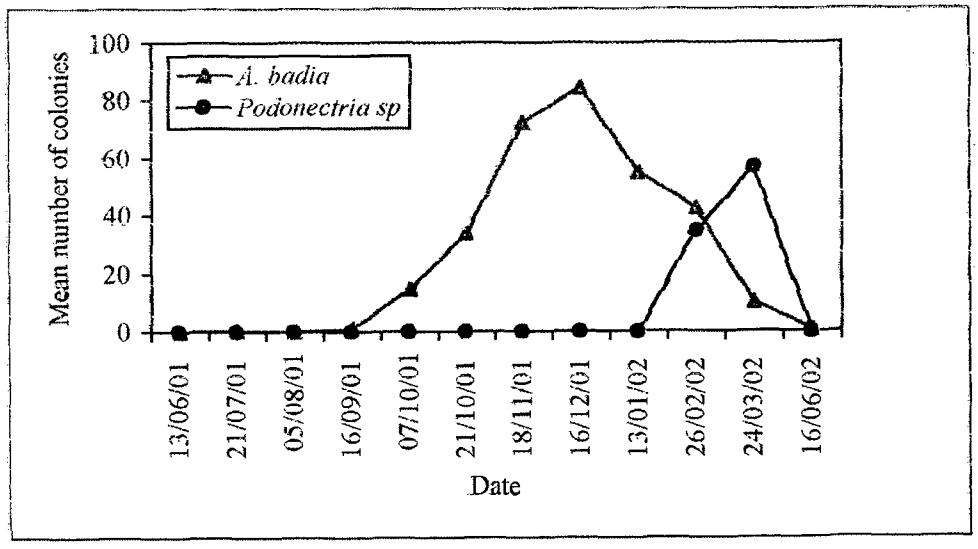


Fig. 4.7.3. a-j: Schematic life cycle of *Aschersonia badia* - *Podonectria* sp.

a. Mature green leaf

b. Insect pupae on green leaf

c. Fungi growing on pupae from sides and within

d. Exosclerotium (stroma) of the fungus covering the host

e. Development of pycnidia in the stroma

f. Conidial ooze from ostiole of pycnidia

g. Mature pycnidia at a deeper level in the stroma

h. Development of ascocarps at the periphery of stroma

i. Mature ascocarps at a deeper level in the stroma; pycnidia obliterated

j. Senescent leaves along with fungal stroma fall and become part of litter

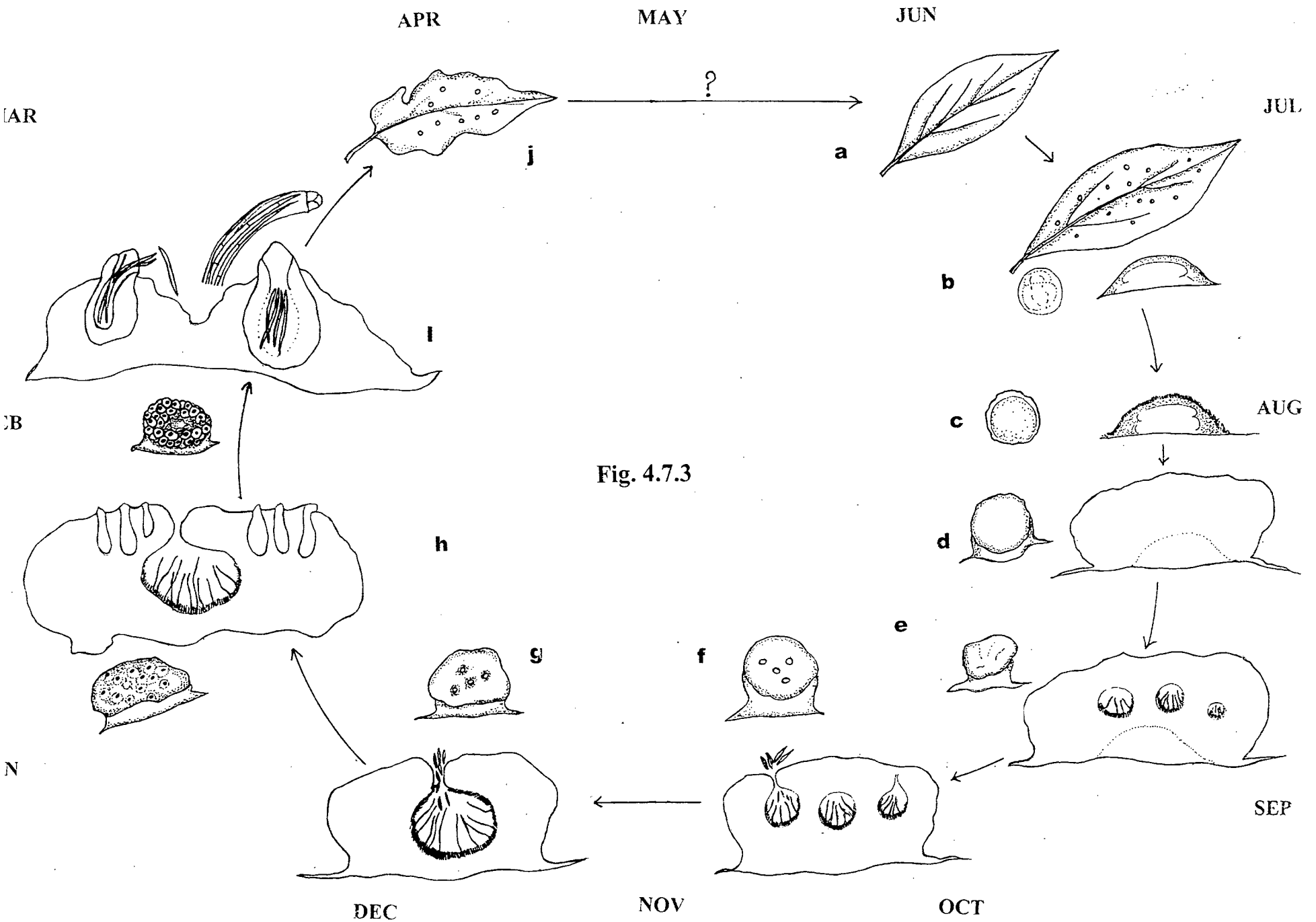


Fig. 4.7.3

Fig. 4.7.4. *In situ* seasonal variation in mean number of individuals of *A. indica* and *Hypocrella* sp. (anamorph- teleomorph).

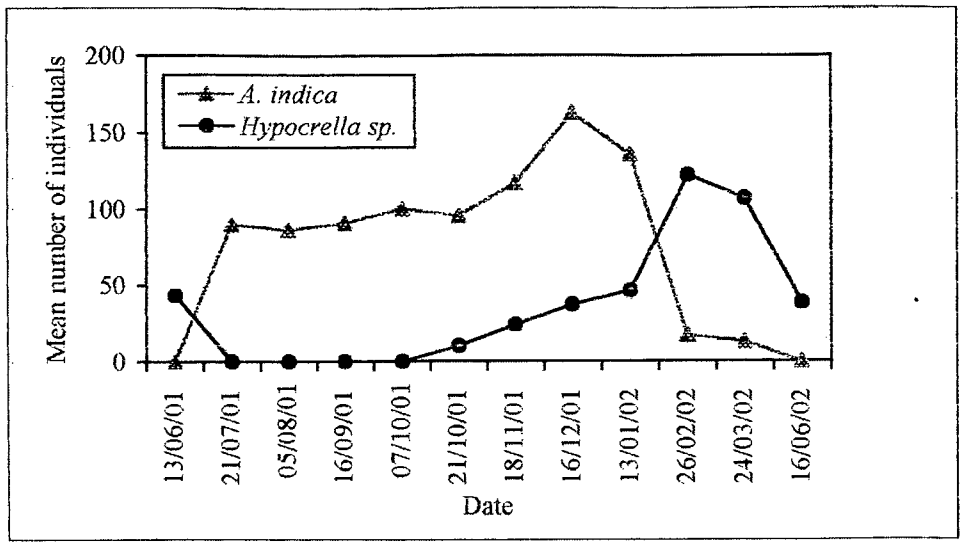


Fig. 4.7.6. *In situ* mean colony size of the entomogenous fungi during study period

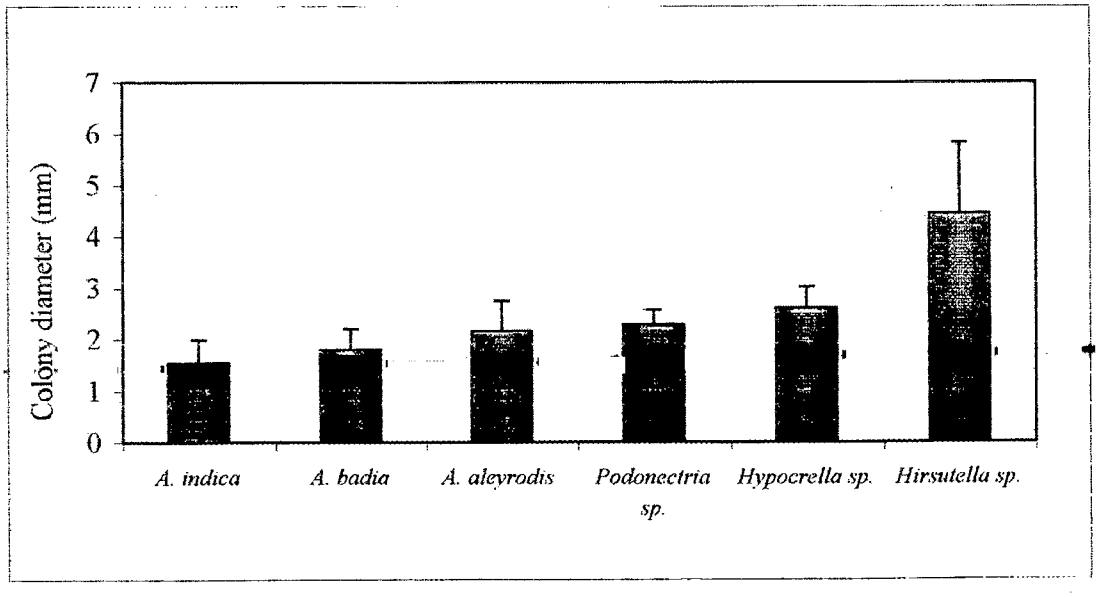


Fig. 4.7.5. a-i: Schematic life cycle of *Aschersonia indica* - *Hypocrella* sp.

a. Mature green leaf

b. Insect pupa on mature leaf

c. Fungi growing from sides and within the pupae

d. Exosclerotium (stroma) of the fungus covering the host with incipient pycnidium

e. Conidial ooze on ostiole of pycnidium with juvenile ascocarps

f. Mature pycnidium with ascocarps developing at periphery

g. Mature ascocarps, asci and ascospores

h. Ascocarps fall off; pycnidial stroma disintegrates

i. Senescent leaves with stromatic ascocarps becoming part of litter

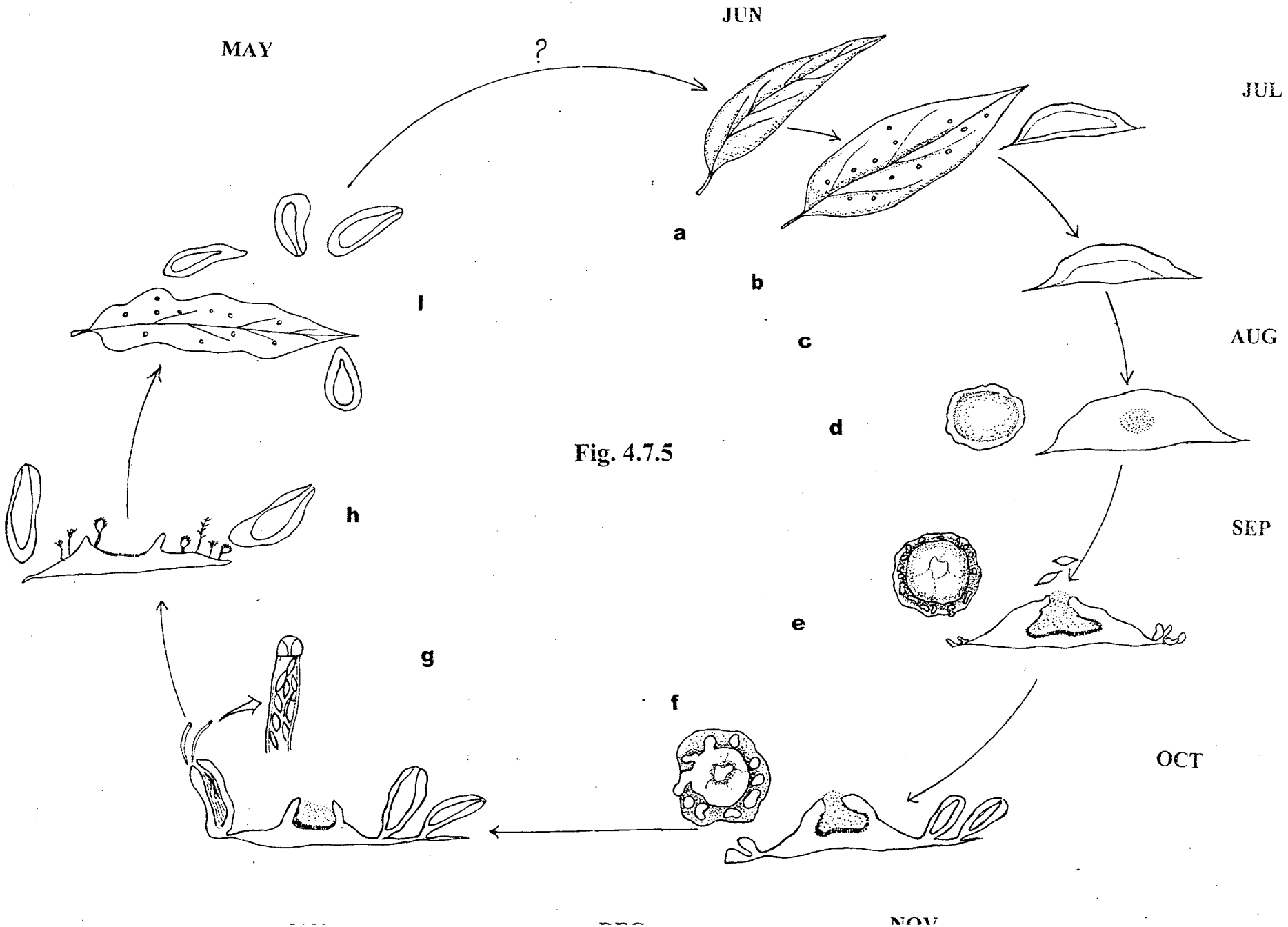


Fig. 4.7.5

Fig. 4.7.7. *In situ* seasonal variation in colony diameter of the entomogenous fungi

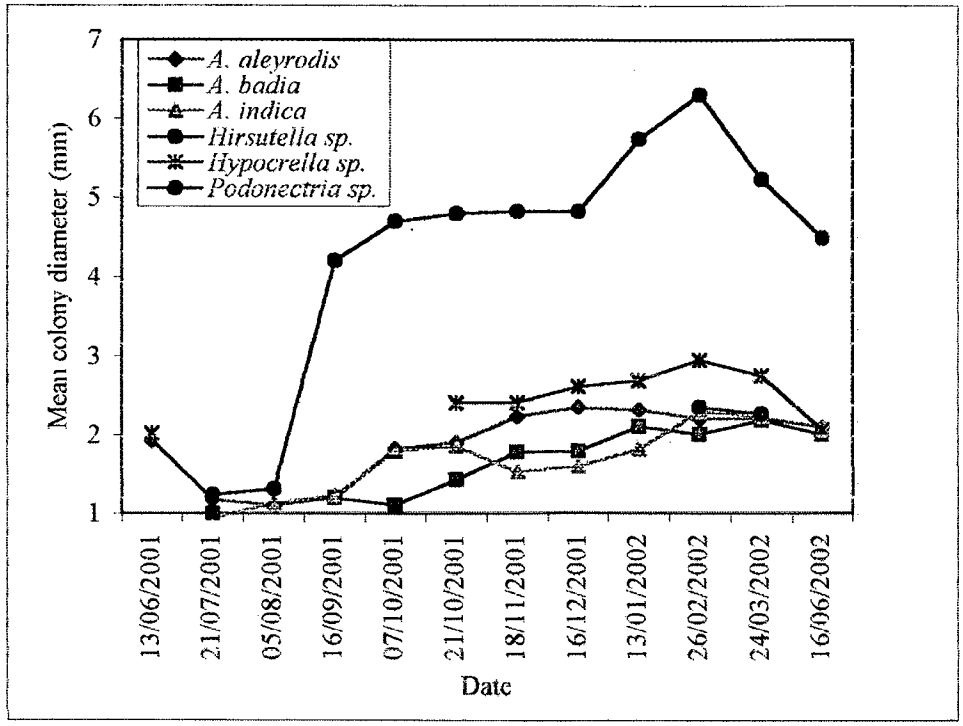


Table 4. 1. Fungi isolated from insects and arachnid hosts/substrates.

	Host/substrate	Number of Samples	Infested Samples	Fungal Isolates Recovered	% Infection	Fungal Diversity Index (FDI)
		a	b	c	d= b/a x 100	e= c/b x 100
i	**MOSQUITO LARVAE & PUPAE**					
	Anopheles sp.	1212	134	26	11.06	19.40
	Culex sp.	1843	164	27	8.90	16.46
	Aedes sp.	472	11	1	2.33	9.09
	Unidentified	145	80	3	55.17	3.75
	MOSQUITO ADULTS	58	0	0	0.00	
	TOTAL	3730	389	57	10.43	14.65
ii	**OTHER INSECTS & ARACHNIDS**					
	Ants (Order : Hymenoptera)	123	16	5	13.01	31.25
	Aphids (Order Hemiptera; Sub order Homoptera)	4196	438	26	10.44	5.94
	Coleopteran adult	124	8	3	6.45	37.50
	Dipteran	143	49	19	34.27	38.78
	Drosophila sp. (Order : Diptera)	94	5	6	5.32	120.00
	Homopteran larvae (Order Hemiptera; Sub order Homoptera)	5206	1489	43	28.60	2.89
	Coccids (Order Hemiptera; Sub order Homoptera)	221	36	5	16.29	13.89
	Scale insects (Order Hemiptera; Sub order Homoptera)	2635	134	32	5.09	23.88
	Mealy bugs (Order Hemiptera; Sub order Homoptera)	56	8	2	14.29	25.00
	Lepidopteran eggs	287	18	7	6.27	38.89
	Lepidopteran larvae	258	32	15	12.40	46.88
	Lepidopteran adults	126	14	1	11.11	7.14
	Unidentified insect eggs	17	4	4	23.53	100.00
	Unidentified insects	5786	51	28	0.88	54.90
	Foam trapped water insects	25	5	3	20.00	60.00
	Mites (Class : Arachnida)	492	14	6	2.85	42.86
	Spiders (Class : Arachnida)	166	34	17	20.48	50.00
	TOTAL	19955	2355	222	11.80	9.43
iii	**LARVAL BAITS IN WATER SAMPLES**	320	17	7	5.31	41.18
iv	**LARVAL BAITS IN SIMULATION FLOATS**	60	0	0	-	-
	NET TOTAL	24065	2761	286	11.47	10.36

Table: 4. 2. Fungal isolates recovered from insects and arachnids found associated with different plant hosts.

Host Plant	Code	Isolates of Fungi Recovered
Ageratum sp.	Asp	1
Anacardium occidentale	Aoc	4
Calotropis gigantea	Cgi	5
Careya arborea	Car	6
Cassia tora	Cto	4
Chromolaena odorata	Cod	20
Cinnamomum zeylanicum	Cze	5
Crotalaria sp.	Csp	2
Dillenia indica	Din	9
Flacourtia montana	Fmo	5
Gnetum ula	Gul	4
Hibiscus esculentus	Hes	1
Holarrhena antidysenterica	Han	18
Holigarna arnottiana	Har	4
Hopea ponga	Hpo	22
Hopea wightiana	Hwi	3
Ixora brachiata	Ibr	5
Ixora coccinea	Ico	7
Leea macrophylla	Lma	4
Mangifera indica	Min	3
Microcos paniculata	Mpa	4
Myristica malabarica	Mma	2
Phyllanthus sp	Psp	2
Psychotria dalzellii	Pda	7
Rauvolfia serpentina	Rse	1
Saraca asoca	Sas	1
Solanum melanogaster	Sme	4
Strobilanthus ixiocephalus	Six	4
Syzygium zeylanicum	Sze	3
Terminalia bellirica	Tbe	2
Terminalia paniculata	Tpa	4
Others	Oth	56
TOTAL		**222**

Table 4. 3. Locality-wise recovery of entomogenous fungi from different sources.

Locality	Fungal isolates recovered from different source types				
	i	ii	iii	iv	Total
Alonrna	-	4	-	-	4
Amboli	-	-	-	-	0
Ambe	-	10	-	-	10
Anmod	-	5	-	-	5
Bicholim	6	-	-	-	6
Bondla	-	10	3	-	13
Chandreshwar	-	2	-	-	2
Chorlem	-	3	-	-	3
Codal	-	4	-	-	4
Colem	-	4	-	-	4
Cotigao	-	5	4	-	9
Cudnem	-	4	-	-	4
Cuncolim	8	-	-	-	8
Curchorem	4	4	-	-	8
Dhudhsagar	-	8	-	-	8
Edda	-	2	-	-	2
Endrem	-	6	-	-	6
GU campus	-	12	-	-	12
Karmali	-	2	-	-	2
Kasaragod	-	9	-	-	9
Kesarval	-	-	-	-	0
Kodachadri	-	7	-	-	7
Kollur	-	17	-	-	17
Melka	-	1	-	-	1
Molem	-	47	-	-	47
Panjim	8	-	-	-	8
Pernem	6	9	-	-	15
Porvorim	11	-	-	-	11
Sonauli	-	1	-	-	1
Subrahmanya	-	1	-	-	1
Taleigao	2	17	-	-	19
Tambdi Surla	-	25	-	-	25
Valpoi	-	3	-	-	3
Vasco	12	-	-	-	12
Total	**57**	**222**	**7**	**0**	**286**

Note: Source types: i) Mosquito larvae & pupae; ii) other insects & arachnids; iii) larval baits in water samples and iv) larval baits in simulation floats.

Table 4. 4. Promising isolates of fungi and their mosquito larvicidal activity.

	Fungus Name	Culture Code No.	Bioactivity (Mortality %)	Duration (h)
1.	*Acremonium* sp.	E29	100	72
2.	*Aspergillus* sp.	C15	60	120
3.	*Aspergillus* sp.	E21	50	120
4.	*Aspergillus* sp.	W5	100	120
5.	*Chaetomella* sp.	E35	94	120
6.	*Gliocladium* sp.	E6	100	24
7.	*Gliocladium* sp.	E13	100	24
8.	*Gliocladium* sp.	E15	100	24
9.	*Gliocladium* sp.	E16	100	24
10.	*Gliocladium* sp.	E19	100	24
11.	*Gliocladium* sp.	E20	100	24
12.	*Gliocladium* sp.	E23	95	120
13.	*Gliocladium* sp.	E48	100	24
14.	*Penicillium* sp.	C2	100	48
15.	*Penicillium* sp.	C4	94	48
16.	*Penicillium* sp.	C5	85	48
17.	*Penicillium* sp.	C20	100	72
18.	*Penicillium* sp.	C26	100	120
19.	*Penicillium* sp.	C29	100	48
20.	*Penicillium* sp.	C42	100	48
21.	*Penicillium* sp.	C44	100	48
22.	*Penicillium* sp.	C51	100	48
23.	*Penicillium* sp.	E2	100	48
24.	*Penicillium* sp.	E9	100	24
25.	*Penicillium* sp.	E11	100	48
26.	*Penicillium* sp.	E26	100	48
27.	*Trichoderma* sp.	C13	100	120
28.	*Trichoderma* sp.	C36	70	120
29.	*Trichoderma* sp.	C37	65	120
30.	*Trichoderma* sp.	C48	60	120
31.	*Trichoderma* sp.	C52	95	120
32.	*Trichoderma* sp.	C53	80	120
33.	*Trichoderma* sp.	C54	100	96
34.	*Trichoderma* sp.	E37	100	96
35.	*Trichoderma* sp.	E39	92.1	120
36.	*Trichoderma* sp.	E42	90	120
37.	*Trichoderma* sp.	W6	60	120
38.	Undetermined sp.	C7	100	48
39.	Undetermined sp.	C17	100	48
40.	Undetermined sp.	C27	100	72

Table 4. 5. Mass production of conidia in different media for *Gliocladium* sp. (E16) (Incubation period 20 days).

Bottle No	Total number of conidia recovered from 50 ml of media spread in 117 cm^2			
	1% corn	4% corn	4% corn + sugar	Czapek Malt
1	9,860	6,18,000	4,25,000	29,90,800
2	11,200	7,20,000	4,18,000	32,10,600
3	11,500	5,10,000	4,30,000	28,92,000
4	10,200	5,30,000	3,56,000	29,10,200
5	10,800	5,65,750	4,16,700	29,24,300
6	11,900	7,15,000	3,48,600	30,10,200
7	12,200	4,95,950	3,92,700	31,20,000
8	10,600	5,96,000	4,02,800	28,40,400
9	9,800	6,80,000	3,60,000	30,86,000
10	9,100	6,40,000	3,87,600	32,16,600
Mean	**10,716**	**6,07,070**	**3,93,740**	**30,20,110**
SD	**± 995.7**	**± 81965.2**	**± 30053.4**	**± 1,33,391.3**

Table 4. 6. Mass production of conidia in different media for *Penicillium* sp. (E9) (Incubation period 25 days).

Bottle No	Total Conidia recovered from 50 ml of media spread in 117 cm^2			
	1% corn	4% corn	4% corn + sugar	Czapek Malt
1	67000	598600	1609800	11452500
2	93500	569800	1458700	12432000
3	176000	456700	1627800	10946000
4	89000	617800	2190000	9804560
5	86500	623400	1986750	9407800
6	83500	499800	1889600	9567900
7	74700	610000	2067000	10005000
8	76000	489600	1578000	10634800
9	78000	768900	2178000	12458000
10	98000	475600	1982000	11450000
Mean	**92220**	**571020**	**1856765**	**10815856**
SD	**± 30878.9**	**± 94425.55**	**± 267015.7**	**± 1124769**

Table 4. 7. 'Dose-range' test of fungal spores against different mosquito larvae.

Fungus	Mosquito larvae	Range (conidia/ml)
Acremonium sp. (E29)	*Culex quinquefasciatus*	1000-50000
	Anopheles stephensi	1000-50000
	Aedes aegypti	10000-100000
Gliocladium sp.(E16)	*Culex quinquefasciatus*	100-10000
	Anopheles stephensi	100-10000
	Aedes aegypti	10000-50000
Penicillium sp. (E9)	*Culex quinquefasciatus*	1000-50000
	Anopheles stephensi	1000-50000
	Aedes aegypti	10000-100000
Trichoderma sp.(C54)	*Culex quinquefasciatus*	10000-100000
	Anopheles stephensi	10000-100000
	Aedes aegypti	10000-100000

Table 4. 8. ANOVA values for bioassay of fungi against mosquito larvae in 250 ml of water for 24, 48 and 72 h.

Fungus	Mosquito larvae	Source of variation	F	DFn	DFd	P value	Significance
Acremonium sp. (E29)	*Cx. quinquefasciatus*	Interaction	0.16	10	72	0.9985	ns
		Treatment duration	1.13	2	72	0.3301	ns
		Dose (spores/ml)	1316.08	5	72	<0.0001	***
	An. stephensi	Interaction	1.16	10	72	0.3332	ns
		Treatment duration	2.05	2	72	0.1364	ns
		Dose (spores/ml)	1536.4	5	72	<0.0001	***
	Ae. Aegypti	Interaction	0.04	10	72	1	ns
		Treatment duration	0.15	2	72	0.8581	ns
		Dose (spores/ml)	264.65	5	72	<0.0001	***
Gliocladium sp. (E16)	*Cx. quinquefasciatus*	Interaction	0.18	10	72	0.9973	ns
		Treatment duration	1.49	2	72	0.2333	ns
		Dose (spores/ml)	2451.04	5	72	<0.0001	***
	An. stephensi	Interaction	2.04	10	72	0.0415	*
		Treatment duration	3.27	2	72	0.0437	*
		Dose (spores/ml)	3421.45	5	72	<0.0001	***
	Ae. Aegypti	Interaction	0.36	10	72	0.9577	ns
		Treatment duration	0.58	2	72	0.5614	ns
		Dose (spores/ml)	732.69	5	72	<0.0001	***
Penicillium sp. (E9)	*Cx. quinquefasciatus*	Interaction	1.14	10	72	0.3475	ns
		Treatment duration	1.14	2	72	0.3264	ns
		Dose (spores/ml)	132.41	5	72	<0.0001	***
	An. stephensi	Interaction	0.35	10	72	0.9629	ns
		Treatment duration	1.64	2	72	0.2012	ns
		Dose (spores/ml)	931.25	5	72	<0.0001	***
	Ae. Aegypti	Interaction	0.03	10	72	1	ns
		Treatment duration	0.03	2	72	0.9698	ns
		Dose (spores/ml)	319.14	5	72	<0.0001	***
Trichoderma sp. (C54)	*Cx. quinquefasciatus*	Interaction	11.79	20	120	<0.0001	***
		Treatment duration	70.23	4	120	<0.0001	***
		Dose (spores/ml)	432.34	5	120	<0.0001	***
	An. stephensi	Interaction	19.14	20	120	<0.0001	***
		Treatment duration	95.84	4	120	<0.0001	***
		Dose (spores/ml)	99.83	5	120	<0.0001	***
	Ae. Aegypti	Interaction	0.24	20	120	0.9997	ns
		Treatment duration	1.6	4	120	0.1787	ns
		Dose (spores/ml)	13.06	5	120	<0.0001	***

Note: * significant; ** very significant; *** extremely significant; ns- not significant.

Table 4. 9. Bonferroni post t-tests for dose response relationship of *Trichoderma* sp. (C54) against mosquito larvae.

	Cx. quinquefasciatus			*An. stephensi*			*Ae. aegypti*		
Dose (spores/ml)	**t**	**P value**	**Summary**	**t**	**P value**	**Summary**	**t**	**P value**	**Summary**
24 h vs. 48 h									
0	0	P > 0.05	ns	0	P > 0.05	ns	0.401	P > 0.05	ns
10000	0	P > 0.05	ns	0	P > 0.05	ns	0.201	P > 0.05	ns
20000	0	P > 0.05	ns	0.371	P > 0.05	ns	0	P > 0.05	ns
40000	0	P > 0.05	ns	0	P > 0.05	ns	0	P > 0.05	ns
60000	4.456	P<0.001	***	0.742	P > 0.05	ns	0	P > 0.05	ns
120000	4.456	P<0.001	***	0.742	P > 0.05	ns	0.201	P > 0.05	ns
48 h vs. 72 h									
0	0.297	P > 0.05	ns	0.742	P > 0.05	ns	0	P > 0.05	ns
10000	0	P > 0.05	ns	1.855	P > 0.05	ns	0	P > 0.05	ns
20000	0	P > 0.05	ns	0.371	P > 0.05	ns	0	P > 0.05	ns
40000	0	P > 0.05	ns	2.597	P > 0.05	ns	0	P > 0.05	ns
60000	0.5941	P > 0.05	ns	4.823	P<0.001	***	0	P > 0.05	ns
120000	1.634	P > 0.05	ns	5.935	P<0.001	***	0	P > 0.05	ns
72 h vs. 96 h									
0	0	P > 0.05	ns	0.7419	P > 0.05	ns	0.201	P > 0.05	ns
10000	0	P > 0.05	ns	0	P > 0.05	ns	0	P > 0.05	ns
20000	0.5941	P > 0.05	ns	0	P > 0.05	ns	0.201	P > 0.05	ns
40000	3.862	P<0.01	**	0.371	P > 0.05	ns	0	P > 0.05	ns
60000	4.159	P<0.001	***	3.71	P<0.01	**	1.605	P > 0.05	ns
120000	4.307	P<0.001	***	5.564	P<0.001	***	0.602	P > 0.05	ns
96 h vs. 120 h									
0	0.297	P > 0.05	ns	0.742	P > 0.05	ns	0.401	P > 0.05	ns
10000	0.297	P > 0.05	ns	0	P > 0.05	ns	0	P > 0.05	ns
20000	1.782	P > 0.05	ns	0.371	P > 0.05	ns	0	P > 0.05	ns
40000	4.456	P<0.001	***	0	P > 0.05	ns	0.803	P > 0.05	ns
60000	1.782	P > 0.05	ns	3.339	P<0.01	**	0	P > 0.05	ns
120000	4.159	P<0.001	***	6.306	P<0.001	***	0.602	P > 0.05	ns

Note: * significant; ** very significant; *** extremely significant; ns- not significant

Table 4. 10. Median Lethal Dose, LD_{50} (spores/ml) value for fungi against 3rd instar larvae of mosquitoes in 250 ml of water.

		Culex quinquefasciatus			*Anopheles stephensi*			*Aedes aegypti*		
Fungus Species		24h	48h	72 h	24h	48h	72 h	24h	48h	72 h
Acremonium sp.	LD_{50}	19712	18992	18885	26385	25341	24561	108638	107026	104974
	S.E.	1.045	1.037	1.031	1.042	1.030	1.016	1.039	1.041	1.047
	95% CI	17457 to 22257	17165 to 21013	17328 to 20581	23556 to 29554	23365 to 27484	23496 to 25675	97731 to 120762	95663 to 119738	92352 to 119321
	R^2	0.992	0.994	0.996	0.993	0.997	0.999	0.989	0.989	0.985
Gliocladium sp.	LD_{50}	4373	4250	4147	5324	5039	5039	27406	27653	27738
	S.E.	1.125	1.124	1.123	1.101	1.098	1.098	1.054	1.067	1.072
	95% CI	3150 to 6071	3072 to 5880	3008 to 5717	4075 to 6956	3892 to 6524	3892 to 6524	23665 to 31739	23120 to 33074	22844 to 33680
	R^2	0.953	0.955	0.957	0.966	0.968	0.968	0.996	0.984	0.980
Penicillium sp.	LD_{50}	1828	1536	1288	1822	1723	1637	31733	30804	30503
	S.E.	1.062	1.064	1.105	1.124	1.093	1.084	1.077	1.068	1.065
	95% CI	1549 to 2157	1294 to 1823	976.8 to 1700	1317 to 2519	1347 to 2203	1307 to 2049	25857 to 38944	25687 to 36941	25624 to 36312
	R^2	0.999	0.999	0.999	0.977	0.986	0.988	0.987	0.990	0.990

		Cx. quinquefasciatus		*An. stephensi*		*Ae. aegypti*	
		96 hrs	120 hrs	96 hrs	120 hrs	96 hrs	120 hrs
Trichoderma sp.	LD_{50}	106936	84332	247114	142921	4.35E+06	2.24E+06
	S.E.	1.067	1.023	1.183	1.183	33.729	9.378
	95% CI	89391 to 127925	79268 to 89720	107489 to 568106	89729 to 227644	248.8 to 7.5996e+010	4478 to 1.1172e+009
	R^2	0.9794	0.9977	0.8866	0.9069	0.2235	0.2852

Table 4. 11. Bioassay of *Penicillium* sp. (E9) against mosquito larvae.

Cx. quinuefasciatus Goa strain

Dose	24 h	SD	48 h	SD
control	0	0	0	0
2570	65.6	2.19	72.8	3.35
6425	89.6	4.56	92.8	6.57
12850	100	0	100	0
25700	100	0	100	0
51400	100	0	100	0

Cx. quinuefasciatus (Pune Strain)

Dose	24 h	SD	48 h	SD
control	0	0	0	0
1000	0	0	6.67	2.89
5000	83.33	2.89	83.33	2.89
10000	95	0	100	0
20000	95	5	100	0
30000	91.67	2.89	100	0

An. stephensi (Goa strain)

Dose	24 h	SD	48 h	SD
0	0.8	1.79	0.8	1.79
878	18.4	4.56	20	4.90
1789	57.6	4.56	57.6	4.56
3557	71.2	5.93	75.2	6.57
8944	84.8	5.22	87.2	8.67
17887	100	0	100	0

An. stephensi (Pune Strain)

Dose	24 h	SD	48 h	SD
control	0	0	0	0
1000	0	0	6.67	7.64
5000	100	0	100	0
10000	100	0	100	0
20000	100	0	100	0
30000	98.33	2.89	100	0

Ae. aegypti Goa Strain

control	0	0	0	0
3244	0	0	0	0
8111	0	0	0	0
16222	22.4	11.52	22.4	11.52
32444	47.2	19.27	49.6	17.34
98444	100	0	100	0

Ae. aegypti Pune strain

control	0.00	0.00	0.00	0.00
25000	5.00	8.66	10.00	8.66
50000	30.00	5.00	30.00	5.00
75000	28.33	7.64	30.00	5.00
125000	31.67	7.64	33.33	5.77
150000	28.33	5.77	41.67	2.89

Table: 4. 12. Values from regression analyses of dose response relationship of Goa and Pune strains of mosquito larvae against spores of *Penicillium* sp. (E9). (Note: Dose response relationship between strains compared at 24 and 48 h separately)

	24 hours		48 hours	
Cx. quinquefasciatus	Goa Strain	Pune Strain	Goa Strain	Pune Strain
Best-fit values				
Slope	0.001185 ± 0.0008268	0.002860 ± 0.001398	0.001115 ± 0.0008496	0.003048 ± 0.001354
Y-intercept	56.32 ± 20.01	29.38 ± 21.55	59.21 ± 20.57	31.48 ± 20.88
X-intercept	-47520	-10270	-53110	-10330
1/slope	843.7	349.7	896.9	328.1
Goodness of Fit				
r^2	0.3394	0.5114	0.3009	0.5586
Sy.x	35.88	36.98	36.88	35.84
Runs test				
P value (runs test)	0.4	0.3	0.4	0.3
Significantly nonlinear?	Not Significant	Not Significant	Not Significant	Not Significant
An. stephensi	Goa Strain	Pune Strain	Goa Strain	Pune Strain
Best-fit values				
Slope	0.004633 ± 0.001608	0.002954 ± 0.001594	0.004603 ± 0.001682	0.002905 ± 0.001532
Y-intercept	29.94 ± 13.40	33.89 ± 24.57	31.44 ± 14.01	35.83 ± 23.62
X-intercept	-6464	-11470	-6831	-12330
1/slope	215.9	338.5	217.3	344.3
Goodness of Fit				
r^2	0.6747	0.4619	0.6519	0.4732
Sy.x	24.63	42.17	25.75	40.54
Runs test				
P value (runs test)	0.3	0.3	0.3	0.3
Significantly nonlinear?	Not Significant	Not Significant	Not Significant	Not Significant
Ae. aegypti	Goa Strain	Pune Strain	Goa Strain	Pune Strain
Best-fit values				
Slope	0.001052 ± 0.0001034	0.0001921 ± 0.00007517	0.001055 ± 0.0001143	0.0002472 ± 0.00005703
Y-intercept	0.4708 ± 4.444	6.946 ± 6.644	0.8154 ± 4.910	6.656 ± 5.041
X-intercept	-447.4	-36150	-773.2	-26920
1/slope	950.2	5205	948.3	4045
Goodness of Fit				
r^2	0.9628	0.6202	0.9552	0.8245
Sy.x	8.588	9.735	9.489	7.386
Runs test				
P value (runs test)	0.4	0.3	0.4	0.4
Significantly nonlinear?	Not Significant	Not Significant	Not Significant	Not Significant

Table 4. 13. Summary of comparison of regression lines of dose response relationship of Goa and Pune strains of mosquito larvae against spores of *Penicillium* sp. (E9). (Note: Dose response relationship between strains compared at 24 and 48 h separately)

Mosquito larvae	Treatment duration (h)	Source of variation	F	DFn	DFd	P value	Significance
Cx. quinquefasciatus	24	Slope	1.078	1	8	0.330	ns
		Intercepts	0.079	1	9	0.785	ns
	48	Slope	1.442	1	8	0.264	ns
		Intercepts	0.027	1	9	0.873	ns
An. stephensi	24	Slope	0.415	1	8	0.538	ns
		Intercepts	0.142	1	9	0.715	ns
	48	Slope	0.439	1	8	0.526	ns
		Intercepts	0.135	1	9	0.722	ns
Ae. Aegypti	24	Slope	42.935	1	8	0.00018	***
		Intercepts*	-	-	-	-	-
	48	Slope	44.065	1	8	0.00016	***
		Intercepts*	-	-	-	-	-

Note: * Since slopes differ very much, it is not possible to test whether the intercepts differ significantly; *** extremely significant; ns – not significant.

Table 4. 15: Bonferroni post t-tests for dose response relationship of fungal spores against mosquito larvae (24 h vs. 48 h).

	Cx. quinquefasciatus				*An. stephensi*				*Ae. aegypti*			
Fungus	Dose (spores/ml)	t	P value	Summary	Dose (spores/ml)	t	P value	Summary	Dose (spores/ml)	t	P value	Summary
Gliocladium sp. (E16)	20000	4.129	P<0.01	**	20000	17.08	P<0.001	***	60000	0	P > 0.05	ns
	15000	2.065	P > 0.05	ns	15000	16.13	P<0.001	***	40000	4	P<0.01	**
	10000	1.032	P > 0.05	ns	10000	10.67	P<0.001	***	20000	4	P<0.01	**
	5000	0.344	P > 0.05	ns	5000	7.589	P<0.001	***	10000	2	P > 0.05	ns
	1000	0	P > 0.05	ns	1000	1.66	P > 0.05	ns	5000	0	P > 0.05	ns
	0	0	P > 0.05	ns	0	0	P > 0.05	ns	0	0	P > 0.05	ns
Penicillium sp. (E9)	20000	4.796	P<0.001	***	20000	10.76	P<0.001	***	120000	10.89	P<0.001	***
	15000	3.07	P < 0.05	*	15000	11.65	P<0.001	***	80000	13.83	P<0.001	***
	10000	4.413	P<0.001	***	10000	11.29	P<0.001	***	50000	7.543	P<0.001	***
	5000	6.715	P<0.001	***	5000	11.47	P<0.001	***	20000	2.198	P > 0.05	ns
	1000	4.413	P<0.001	***	1000	2.869	P < 0.05	*	10000	2.095	P > 0.05	ns
	0	0.192	P > 0.05	ns	0	0.359	P > 0.05	ns	0	0	P > 0.05	ns

Note: * significant; **-very significant; * extremely significant; ns- not significant.**

Table 4. 14. ANOVA values for Dose response relationship of fungi against mosquito larvae in 1500 ml of water for 24, and 48 h.

Fungus	Mosquito larvae	Source of variation	F	DFn	DFd	P value	Significance
Gliocladium sp. (E16)	*Cx. quinquefasciatus*	Interaction	2.59	5	36	0.0423	*
		Treatment duration	9.55	1	36	0.0038	**
		Dose (spores/ml)	50.57	5	36	<0.0001	***
	An. stephensi	Interaction	51.11	5	36	<0.0001	***
		Treatment duration	470.4	1	36	<0.0001	***
		Dose (spores/ml)	146.56	5	36	<0.0001	***
	Ae. Aegypti	Interaction	3.87	5	36	0.0066	**
		Treatment duration	16.67	1	36	0.0002	***
		Dose (spores/ml)	1998.4	5	36	<0.0001	***
Penicillium sp. (E9)	*Cx. quinquefasciatus*	Interaction	4.74	5	36	0.002	**
		Treatment duration	92.82	1	36	<0.0001	***
		Dose (spores/ml)	101.31	5	36	<0.0001	***
	An. stephensi	Interaction	25.71	5	36	<0.0001	***
		Treatment duration	390.54	1	36	<0.0001	***
		Dose (spores/ml)	101.98	5	36	<0.0001	***
	Ae. Aegypti	Interaction	29.97	5	36	<0.0001	***
		Treatment duration	218.83	1	36	<0.0001	***
		Dose (spores/ml)	323.62	5	36	<0.0001	***

Note: * significant; ** very significant; *** extremely significant.

Table 4. 16. Median Lethal Dose, LD_{50} (spores/ml) values for fungi against 3rd instar larvae of mosquitoes in 1500 ml of water along with other standard indices.

Species		*Cx. quinquefasciatus*		*An. stephensi*		*Ae. aegypti*	
		24h	48h	24h	48h	24h	48h
Gliocladium sp. (E16)	LD_{50}	27992	24439	26334	8297	24069	21349
	S.E.	1.048	1.015	1.067	1.151	1.050	1.063
	95% CI	24560 to 31904	23436 to 25484	22019 to 31495	5618 to 12255	21042 to 27531	18004 to 25316
	R^2	0.9884	0.9978	0.976	0.9413	0.9919	0.9874
Penicillium sp. (E9)	LD_{50}	17347	7631	25404	3070	122653	89727
	S.E.	1.043	1.439	1.042	1.098	1.019	1.091
	95% CI	15425 to 19509	2778 to 20957	22675 to 28462	2369 to 3977	116500 to 129132	70416 to 114335
	R^2	0.9797	0.8294	0.995	0.9933	0.9761	0.9335

Table 4. 17: Larvicidal activity of cell-free extracts of 4, 7 and 20 day old cultures of promising isolates.

	Fungus	Code	Mortality (%)		
			4 day	7day	20day
1.	*Acremonium* sp.	E29	0	10	100
2.	*Aspergillus* sp.1	C15	0	0	10
3.	*Aspergillus* sp.2	E21	0	0	100
4.	*Aspergillus* sp.3	W5	0	0	60
5.	*Chaetomella* sp.	E35	0	0	0
6.	*Gliocladium* sp.1	E6	0	0	60
7.	*Gliocladium* sp.2	E13	0	0	30
8.	*Gliocladium* sp.3	E15	0	0	10
9.	*Gliocladium* sp.4	E16	0	0	10
10.	*Gliocladium* sp.5	E19	0	0	60
11.	*Gliocladium* sp.6	E20	0	0	20
12.	*Gliocladium* sp.7	E23	0	0	30
13.	*Gliocladium* sp.8	E48	0	0	10
14.	*Penicillium* sp.1	C2	0	20	100
15.	*Penicillium* sp.2	C4	0	0	0
16.	*Penicillium* sp.3	C5	0	0	0
17.	*Penicillium* sp.4	C20	0	0	0
18.	*Penicillium* sp.5	C26	0	0	10
19.	*Penicillium* sp.6	C29	0	0	20
20.	*Penicillium* sp.7	C42	0	0	0
21.	*Penicillium* sp.8	C44	0	0	100
22.	*Penicillium* sp.9	C51	0	0	20
23.	*Penicillium* sp.10	E2	0	0	90
24.	*Penicillium* sp.11	E9	0	0	80
25.	*Penicillium* sp.12	E11	0	0	10
26.	*Penicillium* sp.13	E26	0	0	100
27.	*Trichoderma* sp.1	C13	0	0	0
28.	*Trichoderma* sp.2	C36	0	0	100
29.	*Trichoderma* sp.3	C37	0	0	50
30.	*Trichoderma* sp.4	C48	0	0	0
31.	*Trichoderma* sp.5	C52	0	0	0
32.	*Trichoderma* sp.6	C53	0	0	100
33.	*Trichoderma* sp.7	C54	0	0	50
34.	*Trichoderma* sp.8	E37	0	0	70
35.	*Trichoderma* sp.9	E39	0	0	10
36.	*Trichoderma* sp.10	E42	0	0	100
37.	*Trichoderma* sp.11	W6	0	10	70
38.	Undetermined sp. 1	C7	0	0	20
39.	Undetermined sp. 2	C17	0	0	20
40.	Undetermined sp. 3	C27	0	0	30

Table 4. 18. Qualitative study of gelatinase, esterase and chitin utilization activity of pure cultures of fungi isolated during the study.

Name of the sp.	Code	Substrate	Substrate's Host plant/ habitat	Locality	Activity (%)	Gelatinase	Esterase	Chitinase
Acremonium charticola	E187	LnL	*Hopea ponga*	Ambe	0	-	-	-
Acremonium charticola	E189	Spiders	*Holarrhena antidysenterica*	Ambe	0	+1	+	-
Acremonium charticola	E192	LnL	*Hopea ponga*	Ambe	0	+5	+++	+
Acremonium sp.1	E29	Spider cadavers	*Ixora brachiata*	Molem	100	+1	+++	-
Acremonium sp.2	E51	*Drosophila* sp.	*I. coccinea*	GU Campus	35	+5	+++	+
Acremonium sp.3	E53	HnL	Un. dicot	Tambdi Surla	0	-	-	-
Acremonium sp.3	E54	HnL	Un. dicot	Tambdi Surla	0	-	-	-
Acremonium sp.3	E57	HnL	Un. dicot	Tambdi Surla	0	+5	+++	-
Acremonium sp.3	E58	HnL	Un. dicot	Tambdi Surla	0	-	-	-
Acremonium sp.4	E91	Ants	Log of wood	Taleigao	30	-	-	-
Acremonium sp.	E92	LnL	Un. dicot herb.	Taleigao	0	+5	-	-
Acremonium sp.5	E115	HnL	*Holigarna arnottiana*	Bondla	0	-	-	-
Acremonium sp.6	E188	LnL	*Hopea ponga*	Ambe	15	+5	+++	-
Acremonium sp.6	E216	Un. insects	*Careya arborea*	Sonolim	0	+1	+	+
Aschersonia aleyrodis	E71	Spiders	*Strobilanthus ixiocephalus*	Molem	0	-	-	-
Aschersonia badia	E196	HnL	*Dillenia indica*	Molem	0	+1	-	-
Aschersonia sp.	E125	HnL	*Syzygium zeylanicum*	Tambdi Surla	10	-	-	-
Aschersonia sp.	E126	HnL	*Holarrhena antidysenterica*	Tambdi Surla	0	-	-	-
Aschersonia sp.	E127	HnL	*Hopea ponga*	Tambdi Surla	0	+2	+++	-
Aschersonia sp.	E176	Coccids	*Leea macrophylla*	Molem	0	-	-	-
Aschersonia sp.	E212	Un. insects	*Hopea ponga*	Colem	30	-	+	-
Aspergillus clavatus	C33	*Anopheles* sp. 4L	Container	Vasco	5	+1	-	-
A. fumigatus	C1	*Anopheles* sp. 4L	Curing water	Panjim	0	-	-	-
A. fumigatus	C8	*Anopheles* sp. 2L	Curing water	Panjim	0	-	-	-
A. fumigatus	C15	*Culex* pupae	Curing water	Curchorem	60	+1	+++	+
A. fumigatus	C18	*Anopheles* sp. 2L	Water collection	Porvorim	0	-	-	-
A. japonicus var. *aculeatus*	C3	*Culex* sp. 2L	Asbestos tank	Panjim	0	+5	-	-
A. niger var. awmori	C46	*Culex* sp. 3L	Curing water	Cuncolim	0	-	-	-

A. niger var. *awmori*	E174	Coccids	*Holarrhena antidysenterica*	Molem	10	+1	-	-
A. niger var. *awmori*	E195	Spiders	*Dillenia indica*	Ambe	35	+1	-	-
A. oryzae var. *oryzae*	E135	Lepidoptera n eggs	*Holarrhena antidysenterica*	Tambdi Surla	0	-	-	-
A. restrictus	C9	*Culex* sp. 2L	Curing water	Curchorem	0	-	-	-
A. restrictus	E64	*Drosophila* sp.	*Holarrhena antidysenterica*	GU campus	15	-	+++	-
Aspergillus sp.1	C45	*Culex* sp. 2L	Curing water	Cuncolim	0	-	-	+
Aspergillus sp.2	E21	Aphids	*Chromolaena odorata*	Curchorem	50	+2	+++	-
Aspergillus sp.3	W5	Larval baits	Pond water	Bondla	100	+5	+	-
Aspergillus sp.4	C11	*Anopheles* sp. 2L	Curing water	Porvorim	0	-	-	-
Aspergillus sp.5	C14	*Anopheles* sp. 2L	Water collection	Porvorim	0	-	-	-
Aspergillus sp.5	E50	Ants	Log of wood	GU campus	34.8	-	+++	-
Aspergillus sp.5	E65	Scale insects	*Microcos paniculata*	GU campus	20	-	++	-
Aspergillus sp.6	E22	Aphids	*Chromolaena odorata*	Curchorem	14	-	-	-
Aspergillus sp.7	E86	Scale insects	*Psychotria dalzellii*	Cotigao	0	+2	+++	-
Aspergillus sp.7	E97	Scale insects	Un. grass	Taleigao	30	+5	+++	-
Aspergillus sp.	E104	Scale insects	Un. grass	Taleigao	40	-	+++	-
Aspergillus sp.	E114	HnL	*Gnetum ula*	Bondla	0	+1	+++	-
Aspergillus sp.	E129	Aphids	*Holarrhena antidysenterica*	Tambdi Surla	0	-	-	-
Aspergillus sp.	E149	LnL	*Terminalia bellirica*	Kollur	0	+1	-	-
Beltrania sp.	E81	Scale insects	*Microcos paniculata*	Molem	20	-	+++	+
Chaetomella sp.	E35	Spider cadavers	*Dillenia indica*	Molem	94.4	-	-	-
Cladosporium sp.1	E143	HnL	*Hopea ponga*	Anmod	30	+5	+	-
Cladosporium sp.2	E210	Un. insects	*H. ponga*	Dhudhsagar	0	-	+++	-
Cladosporium sp.3	E1	Coccids	*Chromolaena odorata*	Kasaragod	0	-	-	-
Cladosporium sp.4	E46	Aphids	*Cassia tora*	Alorna	0	+1	-	-
Cladosporium sp.4	E121	Lepidoptera n eggs	Un. herb	Codal	0	-	-	-
Cladosporium sp.5	C19	*Anopheles* sp. 2L	Water collection	Porvorim	0	-	-	-
Cladosporium sp.5	E144	Scale insects	Un. dicot creeper	Anmod	0	+1	+++	-
Cladosporium sp.	E7	DnA	*Phyllanthus* sp	Kasaragod	0	+5	-	-
Cladosporium sp.	E177	Coccids	*Leea macrophylla*	Molem	0	+1	+++	-
Conidiobolus obscurus	E172	DnA	*Hopea ponga*	Molem	15	+5	+++	-
Curvularia sp.1	C25	*Culex* sp. pupae	Drain	Vasco	0	-	-	-

Curvularia sp.2	E79	HnL	*Terminalia paniculata*	Molem	0	-	-	-
Curvularia sp.2	E141	HnL	Un. dicot plant	Chandreshwar	40	-	-	-
Cylindrocladium sp.	C21	*Anopheles* sp. 2L	Water collection	Porvorim	0	-	-	-
Cylindrocladium sp.	E87	Water insects	Foam in stream	Chorlem	0	-	-	-
Cylindrocladium sp.	W1	Larval baits	Puddle	Cotigao	0	-	-	-
Fusarium sp.1	C16	*Culex* sp. 2L	Tank	Pernem	0	-	-	-
Fusarium sp.1	C28	*Culex* sp. 4L	Well	Bicholim	20	-	-	-
Fusarium sp.1	C30	*Culex* sp. pupae	Well	Bicholim	0	-	+++	-
Fusarium sp.2	E27	Mealy bugs	*Solanum melanogaster*	Karmali	28	+5	-	-
Fusarium sp.2	E32	Spider cadavers	*Hopea wightiana*	Molem	0	+5	-	-
Fusarium sp.2	E70	DnA	*H. ponga*	Molem	0	-	+++	-
Fusarium sp.3	E43	Aphids	*Chromolaena odorata*	Bondla	0	-	-	-
Fusarium sp.4	E60	HnL	Un. dicot	Tambdi Surla	0	+5	+++	-
Fusarium sp.4	E61	HnL	Un. dicot	Tambdi Surla	0	-	+++	-
Fusarium sp.4	E110	Ants	*Anacardium occidentale*	Cudnem	25	+1	+++	-
Fusarium sp.4	E111	Aphids	Un. herb	Cudnem	25	-	+++	-
Fusarium sp.5	E90	Scale insects	Un. dicot	Molem	0	+1	-	-
Fusarium sp.5	E100	LnL	*Solanum melanogaster*	Taleigao	10	-	+++	-
Fusarium sp.	C12	*Anopheles* sp. 4L	Curing water	Porvorim	10	-	-	-
Fusarium sp.	E3	HnL	*Ixora coccinea*	Kasaragod	100	-	-	-
Fusarium sp.	E72	Scale insects	*Dillenia indica*	Molem	40	-	+++	-
Fusarium sp.	E88	Water insects	Foam in stream	Chorlem	30	-	+++	-
Gliocladiopsis sp.	E66	Scale insects	*Calotropis gigantea*	GU campus	0	+5	+++	-
Gliocladiopsis sp.	E108	Aphids	*Crotalaria* sp.	Cudnem	35	+5	-	-
Gliocladium sp.1	E6	HnL	*Chromolaena odorata*	Kasaragod	100	+5	+++	+
Gliocladium sp.1	E13	Aphids	*C. odorata*	Pernem	100	+1	+++	-
Gliocladium sp.1	E15	Aphids	*Cassia tora*	Pernem	100	+5	+++	-
Gliocladium sp.1	E16	Aphids	*Chromolaena odorata*	Pernem	100	+5	+++	-
Gliocladium sp.1	E19	Aphids	*Calotropis gigantea*	Pernem	100	+5	+++	-
Gliocladium sp.1	E23	Aphids	*Chromolaena odorata*	Curchorem	95	+5	+++	-
Gliocladium sp.2	E20	Aphids	*C. odorata*	Curchorem	100	+5	+++	-
Gliocladium sp.3	E49	Ants	Log of wood	GU campus	35	+5	+++	-
Gliocladium sp.	E25	Mealy bugs	*Solanum melanogaster*	Karmali	0	+5	-	-
Gliocladium sp.	E48	*Drosophila* sp.	*Strobilanthus ixiocephalus*	GU campus	100	+5	+++	+

Gliocladium sp.	E124	HnL	*Crotalaria* sp.	Codal	30	+5	+++	+
Gliocladium sp.	E200	Spiders	*Anacardium occidentale*	GU campus	0	+2	+++	-
Gliocladium sp.	E215	Un. insects	*Hopea ponga*	Colem	0	+5	+++	-
Gliocladium sp.	E217	Un. insects	*H. ponga*	Molem	0	+1	+++	-
Hirsutella sp.1	E175	Coccids	*Holarrhena antidysenterica*	Molem	0	+3	++	-
Hirsutella sp. 1	E203	Un. insects	*Cinnamomum zeylanicum*	Molem	0	-	++	-
Hirsutella sp. 1	E206	Un. insects	*C. zeylanicum*	Dhudhsagar	0	+5	+++	-
Hirsutella sp. 2	E128	HnL	*Tridax procumbens*	Tambdi Surla	0	-	-	-
Hirsutella sp.2	E180	DnA	*Morinda citrifolia*	Molem	0	-	++	-
Mucor sp.	C32	*Culex* sp. pupae	Curing water	Cuncolim	0	-	-	-
Mucor sp.	E28	Spider cadavers	*Hopea wightiana*	Molem	38.8	-	-	-
Mucor sp.	E30	Spider cadavers	*H. wightiana*	Molem	0	-	-	-
Mucor sp.	E36	Spider cadavers	*Holarrhena antidysenterica*	Molem	15	+3	-	-
Mucor sp.	E41	Aphids	*Chromolaena odorata*	Bondla	0	+1	-	-
Mucor sp.	E109	Ants	*Anacardium occidentale*	Cudnem	15	+1	+++	-
Paecilomyces javanicus	E145	LnL	Un. dicot	Anmod	0	-	+++	-
Paecilomyces sp.1	E186	Un. insects	Unidientified grass	Ambe	0	+5	+++	-
Paecilomyces sp.2	E12	Aphids	*Chromolaena odorata*	Pernem	0	-	-	-
Paecilomyces sp.2	E116	Spiders	*Anacardium occidentale*	Alorna	0	+1	-	-
Paecilomyces sp.3	C40	*Anopheles* sp. 2L	Container	Vasco	20	-	-	-
Paecilomyces sp.3	E31	Dead mites	*Chromolaena odorata*	Molem	0	+3	-	-
Paecilomyces sp.3	E112	HnL	*Myristica malabarica*	Bondla	30	+5	+++	+
Paecilomyces sp.3	E142	Coleopteran adults	*Strobilanthus ixiocephalus*	Molem	40	-	+	-
Paecilomyces sp.3	E147	Spiders	*Dillenia indica*	Anmod	0	-	+++	-
Paecilomyces sp.3	E190	Insect eggs	*Hopea ponga*	Ambe	0	+1	+++	-
Paecilomyces sp.3	E193	Insect eggs	*Holarrhena antidysenterica*	Ambe	25	-	+++	-
Paecilomyces sp.3	E194	Insect eggs	*Hopea Ponga*	Ambe	15	-	+++	-
Paecilomyces sp.3	E209	Un. insects	*Dillenia indica*	Dhudhsagar	0	+1	++	-
Paecilomyces sp.3	E219	Un. insects	*Holarrhena antidysenterica*	Molem	0	-	+++	-
Paecilomyces sp.3	E221	Un. insects	*Careya arborea*	Molem	0	+5	+++	-
Paecilomyces sp.4	E59	HnL	Un. dicot	Tambdi Surla	0	+1	+++	-
Paecilomyces sp.4	E93	LnL	Un. dicot	Taleigao	0	+1	-	-

Paecilomyces sp.4	E94	*Drosophila* sp.	Un. dicot	Taleigao	0	+5	++	-
Paecilomyces sp.5	E134	Aphids	*Chromolaena odorata*	Tambdi Surla	0	-	-	-
Paecilomyces sp.6	E191	HnL	*C. odorata*	Ambe	15	+1	+++	+
Paecilomyces sp.7	E181	DnA	*Holarrhena antidysenterica*	Molem	15	-	++	-
Paecilomyces sp.	E74	Scale insects	Un. dicot	Molem	0	+1	++	-
Paecilomyces sp.	E152	DnA	*Mangifera indica*	Kodachadri	0	+1	-	-
Paecilomyces sp.	E173	Un. insects	*Leea macrophylla*	Molem	0	+1	+++	-
Paecilomyces sp.	E211	Un. insects	*Hopea ponga*	Dhudhsagar	0	+1	++	-
Penicillium sp.1	C2	*Anopheles* sp. 2L	Curing water	Panjim	100	+5	+++	+
Penicillium sp.1	C29	*Culex* sp. 2L	Well	Bicholim	100	+5	-	-
Penicillium sp.1	C44	*Anopheles* sp. pupae	Container	Vasco	100	+5	+++	-
Penicillium sp.2	C4	*Anopheles* sp. 3L	Water collection	Porvorim	94.6	+2	-	-
Penicillium sp.3	C20	*Culex* sp. 3L	Drain	Vasco	100	+3	-	-
Penicillium sp.3	C42	Mosquito larval exuviae	Accumulated water on terrace	Panjim	100	+5	+	-
Penicillium sp.3	E9	HnL	*Ixora coccinea*	Kasaragod	100	+5	+++	-
Penicillium sp.3	E26	Scale insects	Anthurium plants	GU campus	100	+5	+++	-
Penicillium sp.4	C5	*Anopheles* sp. 3L	Curing water	Panjim	85	-	+++	-
Penicillium sp.4	C26	*Culex* sp. 2L	Drain	Vasco	60	+1	-	-
Penicillium sp.4	C51	*Anopheles* sp. 3L	Paddy field pond	Taleigao	100	+5	+++	+
Penicillium sp.4	E2	HnL	*Chromolaena odorata*	Kasaragod	100	+5	+++	+
Penicillium sp.4	E11	HnL	*C. odorata*	Pernem	100	+5	+++	+
Penicillium sp.	C24	*Aedes* sp. 4L	Curing water	Bicholim	0	-	-	-
Penicillium sp.	C55	*Culex* sp. 3L	Drain	Vasco	10	+1	-	-
Penicillium sp.	E8	DnA	*Phyllanthus* sp.	Kasaragod	0	-	-	-
Penicillium sp.	E89	Water insects	Foam in stream	Chorlem	0	+1	+++	-
Penicillium sp.	E113	HnL	*Cinnamomum zeylanicum*	Bondla	30	+1	+++	-
Penicillium sp.	E146	Scale insects	Un. dicot	Anmod	0	+5	+++	+
Penicillium sp.	E153	DnA	*Mangifera indica*	Kodachadri	0	+5	+++	-
Penicillium sp.	E204	Un. insects	*Gnetum ula*	Dhudhsagar	0	+5	++	-
Penicillium sp.	E208	Un. insects	*Myristica malabarica*	Dhudhsagar	0	+1	+++	-
Pestilotiopsis sp.	E105	Scale insects	Un. grass	Taleigao	15	-	-	-
Pleurothecium sp.	E55	HnL	Un. dicot	Tambdi Surla	0	-	-	-
Syncephalastrum sp.	C41	Mosquito larval exuviae	Accumulated water on terrace	Panjim	0	-	-	-

Trichoderma sp.1	C13	*Anopheles* sp. 3L	Curing water	Porvorim	100	+5	+++	+
Trichoderma sp.2	C36	*Culex* sp. 3L	Curing water	Cuncolim	70	+5	+++	+
Trichoderma sp.3	C37	*Anopheles* sp. 3L	Curing water	Cuncolim	65	+5	+++	+
Trichoderma sp.3	C48	*Culex* sp. 3L	Container	Vasco	60	+5	+	+
Trichoderma sp.4	C52	*Culex* sp. 3L	Tank	Pernem	95	+5	++	+
Trichoderma sp.4	C53	*Anopheles* sp. 3L	Bottles	Pernem	80	+5	+	+
Trichoderma sp.4	C54	*Culex* sp. 3L	Curing water	Cuncolim	100	+5	++	-
Trichoderma sp.4	E37	Spider cadavers	*Dillenia indica*	Molem	100	+5	++	+
Trichoderma sp.4	E39	Dead mites	*Terminalia paniculata*	Molem	92	+5	-	-
Trichoderma sp.4	E42	Aphids	*Chromolaena odorata*	Bondla	90	+2	+	-
Trichoderma sp.4	W6	Larval baits	Pond water	Bondla	60	+5	+	-
Trichoderma sp.5	E106	Scale insects	Un. grass	Taleigao	15	-	-	-
Trichoderma sp.	C6	*Anopheles* sp. 2L	Curing water	Porvorim	40	-	-	-
Trichoderma sp.	C39	*Anopheles* sp. 2L	Barrel	Vasco	30	-	-	+
Trichoderma sp.	E5	HnL	*Ixora coccinea.*	Kasaragod	0	-	-	-
Trichoderma sp.	E14	Aphids	*Chromolaena odorata*	Pernem	0	+5	-	-
Trichoderma sp.	E38	Dead mites	*Holarrhena antidysenterica*	Molem	41.67	+5	-	-
Trichoderma sp.	E45	Scale insects	*Strobilanthus ixiocephalus*	Alorna	0	+5	-	-
Trichoderma sp.	E69	DnA	*Hopea ponga*	Molem	0	+5	+++	+
Trichoderma sp.	E101	LnL	*Hibiscus esculentus*	Taleigao	0	+5	+++	-
Trichoderma sp.	E103	Scale insects	Un. grass	Taleigao	25	-	-	-
Trichoderma sp.	E107	Scale insects	Un. grass	Taleigao	0	+5	+++	-
Trichoderma sp.	E140	HnL	Un. dicot	Chandreshwar	0	-	+	-
Trichoderma sp.	E183	Coleopteran head	*Cinnamomum zeylanicum*	Endrem	0	+5	+	-
Trichoderma sp.	E184	LnL	*Hopea ponga*	Endrem	0	+1	+	-
Trichoderma sp.	W2	Larval baits	Puddle	Cotigao	0	-	-	-
Trichoderma sp.	W3	Larval baits	Paddy field	Cotigao	0	-	-	-
Trichoderma sp.	W4	Larval baits	Stagnant water	Cotigao	10	-	-	-
Trichoderma sp.	W7	Larval baits	Pond	Bondla	0	+5	+	-
Undetermined sp. (phialidic)	C10	*Anopheles* sp. 2L	Pond	Taleigao	0	-	-	-
Undetermined sp. (phialidic)	C43	Mosquito larval exuviae	Accumulated water on terrace	Panjim	0	-	-	-
Undetermined sp. (phialidic)	C56	*Culex* sp. 3L	Tank	Pernem	0	+1	-	-
Undetermined sp. (phialidic)	C57	*Culex* sp. 3L	Tank	Pernem	0	+1	-	-
Undetermined sp. (phialidic)	E10	Spiders	*Careya arborea*	Molem	0	+5	-	-

Undetermined sp. (phialidic)	E62	HnL	Un. dicot	Tambdi Surla	0	-	-	-
Undetermined sp. (phialidic)	E63	HnL	Un. Dicot	Tambdi Surla	0	+5	-	-
Undetermined sp. (phialidic)	E68	DnA	*Hopea ponga*	Molem	0	-	-	-
Undetermined sp. (phialidic)	E77	Scale insects	*Terminalia paniculata*	Molem	0	+4	+++	-
Undetermined sp. (phialidic)	E82	Scale insects	*Hopea ponga*	Cotigao	0	-	+++	-
Undetermined sp. (phialidic)	E83	Scale insects	*Holarrhena antidysenterica*	Cotigao	0	+2	-	-
Undetermined sp. (phialidic)	E85	Mites	*Psychotria dalzellii*	Cotigao	0	-	+++	-
Undetermined sp. (phialidic)	E99	Scale insects	Un. grass	Taleigao	30	-	-	-
Undetermined sp. (phialidic)	E119	DnA	*Careya arborea*	Valpoi	10	+5	-	-
Undetermined sp. (phialidic)	E168	Un. insects	Un. dicot	Kollur	0	+5	+++	-
Undetermined sp. (phialidic)	E205	Un. insects	*Gnetum ula*	Dhudhsagar	0	-	++	-
Undetermined sp. (phialidic)	E213	Un. insects	*Hopea ponga*	Colem	0	-	-	-
Undetermined sp. (phialidic)	E222	Un. insects	*H. ponga*	Molem	0	+2	-	+
Undetermined sp. (Pycnidial)	C31	*Anopheles* sp. 2L	Container	Vasco	100	-	-	-
Undetermined sp. (Pycnidial)	C34	*Anopheles* sp. 3L	Container	Vasco	5	-	-	-
Undetermined sp. (Pycnidial)	C35	*Anopheles* sp. pupae	Container	Vasco	0	-	-	-
Undetermined sp. (Pycnidial)	C50	*Culex* sp. 3L	Curing water	Cuncolim	0	-	-	-
Undetermined sp. (pycnidial)	E24	Aphids	*Ageratum* sp.	GU campus	0	-	-	-
Undetermined sp. (Pycnidial)	E40	Aphids	*Holarrhena antidysenterica*	Bondla	15	-	-	-
Undetermined sp. (Pycnidial)	E202	Coleopteran adults	*Gnetum ula*	Subrahmanya	40	+1	++	-
Undetermined sp. (Thallic)	C38	*Culex* sp. pupae	Curing water	Cuncolim	0	-	-	-
Undetermined sp. (Thallic)	E56	HnL	Un. dicot	Tambdi Surla	0	+5	+	-
Undetermined sp. (Thallic)	E182	Insect eggs	Un. dicot	Endrem	0	+5	-	-
Non-sporulating	C7	*Anopheles* sp. 2L	Curing water	Porvorim	100	+5	-	+
Non-sporulating	C17	*Anopheles* sp. 2L	Water collection	Porvorim	100	+5	-	+
Non-sporulating	C22	*Culex* sp. pupae	Sump tank	Bicholim	20	-	-	-
Non-sporulating	C23	*Culex* sp. 3L	Cement tank	Bicholim	0	-	-	+
Non-sporulating	C27	*Culex* sp. 2nd instar	Well	Pernem	100	+5	-	+

Non-sporulating	C47	*Culex* sp. 2L	Curing water	Curchorem	0	-	-	-
Non-sporulating	C49	*Culex* sp. 3L	Curing water	Curchorem	0	-	-	-
Non-sporulating	E4	HnL	*Ixora coccinea*	Kasaragod	0	-	-	-
Non-sporulating	E17	Aphids	*Calotropis gigantea*	Pernem	0	-	-	-
Non-sporulating	E18	Aphids	*C. gigantea*	Pernem	0	+5	-	-
Non-sporulating	E33	Dead mites	*Chromolaena odorata*	Molem	0	-	-	-
Non-sporulating	E34	Spider cadavers	*Psychotria dalzellii*	Molem	0	+1	-	-
Non-sporulating	E44	Aphids	*Chromolaena odorata*	Bondla	0	-	-	-
Non-sporulating	E47	Aphids	*Cassia tora*	Alorna	0	-	-	-
Non-sporulating	E52	*Drosophila*	*Ixora brachiata*	GU campus	40	-	-	-
Non-sporulating	E67	*Drosophila*	*Microcos paniculata*	GU campus	0	+5	-	-
Non-sporulating	E73	Scale insects	Un. dicot	Molem	0	-	-	-
Non-sporulating	E75	Scale insects	Un. dicot	Molem	0	+5	-	-
Non-sporulating	E76	Scale insects	*Microcos paniculata*	Molem	0	-	+++	-
Non-sporulating	E78	Scale insects	*Terminalia paniculata*	Molem	0	-	-	-
Non-sporulating	E80	Scale insects	*Holarrhena antidysenterica*	Molem	45	+5	+++	-
Non-sporulating	E84	Scale insects	*Psychotria dalzellii*	Cotigao	0	-	+++	-
Non-sporulating	E95	Scale insects	Un. grass	Taleigao	20	+5	+++	-
Non-sporulating	E96	Scale insects	Un. grass	Taleigao	55	+5	++	-
Non-sporulating	E98	Scale insects	Un. grass	Taleigao	10	-	-	-
Non-sporulating	E102	LnL	*Solanum melanogaster*	Taleigao	10	-	-	-
Non-sporulating	E117	Spiders	*Dillenia indica*	Valpoi	25	-	-	-
Non-sporulating	E118	Spiders	*D. indica*	Valpoi	10	+5	+	-
Non-sporulating	E120	Aphids	*Holarrhena antidysenterica*	Bondla	0	+5	-	-
Non-sporulating	E122	Lepidopteran eggs	Un. dicot	Codal	10	-	-	-
Non-sporulating	E123	HnL	Un. grass	Codal	25	+5	+	-
Non-sporulating	E130	Aphids	*Calotropis gigantea*	Tambdi Surla	0	+2	-	-
Non-sporulating	E131	HnL	*Holarrhena antidysenterica*	Tambdi Surla	0	-	-	-
Non-sporulating	E132	Scale insects	Un. dicot	Tambdi Surla	0	+5	-	-
Non-sporulating	E133	Scale insects	*Hopea ponga*	Tambdi Surla	0	+5	-	-
Non-sporulating	E136	Lepidopteran eggs	*Flacourtia montana*	Tambdi Surla	0	-	-	-
Non-sporulating	E137	Lepidopteran eggs	*F. montana*	Tambdi Surla	0	-	-	-
Non-sporulating	E138	Lepidopteran eggs	*F. montana*	Tambdi Surla	10	+1	+	-
Non-sporulating	E139	Lepidopteran eggs	*F. montana*	Melka	0	-	-	-

Non-sporulating	E148	HnL	*Terminalia bellirica*	Kollur	0	-	+++	-
Non-sporulating	E150	HnL	*Holarrhena antidysenterica*	Kodachadri	0	+5	+	-
Non-sporulating	E151	LnL	*Mangifera indica*	Kollur	0	-	-	-
Non-sporulating	E154	HnL	*Syzygium zeylanicum*	Kodachadri	0	+5	-	-
Non-sporulating	E155	LnL	*Cassia tora*	Kodachadri	0	+5	-	-
Non-sporulating	E156	HnL	*Psychotria dalzellii*	Kodachadri	0	+5	+++	-
Non-sporulating	E157	HnL	*P. dalzellii*	Kodachadri	0	-	-	-
Non-sporulating	E158	DnA	*Ixora coccinea*	Kollur	0	-	-	-
Non-sporulating	E159	DnA	*I. coccinea*	Kollur	0	-	+++	-
Non-sporulating	E160	DnA	*Syzygium zeylanicum*	Kollur	10	+5	+	-
Non-sporulating	E161	DnA	*Holigarna arnottiana*	Kollur	15	+5	-	-
Non-sporulating	E162	DnA	*H. arnottiana*	Kollur	0	+1	+++	-
Non-sporulating	E163	DnA	*Terminalia Billerica*	Kollur	0	+5	+	-
Non-sporulating	E164	DnA	*Ixora brachiata*	Kollur	10	+5	+++	-
Non-sporulating	E165	HnL	*I. brachiata*	Kollur	19.8	+5	+++	-
Non-sporulating	E166	HnL	*I. brachiata*	Kollur	10	+1	+++	-
Non-sporulating	E167	Dipteran larvae	*Holigarna arnottiana*	Kollur	25	+5	-	-
Non-sporulating	E169	Un. insects	Un. dicot	Kollur	10	-	+	-
Non-sporulating	E170	Un. insects	Un. dicot	Kollur	5	+5	++	-
Non-sporulating	E171	Un. insects	Un. dicot	Kollur	15	+1	+	-
Non-sporulating	E178	HnL	*Leea macrophylla*	Molem	0	-	-	-
Non-sporulating	E179	LnL	*Morinda citrifolia*	Molem	0	+5	+++	-
Non-sporulating	E185	LnL	*Hopea ponga*	Endrem	0	-	-	-
Non-sporulating	E197	Un. insect pupae	*Cinnamomum zeylanicum*	Endrem	0	+4	+	-
Non-sporulating	E198	Un. insect pupae	*Saraca asoca*	Endrem	0	-	++	-
Non-sporulating	E199	Lepidopteran adult	*Flacourtia montana*	Edar	0	+5	++	-
Non-sporulating	E201	Spiders	*Psychotria dalzellii*	Edar	0	-	-	-
Non-sporulating	E207	Un. insects	*Rauvolfia serpentina*	Dhudhsagar	0	-	-	-
Non-sporulating	E214	Un. insects	*Hopea ponga*	Colem	0	+1	+	-
Non-sporulating	E218	Un. insects	*Careya arborea*	Molem	0	+5	++	-
Non-sporulating	E220	Un. insects	*Careya arborea*	Molem	0	-	-	-

Note: 2L = 2nd instar larvae; 3L = 3rd instar larvae; 4L = 4th instar larvae; Un. = unidentified; HnL =Homopteran larvae; LnL = Lepidopteran larvae; DnA = Dipteran adults.

Table 4. 19: Field trial of *Penicillium* sp. (E9) against *Anopheles stephensi* larvae.

Replicate	Dose Spores/ml	n	Mortality 24 h	48 h	Mean mortality (%)
1	20000	100	53	61	53.8 ± 8.53% (24h)
2	20000	100	41	54	
3	20000	100	60	62	66 ± 10.56% (48h)
4	20000	100	52	72	
5	20000	100	63	81	
control	0	100	4	4	

Table 4. 20: Occurrence of entomogenous fungi on different host plant species.

Plant species	*A. aleyrodis*	*A. badia*	*A. indica*	*Hirsutella* sp.	*Hypocrella* sp.	*Podonectria* sp.
Calycopteris floribunda	+	+	-	-	-	+
Dillenia indica	+	+	-	-	-	+
Holarrhena antidysenterica	+	+	-	-	-	+
Hopea ponga	+	+	+	+	+	-
Leea macrophylla	+	+	-	-	-	+
Morinda citrifolia	+	+	-	-	-	-
Strobilanthus ixiocephalus	+	-	-	-	-	-

LEGENDS FOR PLATES

PLATE: I

Mosquito breeding habitats: (a) A slow flowing stream along the roadside; (b) An open drain; (c) A sewage channel; (d) Curing water at the construction site; (e) Inner view of an overhead syntex tank kept open, with decaying materials.

PLATE: II

Mosquito breeding habitats: (a) Mineral water bottles on the roof, which accumulate rainwater; (b) A barrel to store water at a construction site; (c) Abandoned tyres, which accumulate rainwater; (d) A masonry tank at the construction site; (e) A sump tank under construction; (f) An abandoned cement tank

PLATE: III

Mosquito breeding habitats: (a) Stagnant water from a overflowing septic tank; (b) A wetland; (c) Collection of water at excavated site prepared for construction; (d) A pond with. natural springs used for sanitation purposes.

PLATE: IV

Sources of insect fungi: (a) A view of Western Ghats forest at Sri Bhagawan Mahaveer Wildlife Sanctuary, Molem; (b) Aphids on *Chromolaena odorata*; (c) A Lepidopteran infected by *Conidiobolus obscurus* on *H. ponga;* (d) An unidentified insect larva infected by *Paecilomyces javanicus;* (e) An ant infected by an unidentified fungus on *Holarrhena antidysenterica.* (f) Aschersonia aleyrodis colonies growing over unidentified insects.

PLATE: V

Simulation float chamber: (a) A simulation float chamber; (b) A simulation float chamber tied with a nylon rope in the mosquito breeding habitat; (c) Simulation float chambers in a small pond; (d) Simulation float chambers in a large wet land.

PLATE: VI

Sampling and processing mosquito larvae: (a) Sampling mosquito larvae/pupae from a curing water at a construction site; (b) Sampling of mosquito larvae/pupae from a well; (c) Contents of a single dip, a third instar larvae of *Aedes* sp. can be seen; (d) Transferring the mosquito larvae/pupae from the dipper into a screw cap container; (e) Incubation of samples from different breeding habitats.

PLATE: VII

Pure cultures: (a) A 7 day old culture of *Fusarium* sp.2. (E70) on CMA; (b) *Penicillium* sp. (E208) on SDA; (c) *Cladosporium* sp.1 (E143) on SDA; (d) Non-sporulating fungus (E67) on CMA; (e) *Gliocladiopsis* sp. (E108) on SDA; (f) *Penicillium* sp. (E9) on SDA; (g) Pure cultures of fungi on malt extract agar slants (21 day old).

PLATE: VIII

***Aschersonia aleyrodis*:** (a-c) Stromata formed on infected insects, (a) 0.35X; (b) Hyphae growing from the anterior region of host, 50X; (c) Matured colonies with many pycnidia (ostioles can be seen), 10X; (d) Single colony with many pycnidia, 20X; (e) Dry and decaying old colonies, 15X; (f) Vertical section passing through a stroma showing a pycnidium, 60X.

PLATE: IX

***Aschersonia badia*:** (a-c) Stromata formed on infected insects, (a) Colonies on leaves of *Schizyzium* sp., 0.35X; (b) Young colonies, 10x; (c) Colonies with pycnidia (orifices of pycnidia can be seen) 15X; (d) Young colony of *Aschersonia badia* and a *Cladosporium* sp. on insects, 20X; (e) Matured colonies with many pycnidia, 15X; (f) Vertical section passing through a stroma showing pycnidia, 60X.

PLATE: X

***Aschersonia brunnea*:** (a-c) Stromata formed on infected insects showing large ostioles, 20X; (d) Vertical section passing through a stroma showing a pycnidium, 60X.

PLATE: XI

***Aschersonia indica* sp. nov.** (a-c): (a) Stromata formed on infected insects on *Hopea ponga*, 15X; (b) Single colony with central pycnidium (ostiole can be seen) and peripheral tubercle-like structures, 20X; (c) Vertical section passing through a stroma showing a pycnidium, 60X. ***Hypocrella* sp.** (d & e): (d) Young ascocarps developing from the periphery of *Aschersonia indica* colony, 20X; (e) Mature colonies with only ascocarps, 30X.

PLATE: XII

Podonecrtia* sp.** (a & b): (a) Young ascocarps developing at the periphery of *Aschersonia badia* stroma, 15X; (b) matured ascocarps, 15X; ***Cladosporium* sp.** (E143) (c & e): On insects on leaves of unidentified plant, (c) 0.25X; (e) 4X; ***Acremonium charticola (E189) (d & f): On an unidentified insect, (d) 5X; (f) 15X.

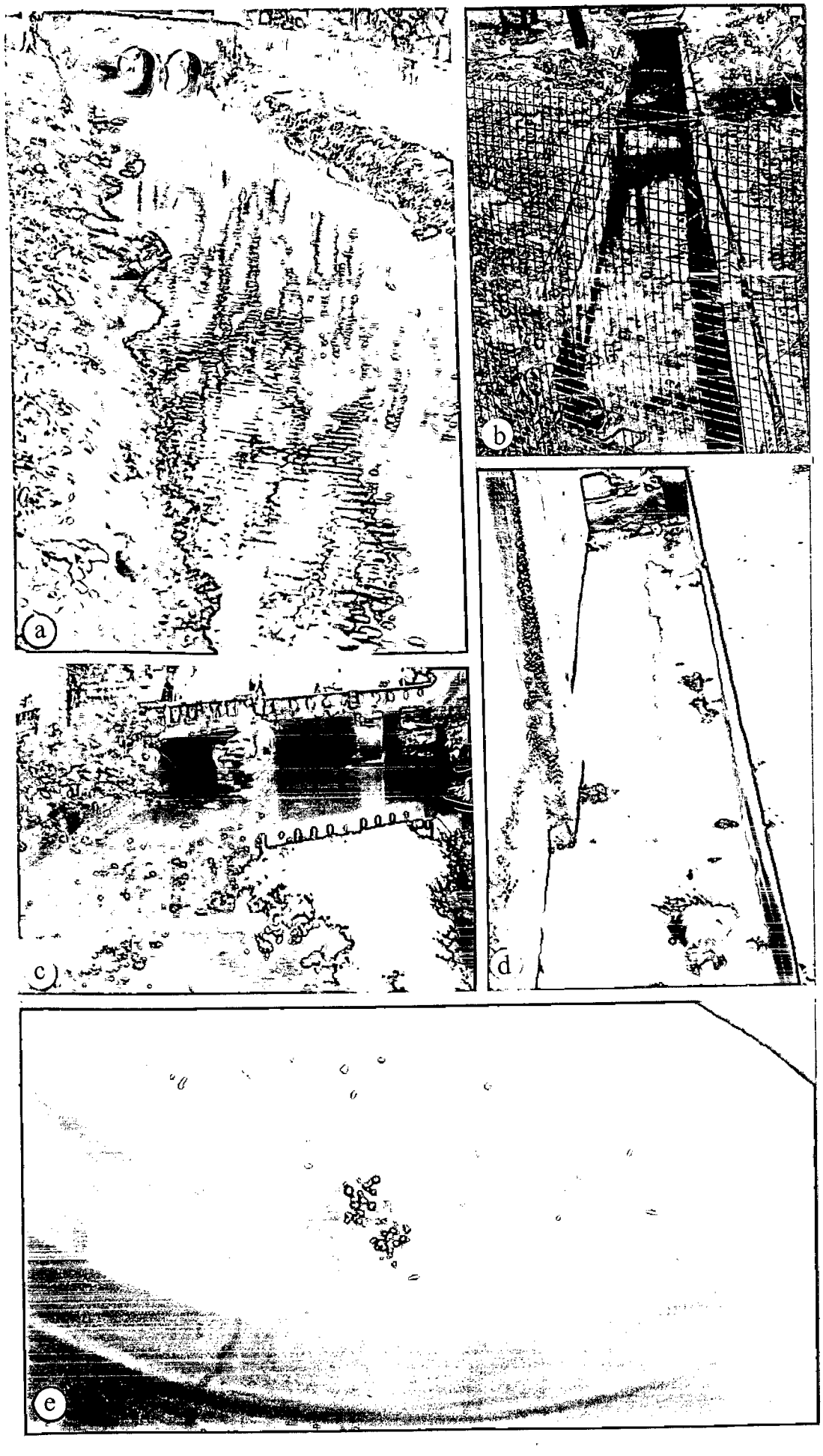
PLATE: XIII

Hirsutella* sp.** (a & b): Colony on insects on *H. ponga* foliage, (a) 10X; (b) 15X; ***Gibellula pulchra (c & d): On a spider on *Dillenia indica* foliage, (c) 5X, (d) 20X.

PLATE: XIV

Bioassay: (a) Cultures of mosquito larvae in the insectory; (b) Introducing healthy larvae in bioassay; (c) A preliminary screening-bioassay set up; (d) Mass production of the fungus by thin layer-liquid medium-still culture method; (e) Concentrated spore suspensions of *Penicillium* sp. (E9) (seen green) and *Gliocladium* sp. (E16) (seen colourless).

PLATE : I



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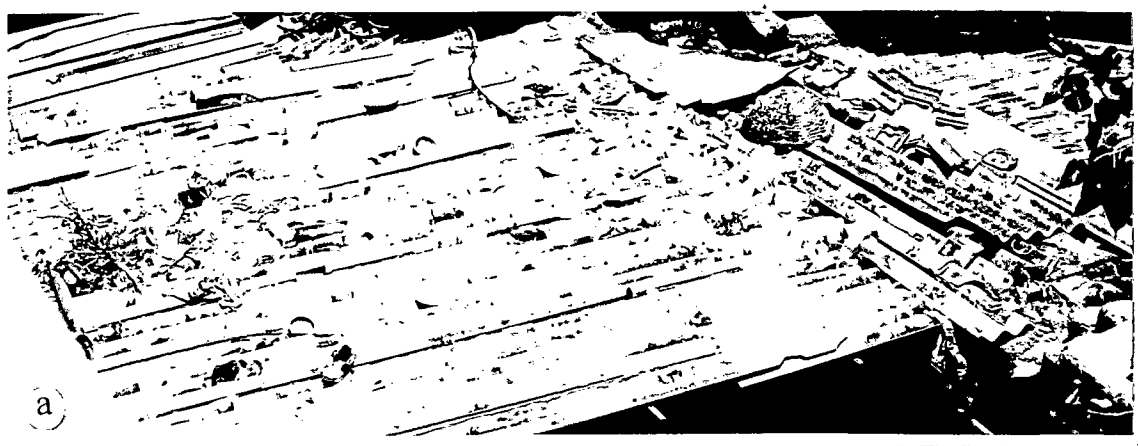
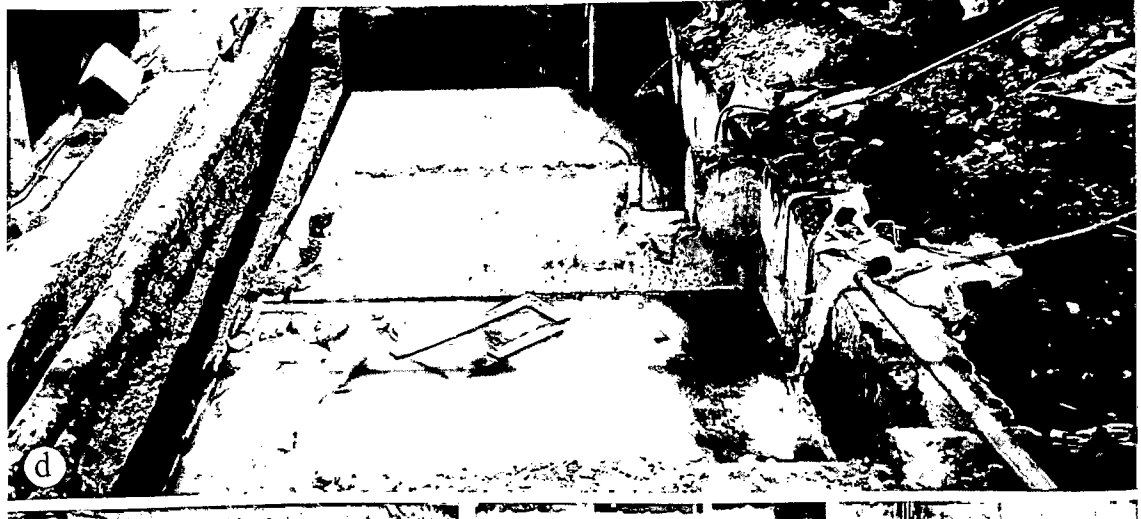

a)


b)


c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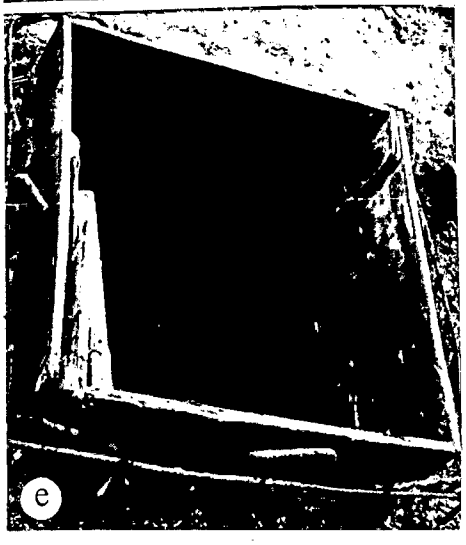
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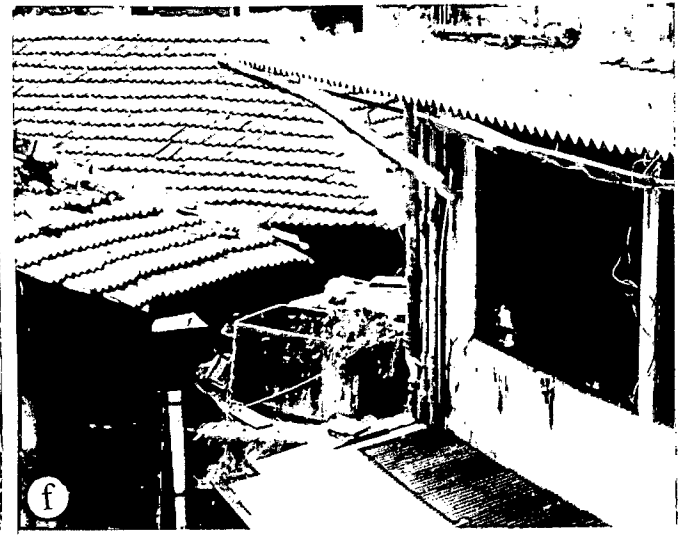
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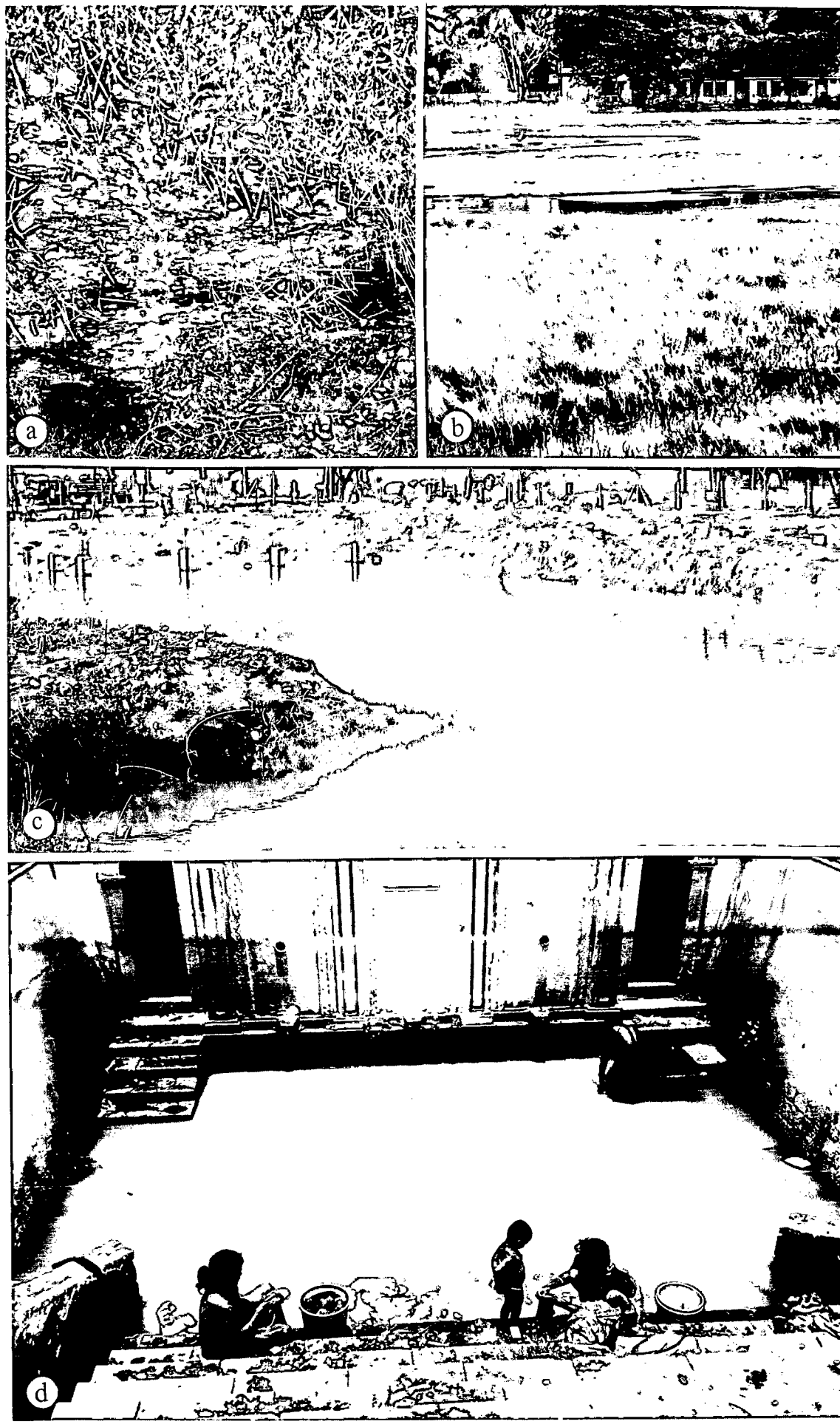

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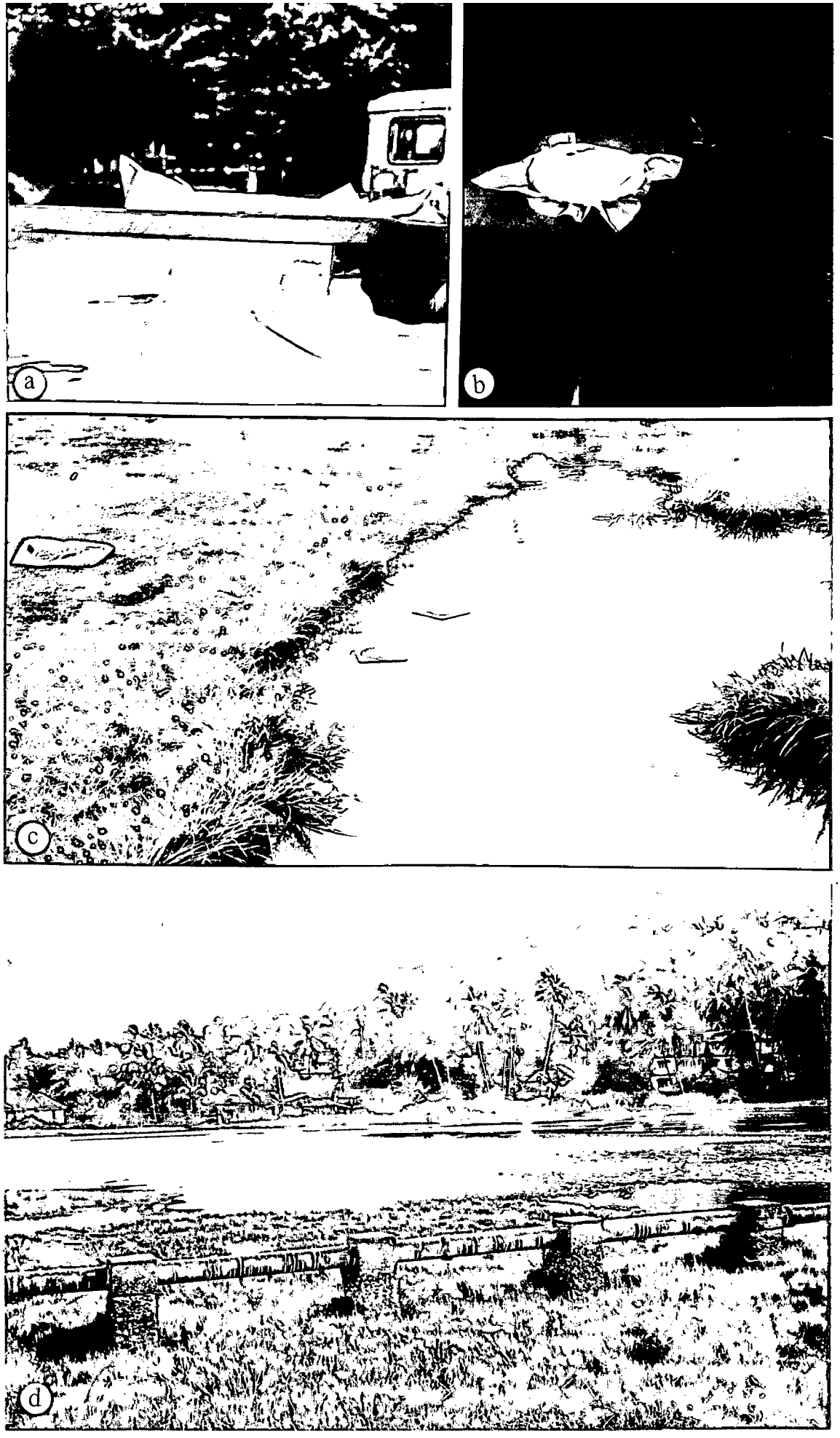
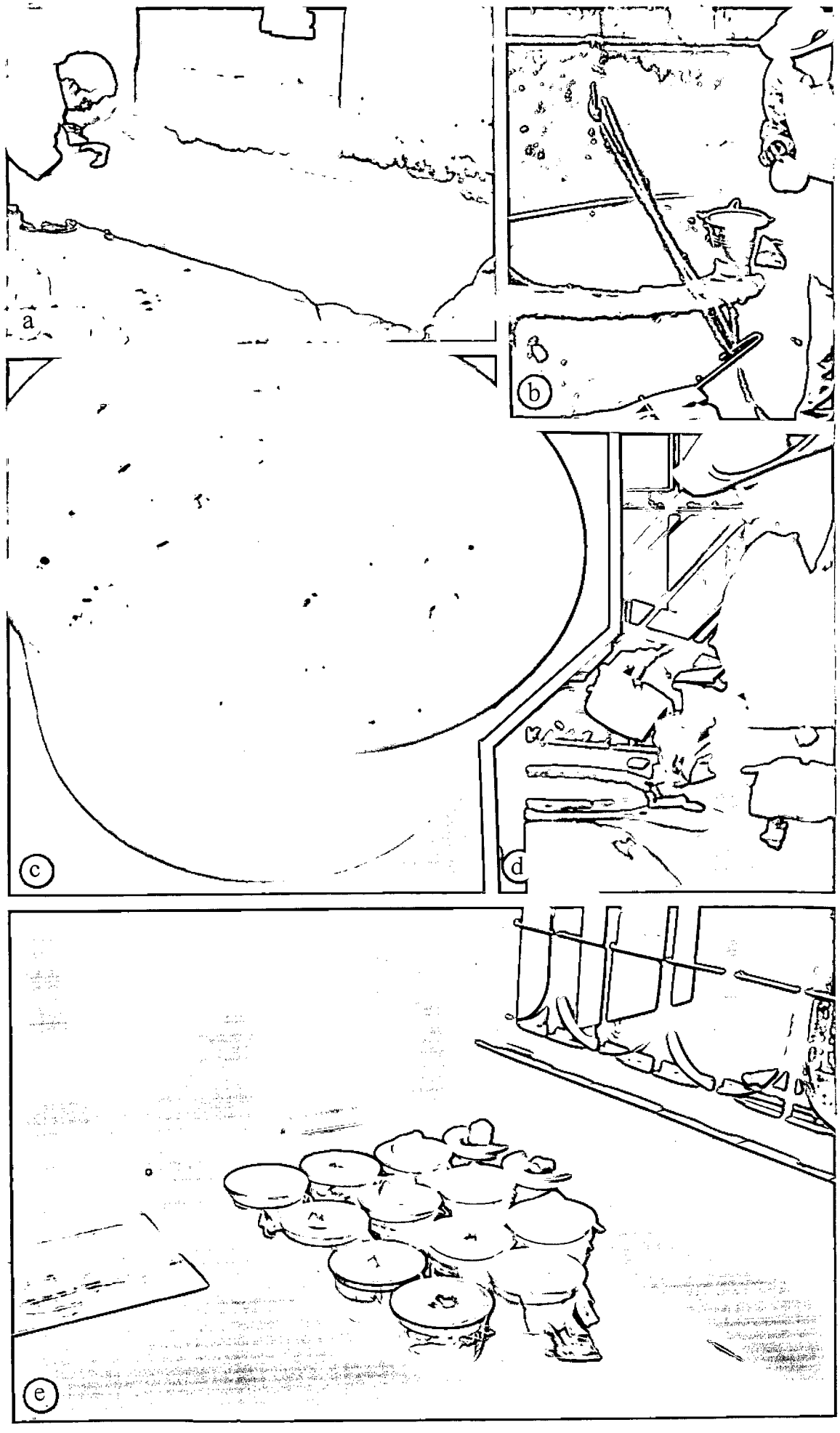

a
b
c
d

PLATE : VI



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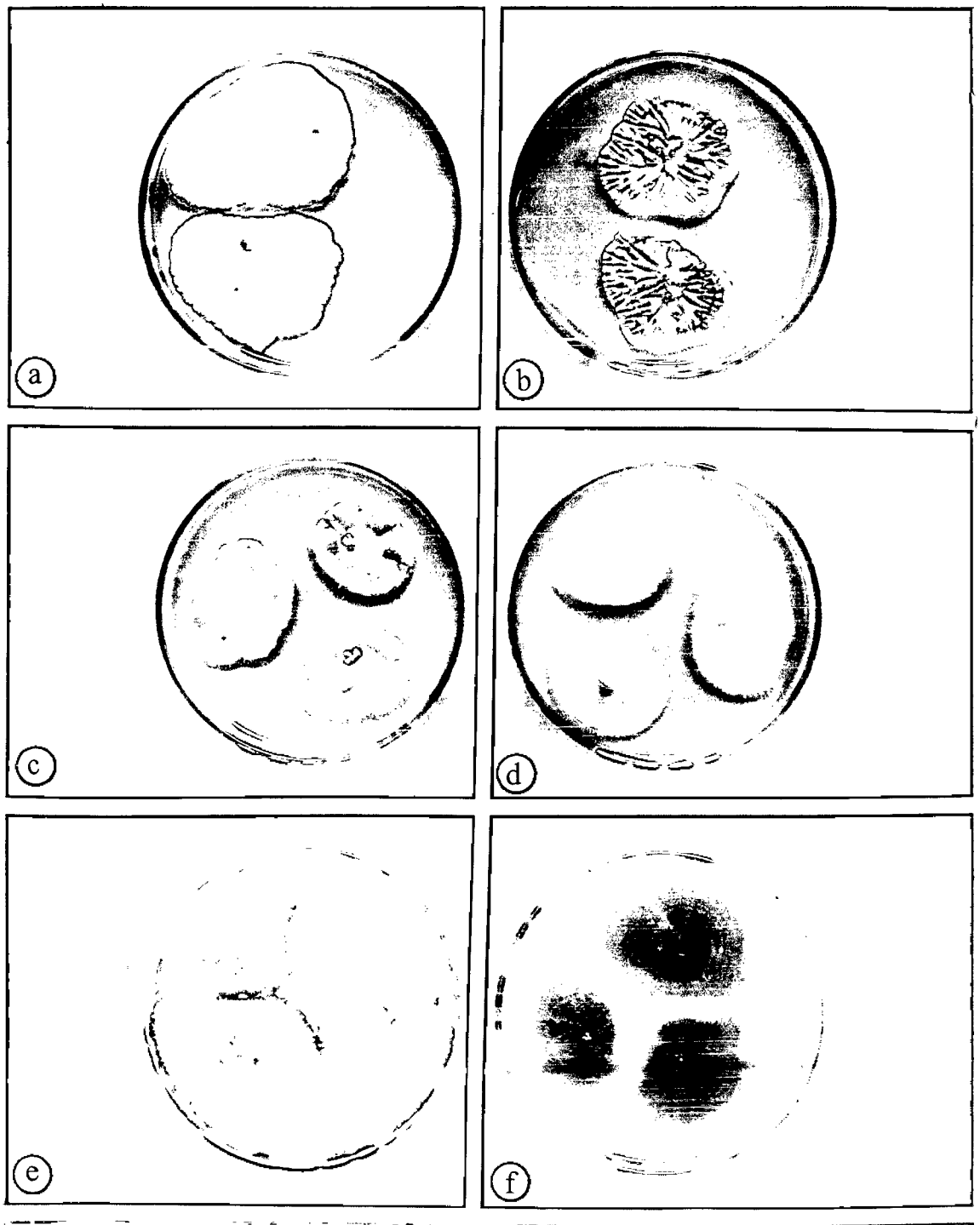

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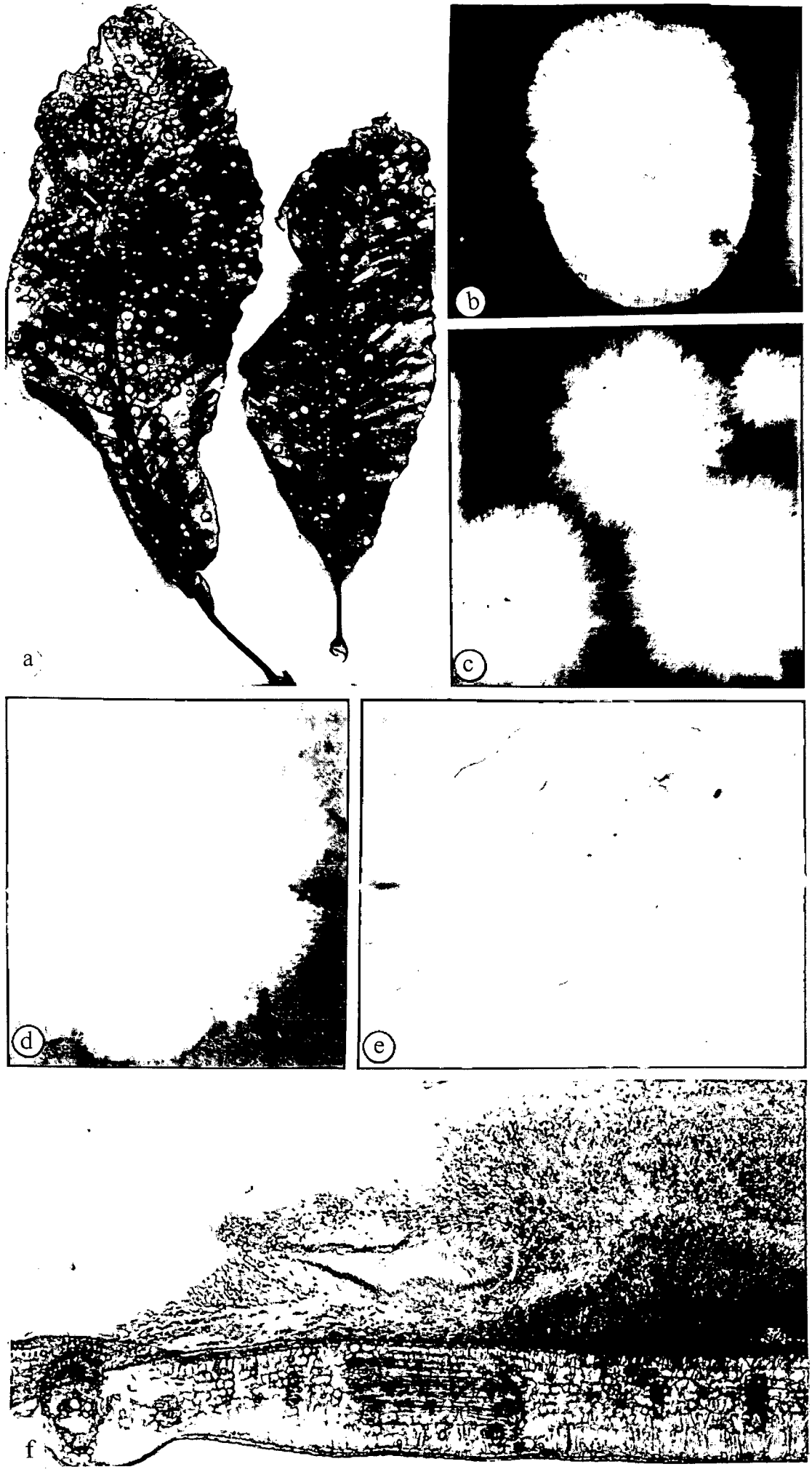

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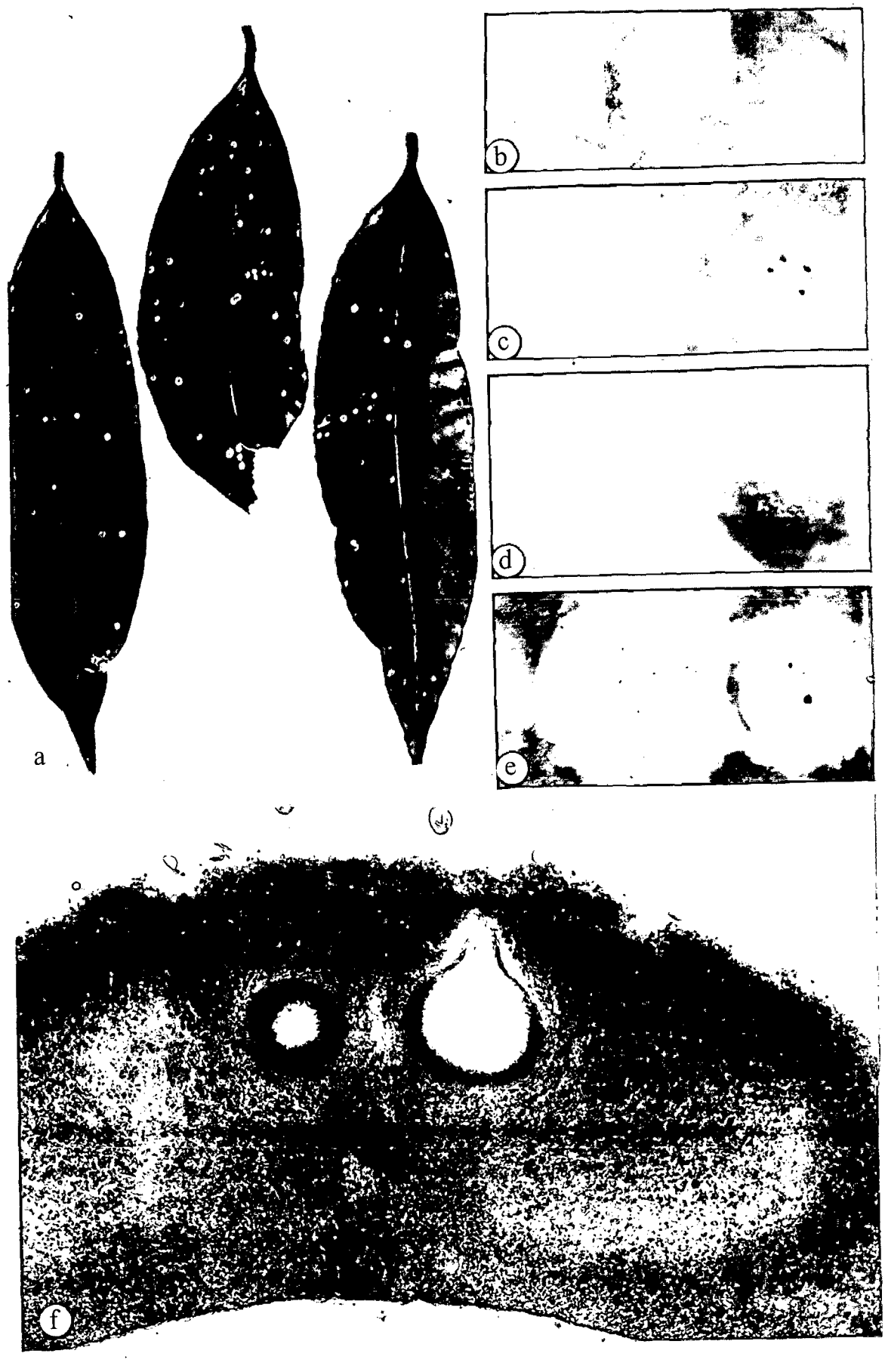

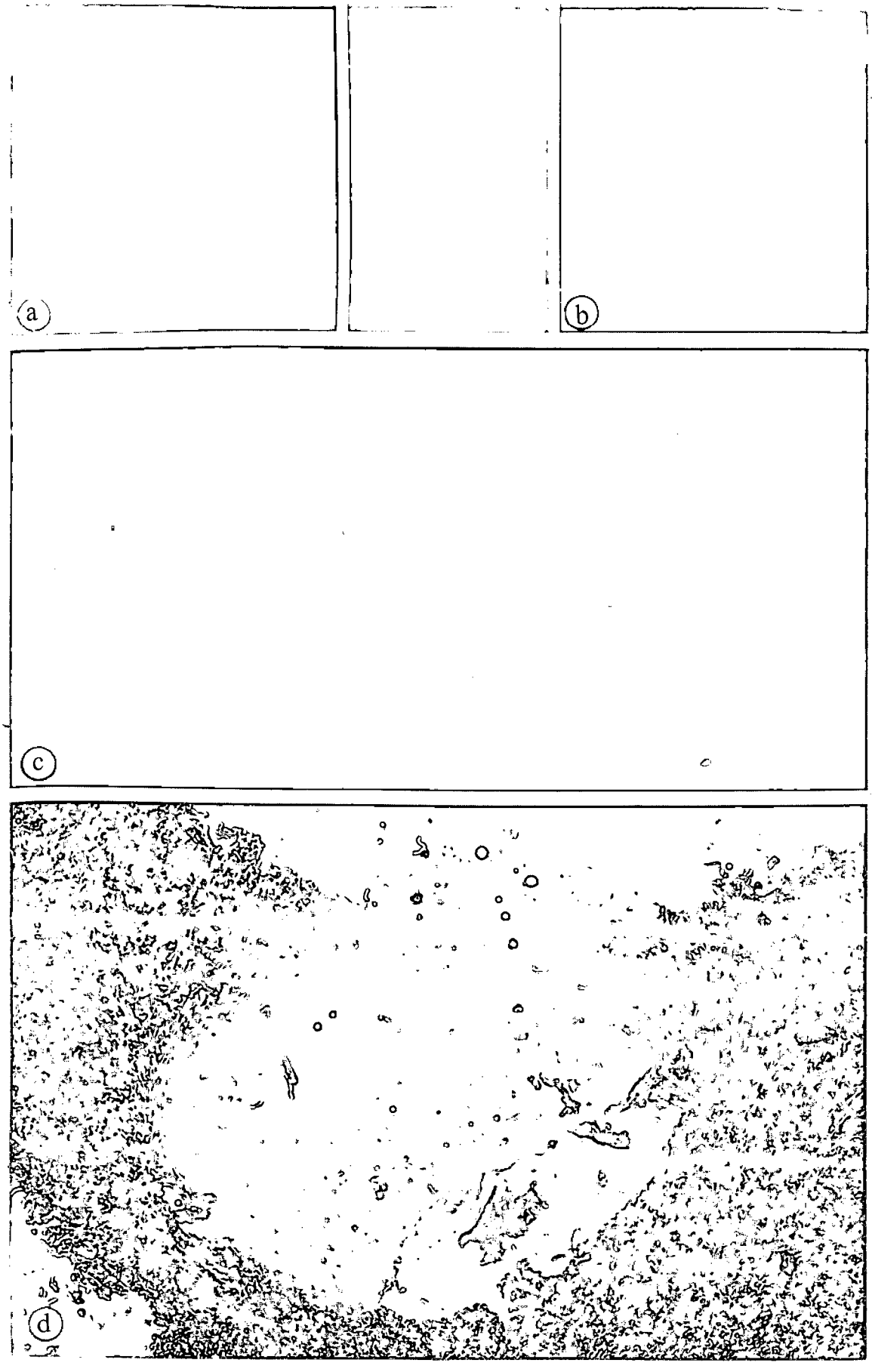
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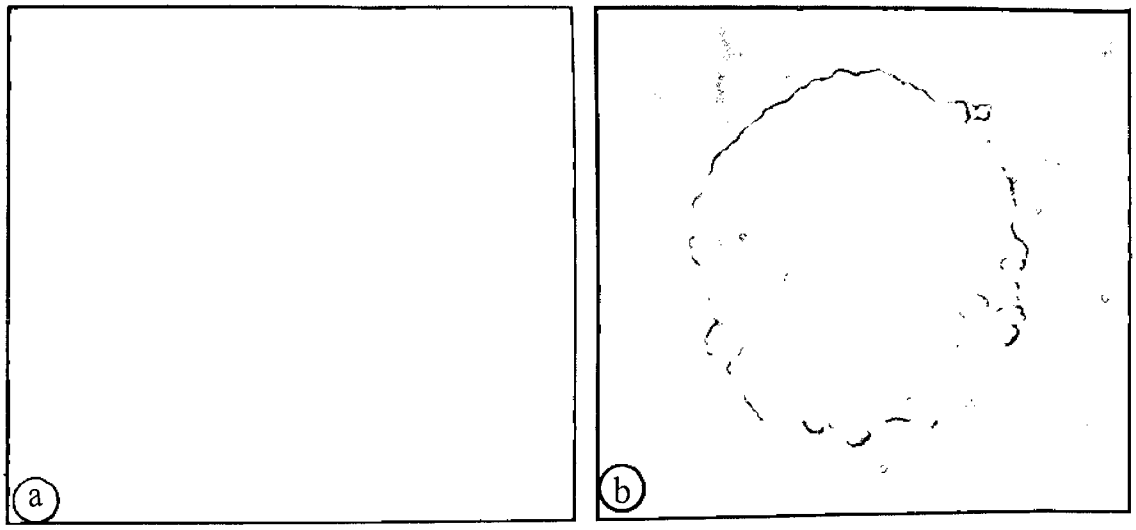
PLATE : XI



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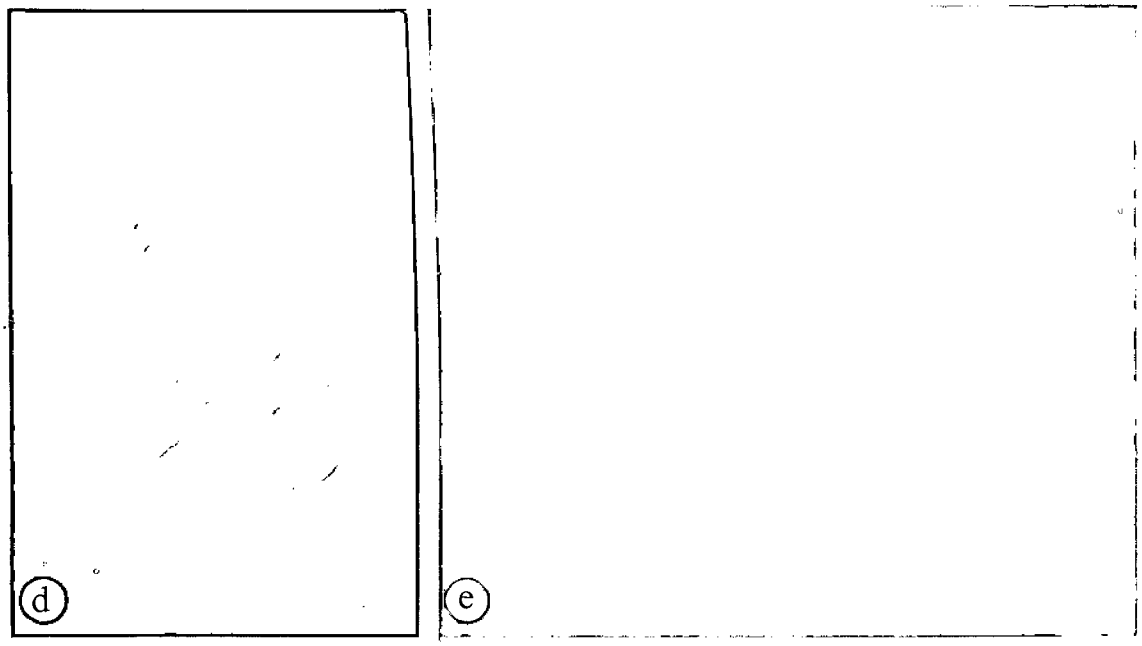

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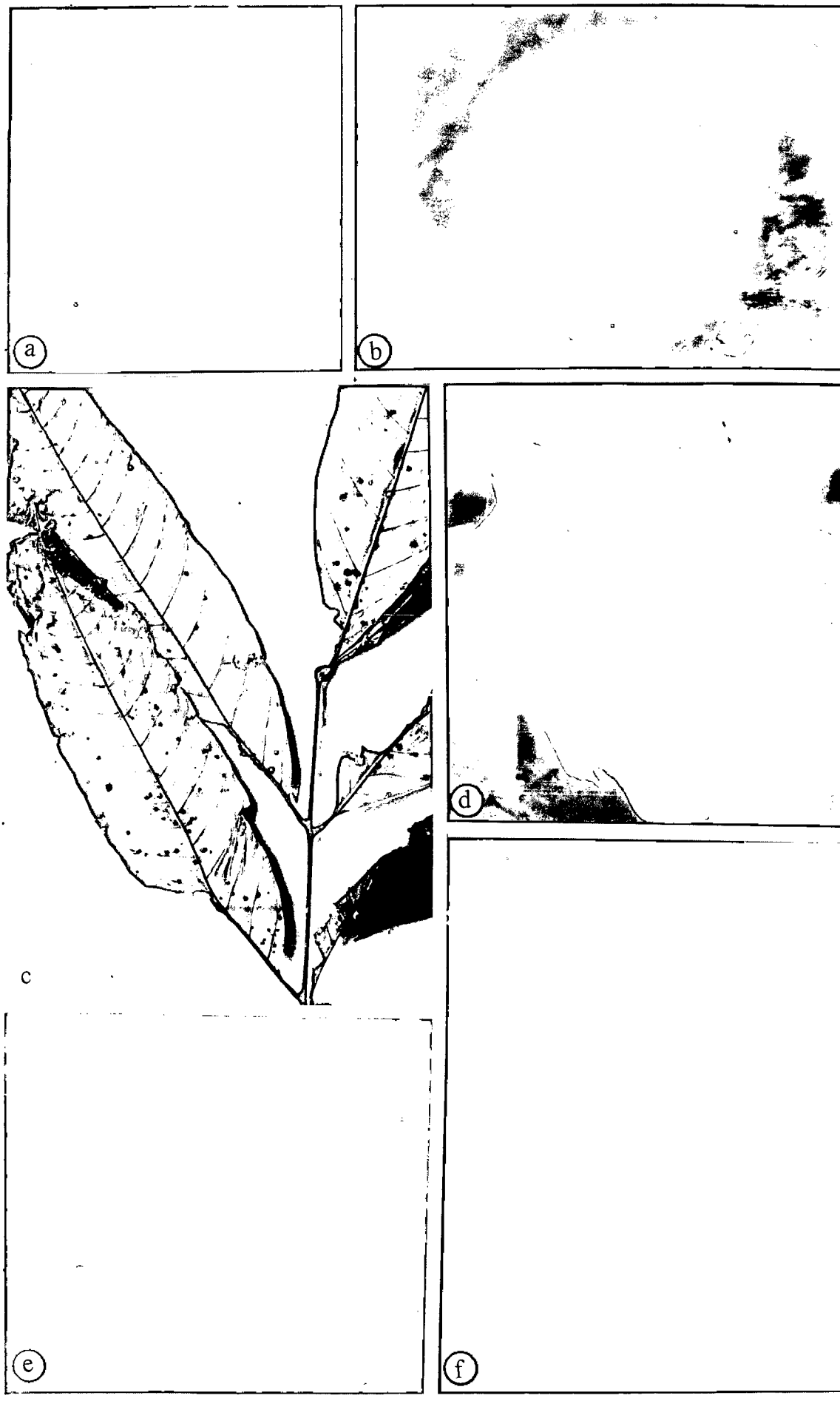


PLATE : XIII

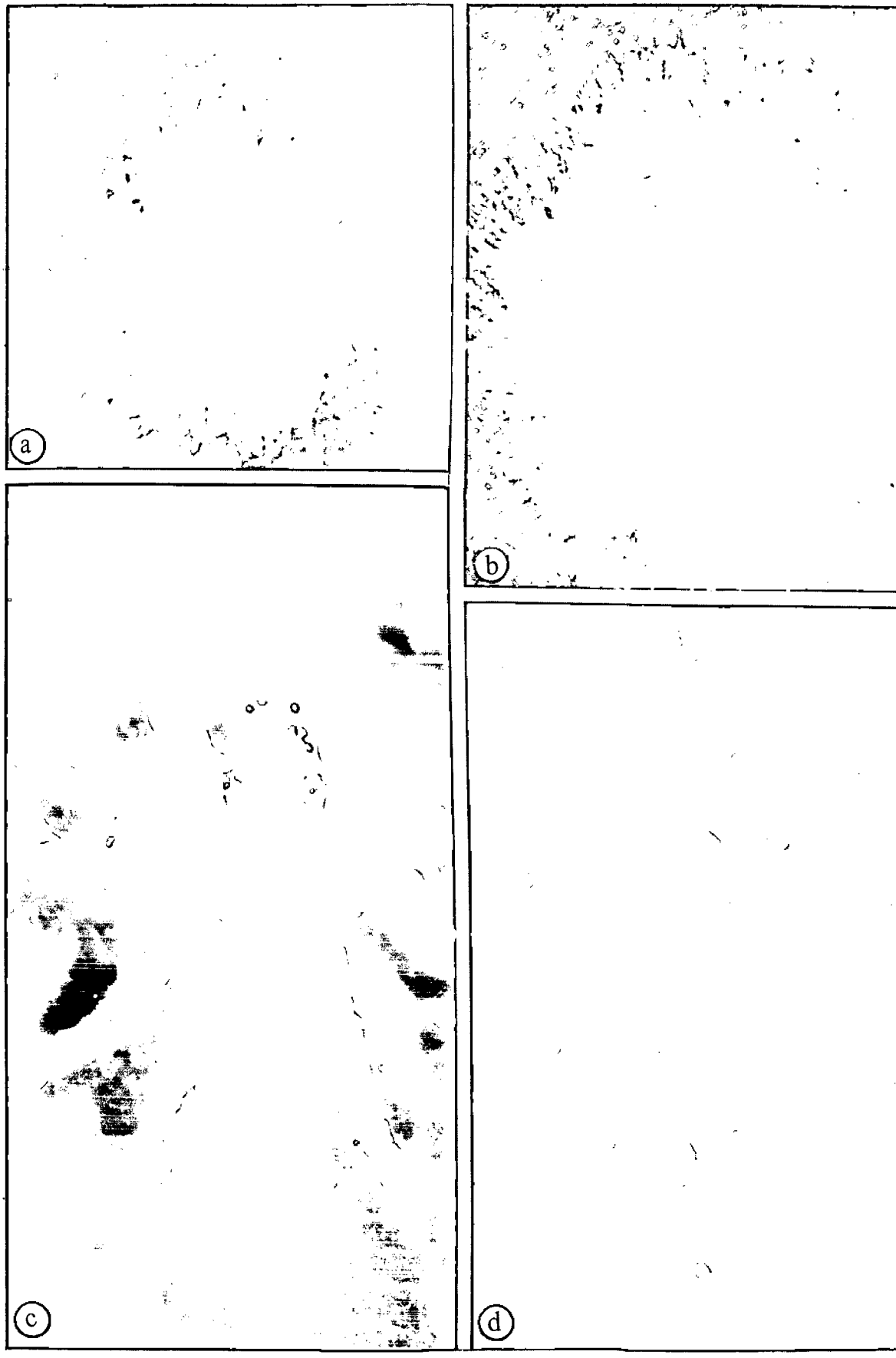
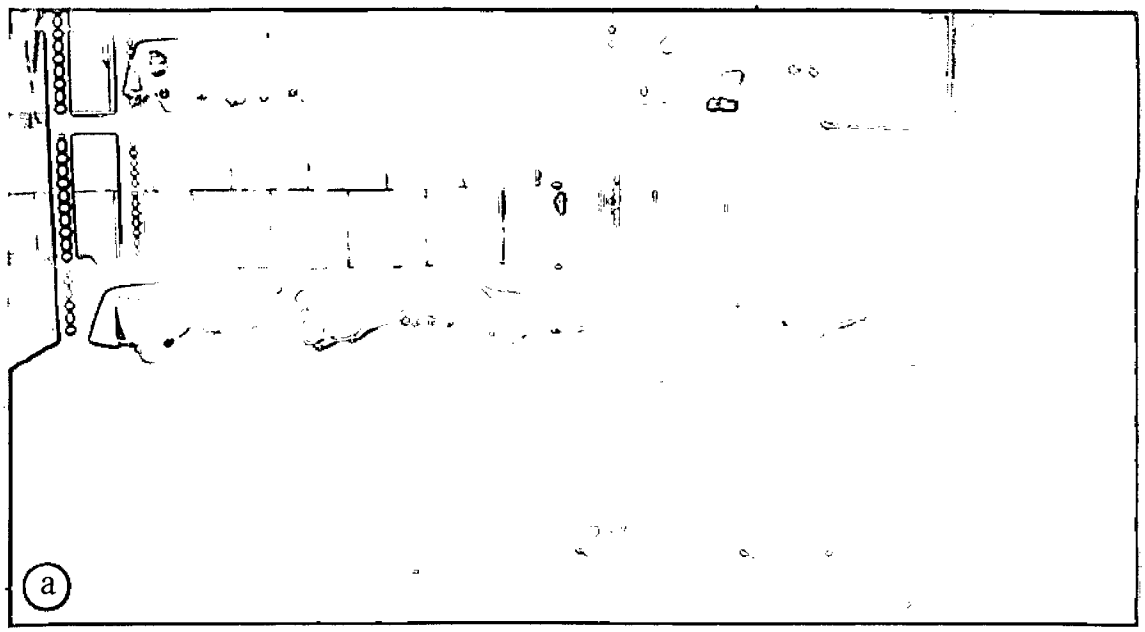
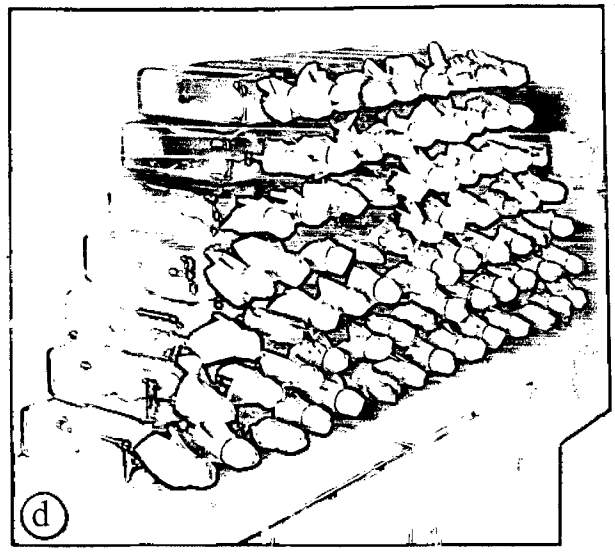
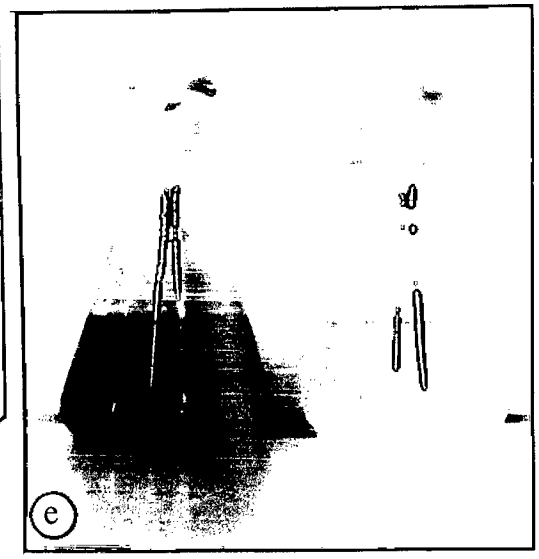


PLATE : XIV







This study on diversity, taxonomy and ecology of entomogenous fungi isolated from a varicty of insect hosts and other substrates in various localities of Goa, Karnataka, Kerala and Maharashtra, and assessment of dose-response relationship of spores and analysis of impact of cell-free extracts of these fungi on developmental stages of mosquito vectors was undertaken during March 1999 to June 2002. The results obtained are discussed below.

4.8. Discussion

4.8.1. Diversity and taxonomy of entomogenous fungi:

In all, 286 isolates assignable to 74 species and 23 genera of entomogenous fungi have been encountered in this work. These included *Acremonium* (7), *Aschersonia* (4), *Aspergillus* (16, *Beltrania* (1), *Chaetomella* (1), *Cladosporium* (5), *Conidiobolus* (1), *Curvularia* (2), *Cylindrocladium* (1), *Fusarium* (5), *Gibellula* (1), *Gliocladiopsis* (1), *Gliocladium* (3), *Hirsutella* (2), *Hypocrella (1), Mucor (6), Paecilomyces* (7), *Penicillium* (4), *Pestilotiopsis* (1), *Pleurothecium* (1), *Podonectria* (1), *Syncephalastrum* (1) and *Trichoderma* (5). Amongst the 28 undetermined taxa, 7 were pycnidial (Coelomycetes) and 21 belonged to Hyphomycetes (thallic, 3 and phialidic, 18), Non-sporulating (70). Seventy isolates did not produce any kind of reproductive propagules and they were designated as 'morphological' types. The host and taxonomic diversity of entomogenous fungi is given in Table: 4.21.

Living and dead insects infected by microfungi can be found in habitats such as live and dead forest trees, undergrowth, terrestrial soil, flowing streams, stagnant ponds, cultivated and barren fields, construction sites and even in laboratory and on commercial insect colonies. It is generally the student who works with fungi first notices colonization of insects by extraneous fungal materials. Visual search of habitats of interest for infected insects, which appear different from normal forms of same

population, is one of the successful ways of collecting fungi infected insects. Although fungal pathogens are usually present at low level of infection in insect populations (as enzootic diseases) they are most easily recognized during epizootics when there is an unusual abundance of diseased insects. Though sure-shot, hand-picking of infected specimens to some extent allows selectivity in isolation. Collection of number of living insects using standard insect collecting techniques and subsequent screening in the field or laboratory for infected specimens (as described in the key given below) is more meaningful method of isolation of entomogenous fungi. Essentially, the tools of collection of infected insects such as baits, handpicking, aquatic nets and dippers are same as those used for survey of healthy insect populations. Insects infected by fungi often exhibit characteristic symptoms such as striking colours, luxuriant growth of the pathogen on surface of the larvae, signs of etiological agent within the host, sluggishness, inability to rise to the surface as in mosquito larvae, swirling of head, lack of feeding, unusual positioning on plant host, mummification, fragility or hardening of the integument and difference in size.

A modified diagnostic key based on Lacey and Brooks (1997) for identification of infected insects for entomopathogenic fungi is given below:

1. Apparent external growth----------2
1. No external growth----------3
2. Growth on the surface of insects, mass of worm-like, red, cream or light brown in colour..... **NEMATODES**
2. Growth on the surface of insects, powdery, slimy (white, green or red) or sometimes limited to intersegmental area or growth club like.....**FUNGI**
3. Normal from outside, metazoans with distinct mandibles and segments, feeding internally or externally on host tissues.....**PARASITOIDS**

3. Normal from outside, absence of metazoan parasites upon dissection, but contain various motile or non motile unsegmented life-cycle stages such as, spores, cysts or filamentous structures----------4

4. Aquatic or other insects with transparent integument----------5

4. Terrestrial insects or those with nontransparent cuticle----------7

5. With a milky haemolymph.....**BACTERIA**

5. With a clear haemolymph----------6

6. Abnormal whitish masses in haemocoel associated with fat body, comprised of ovoid to pyriform bodies.....**PROTOZOA**

6. Haemocoel filled with hyaline filamentous bodies or rust coloured oval spores with sculptured walls..... **FUNGI**

7. Insect body hardened, mummified and filled with hyaline hyphae, hyphal bodies or spherical resting spores..... **FUNGI**

7. Insect body not hardened but stunted with malformed body parts.....
....**PROTOZOA, RICKETTSIAE, NEMATODES, BACTERIA, VIRUSES**

In order to minimise the damage on specimen, diseased insects in the field were collected using fine forceps and/or along with the substrate to which they were attached. Even minute insects were collected along with their host plant foliage. Live insects were incubated with the substrate, for e.g., mosquito larvae in water from a breeding habitat or aphids on host foliage. When an insect was suspected of being infected, it was examined as soon as possible so as to avoid overgrowth by fast growing saprophytic fungi. Surface sterilization of insects including mosquito larvae was found to kill the entomopathogenic fungi inside the host. Therefore, isolation of fungi from insect hosts was done without surface sterilization of the latter. When external growth was apparent, as it was in most cases, successful isolation was achieved by carefully touching the spores on the surface of host with a fine-tipped sterile needle and then streaking on an isolation plate. For entomophthoralean fungi, isolation was most successful when conidia from host cadaver was made to project from the lid of the petri plate into agar medium.

Fungi were the only entomopathogens found in homopterans (insects with sucking food habit). Entomopathogenic fungi generally kill their host after infection. Following death, fungal hyphae entangle the host and infectious spores surface externally. Dead insect cadavers clasp to host plant due to mycoses, get mummified and persist for several weeks. Isolation of fungi from such specimens was achieved even after death of the insects.

Efforts were made from early days to document the taxonomic diversity of entomogenous fungi in India. Several students of mycology by their indefatigable endeavour collected and described fungi found growing on different hosts and substrates in different regions of our country, including those found on insects. In the recent days, major taxonomic work on entomogenous fungi is that emanated from Jabalpur school of Mycology (Agarwal and Rajak, 1988).

From a phyto-geographic point of view, western region of peninsular India, encased by Arabian sea on the west and Western Ghats along the eastern side and covered by varied kinds of vegetation ecosystems, hilly tracks, undulating interior terrains, streams, ponds, coastal plains and beaches, is a complex set up. The plant and fungal communities in the region are very dense and diverse (Pascal, 1989). Although the insect population is said to be extremely high in the region, they have not been explored much (Ananthakrishnan 1996; Saldhana, 1984). The associative entomogenous fungi have also not been studied so far (Bhat, 2000). On this background, the results obtained from biodiversity documentation of entomogenous fungi of some parts of Western Ghats in southern India through this work are very rewarding and most invaluable.

Fungi in culture are now considered to be a treasure. Fungal bioresources can be used for advantage only if pure isolates are available. Industries involved in fungal bioprospecting, enzymology, biofertilizers, biofuels, food, etc. obtain authentic and standard cultures from recognized national or international culture collections such as Microbial Type Culture Collection, IMTECH, Chandigarh, American Type Culture Collection, U.S.A., CABI Bioscience, U.K. and Centraal Beureau vor Schemmilcultures, Baarn, Netherlands. Realizing that fungi from extreme habitats are very potential in their activity, a few industries involved in fungal biotechnology have initiated sourcing fungi on their own from difficult habitats and environments such as marine, mangrove, deep sea, freshwater streams, endophytes, coprophilous and entomogenous. From this angle, a large collection of entomogenous fungi in pure culture form, isolated from diverse habitats and substrates of Goa, Karnataka and Kerala and deposited in Goa University Fungus Culture Collection, is very worthy and useful in the future in many ways.

4.8.2. Screening fungi for biocontrol potential against mosquito larvae and assessment of mosquito-larvicidal activity of candidate species:

One of the aims of biocontrol programmes is search for newer isolates of entomopathogens. In the present study, several isolates of *Aspergillus, Gliocladium, Penicillium* and *Trichoderma*, 1 each of *Acremonium* and *Chaetomella* and 3 of an undetermined fungus have shown property of control of mosquito larvae tested. In general, species of *Aspergillus, Gliocladium* and *Penicillium* were found to be fast and those of *Trichoderma* were slow performers. Species of *Gliocladium* and *Trichoderma* are well-recognized biocontrol agents of fungal pathogens of plants (Dubey, 2002; Nasim and Bambawale, 2003). *T. harzianum* parasitize elm bark beetle *Solytus*

(Deshpande, 1999). They are able to do this by production of specific hydrolytic enzymes. Biocontrol property of fungi, especially on mosquito larvae, is a new dimension of their action and holds immense potential for the future.

Fungal isolates such as C24, E26, and E2 of *Penicillium* sp. with similar cultural and morphological characters, acted differently in their mosquito larvicidal activity. At same spore concentration, E2 isolate effected 100% mortality in 24 h, E26 it took 48 h and C24 did not show any sign of action on mosquito larvae tested. Although *Aspergillus fumigatus* isolates C1, C8 and C15 have similar cultural and morphological features, only C15 isolate resulted in 60% mortality under similar experimental conditions. This clearly indicates that properties of fungi, beyond the cultural and morphological, such as host/substrate and site affinities hold the key for their metabolic recognition. This is one of the arguments that calls for deposition of all kinds of isolates of fungi in culture collections with comprehensive database.

Being filter feeders, mosquito larvae accumulate fungal spores in their gut within an hour of exposure even to dilute spore suspension. Larval gut contained abundant conidia of all the *Penicillium* isolates tested and packed spores were visible as a green tube under stereomicroscope. Larvae were unable to eject spores out of the gut. These conidia-fed larvae were unable to surge to the surface and eventually died at the bottom. Conidia of *Penicillium* sp. attenuated by autoclaving as well as spores of other inactive isolates when fed were ejected as pellets without or after digestion. It is therefore deduced that fungal spores of promising isolates apparently extend mechanical blockade gut movement.

Gliocladium sp., another promising fungus, isolated from insects other than mosquito. This fungus provided 100% mortality with in 24 h, with gut of larvae completely lysed at foregut region within 8-10 h of exposure.

In the case of *Trichoderma*, isolates in general effected slow mortality. However, browning (melanisation) and disruption of gut were observed in some larvae within 24-48 h of exposure, though majority of them showed filamentous fungal growth in the haemocoel. The larvae were found with conidia adhering to anal papillae, cervical collar and even inter-segmental regions. Being very versatile, isolates of *Trichoderma* sp., though acted slowly in laboratory conditions, in the course of time when several more trials are conducted with varying combinations might prove as a useful biocontrol agent for field application.

Several fungi isolated in the present study, belonging to the genera such as *Acremonium, Aspergillus, Chaetomella, Gliocladium, Penicillium* and *Trichoderma* and a few unidentified taxa, new records as larvicidal or mosquito-pathogenic fungi. This exercise, sourcing of entomopathogenic fungi from mosquito larvae and non-mosquito insects and arachnids, opens up a new avenue in the search for potential biocontrol agents against vector mosquitoes. Needless to say that use of entomopathogenic fungi should be a reliable advancement in mosquito suppression because, once well established it would remain as a durable, non-polluting and self-perpetuating programme.

4.8.3. Activity of culture filtrates of promising isolates

Vijayan and Balaraman (1991) had showed that crude cell-free extracts of 133 out of 350 fungal isolates tested were active against mosquito larvae. Out of these, a *Paecilomyces* sp. isolate was highly active.

In the present study, cell-free extracts of 20-day old 17 fungal isolates out of 40 tested showed 50% or more mortality effect on mosquito larvae. The isolates belonged to *Acremonium* (1), *Aspergillus* (2), *Gliocladium* (2), *Penicillium* (5) and *Trichoderma* (7). Cell-free extracts of 7-day old cultures of only three isolates showed mortality of 20% or less. It is presumed-that active principles elaborated by the fungi later in growth phase were responsible for the kill. It is also interesting to note that cell free extracts of slow-acting isolates have effected mortality on mosquito larvae than that of fast-acting fungi, except for *Penicillium* sp. From the results obtained it is evident that entomopathogenic fungi are capable of producing secondary metabolites, which have utility value.

4.8.4. Enzymes of entomogenous fungi

St Leger et al. (1986a) observed that fungi produce a range of extracellular cuticle-degrading enzymes corresponding to components of insect cuticles, namely protein, chitin and lipid. St Leger et al. (1987a) extracted protease and aminopeptidase from experimental insects about 16 hours after inoculation with *Metarhizium anisopliae*. Endoprotease activity was attributed to Pr1 and Pr2 proteases. Pr1 appeared to be pathogenicity-determinant as it had high cuticle degrading ability and was produced in large amount during infection (St Leger et al., 1987a,b). Inhibitors of Pr1 drastically reduced the mortality, supporting that Pr1 is important for penetration. The inhibitor also reduced browning, invasion of haemolymph and thereby curtailed insect growth rate.

Esterases generally hydrolyse short-chain fatty acids whereas lipases degrade long-chain esters. Enzyme Pr1 can also degrade p-nitrophenol esters and therefore it is considered to be similar to lipase (Charnley and St Leger, 1991). Though St Leger et al.

(1987a) detected esterase in pregerminating and germinating conidia and appressoria of *Metarhizium. anisopliae* on insect host, Charnley and St Leger (1991) doubted the involvement of lipases in pathogenicity as they failed to extract true lipases from cuticle of insect hosts. However, ultra-structural studies have demonstrated early hydrolysis of wax layer on cuticles just beneath the appressoria and germ tubes and thus attributing it to lipase or esterase activity (Zacharuk, 1970; Charnley and St Leger, 1991).

St Leger et al. (1987a) could not find any evidence of chitinase production by conidia of *Metarhizium anisopliae* during first 40 h after inoculation of hosts. It was suggested that absence chitinase activity during penetration could be due to inhibitors in the cuticle (St Leger et al.,1986d). Absence of products of chitin degradation in infected cuticle was an indication of negligible role of chitinase during invasion. Charnley and St Leger (1991) opined that probable role of chitinase could be nutrition during post-penetrative phase of the fungus in insect cadaver.

In the present study, all the isolates brought into culture were subjected to qualitative analyses of esterase, protease and chitinase. Of the 286 strains tested, 137 were positive for esterase. Based on the grading considered, 90 isolates showed +++ activity, 20 exhibited ++ activity and 27 isolates were with + activity (Table 4.18). Only 125 amongst 286 isolates tested were positive for protease (Table 4.18). Of the 286 isolates tested for chitinase, only 31 showed positive to chitin utilization activity (Table 4.18).

Most of the active isolates, in the present work, are found to be esterase and protease positive. Chitin utilizing activity was observed in very few isolates. Though preliminary and qualitative at this stage, results obtained of the studies of enzymes of entomopathgogenic fungi are very promising.

4.8.5. Preliminary field trials of promising isolate

When preliminary field trial of a laboratory-proven promising isolate of *Penicillium* sp. (E9) was carried out during rainy season, interesting results on performance of the fungus as a biocontrol agent were obtained. Although the set up was small and only a volume of 4 L, the results obtained could vouch on their application value. A larval mortality of 66 ± 10.56% observed in the field trial amply confirms the biocontrol potential of the fungus tested. This result was obtained within 48 h of initiation of the trial.

Fungi such as species of *Penicillium*, produce large quantity of spores even in minimal media on a very short time period. Use of locally available corn grains and simple sugar in the preparation of an indigenous culture medium made the field trial cost-effective and very challenging. As shown in this work, production cost of large quantity of spores was fairly low. Compared to other biocontrol principles, these were easy to handle and comfortable execute in the field.

With such positively performing fungus is in hand and once the isolate tested against non-target species, the entomogenous fungi can be used for field application with an aim to control mosquito vectors.

4.8.6. Field observations on *Aschersonia* and *Hirsutella*

Detailed life cycle studies of fungi associated with insects and other arthropods were not new. Classic examples include several of Hypocrealean, Clavicepetalean and Entomophthoralean fungi (Kendrick, 2001). Besides, fungi such as species of *Coelomomyces* have mosquito larvae and copepods as hosts in their life cycle. Fungi such as *Claviceps purpurea*, need a lepidopteran host for successful completion of its life cycle. Species of *Nectria* appearing on scale insects in some part of their life history

have been observed (Luttrell, 1967). It was these insect-host relationship issues attracted mycologists and entomologists to study the life history of the fungi with an aim further to use them for control of insects pests (Pell et al., 2001, Inglis et al., 2001).

During the course of present study, an effort was made to construct the life history of 4 species of entomogenous fungi, namely *Aschersonia aleyrodis, A. badia, A. indica* and *Hirsutella* sp. based on materials gathered from the field at regular monthly intervals and subsequent careful laboratory observations.

From the results obtained on studies of *Aschersonia indica* (Teleomorph: *Hypocrella* sp.) and *Hirsutella* sp., it is clear that besides the insect host, the fungus is indirectly associated with a specific plant host, *Hopea ponga*. This association may be attributed to the humid climatic condition of riparian habitat where *H. ponga* is generally in abundance. The fungi and associated insects were not observed on any other plant hosts in same region. Efforts made to locate the fungi and their insect host on tree foliage away from the stream were not successful. Moreover these fungi and insects were found distributed only on the banks of the stream. This clearly indicated that the fungi and insect host needed humid environment for their growth. Fungal association with the host plant may be secondary but with the insect host it may be considered as a specific 'fungus-insect-plant' association.

It can also be deduced from the observed facts that these fungi never infected the plant leaves. There were no discernible symptoms on the leaves. On drying, the insects and the associative fungus were detached or peeled off very easily without leaving any mark of infection on leaf surface. Cross section of infected portion on the leaf showed no trail of fungal penetration through leaf cuticle and epidermis. This further confirmed that the fungus is purely entomogenous in its biological behaviour.

Aschersonia aleyrodis and *A. badia* (Teleomorph- *Podonectria* sp.) were found even 1-2 km away from the stream. *A. aleyrodis* along with its insect host was found in all plants examined and in large numbers, on rainy days. *A. badia* was found in most plants but in less numbers than that of *A. aleyrodis.* During dry periods however these fungi were collected only from near the streams.

Aschersonia aleyrodis and *Hirsutella* sp. were found throughout the year in their anamorphic state. Their perfect states were not observed during the study period. *Aschersonia badia* and *A. indica* were present during rain and winter seasons (July to December). *Podonectria* sp., teleomorph phase of *Aschersonia badia*, developed on the same stroma in January-February, the months where warmer summer days commences. In mid-summer (during March), most foliage that harboured the fungus became senescent and were shed off. In May, these fungi therefore became part of the litter flora. Consequent to this, during April-June, tree foliage had very less of these fungi.

Similarly, *Aschersonia indica* was seen from mid–June to December. *Hypocrella* sp., teleomorph of the fungus, developed along sides of the anamorph stroma and matured in October. In January-February, mature fruit bodies of *Hypocrella* were found. Most of these soon got detached from the stroma and found their way into soil. As in *Aschersonia badia, A. indica* also developed teleomorph in the summer.

It is not clearly understood, how the ascospores, survived in summer months in litter and eventually reached the next generation of arboreal insects on fresh foliage. Nevertheless, pycnidiospores of *Aschersonia badia* or *A. indica* were found broadcasted into large population of insects. When a droplet of water was placed on the fructification, the conidia were oozed out through the ostiole. As these spores were slimy they stuck to crawling insects in large numbers. It can be presumed that anamorph

served as propagation unit and teleomorph acted as aestivating unit in the life cycle of these fungi.

Anamorph phase of *Aschersonia indica* were observed from mid–June to December. *Hypocrella* phase developed from periphery of the stroma and matured by October. In January, ascocarps were found matured and got detached from main body into soil. Similar to *Aschersonia badia, A. indica* had teleomorph phase in summer. Hywel-Jones and Evans (1993) while reporting *Hyocrella discoidea* and its anamorph *Aschersonia samoensis*, noted that *H. discoidea* occurs in summer and *Aschersonia samoensis* in other favourable seasons.

This exercise of *in situ* investigation of life cycle of entomogenous fungi provided proof not only of time and duration of occurrence of fungi on insects but also their ability to self-perpetuate and sustain on their own. As discussed above, pycnidial phase of *Aschersonia badia* with abundant spores occurred in monsoon and post-monsoon months. If we take that survival of propagules of this fungus is all well during warm and humid months, there is a possibility of utilizing them for field application against agricultural insect pests.

Table. 4.21. Host and taxonomic diversity of entomogenous fungi collected from Goa, Karnataka, Kerala and Maharashtra

Name of fungus (Genus with no. of spp./ tax. spp.)	Host/substrate (with no. of isolates)	Locality (with no. of sites)	Cultured (Y/N) (if Yes, with no. of isolates)
Acremonium (7)	IN (13), SP (1)	GA (7)	Y (14)
Aschersonia (4)	IN (7)	GA (3)	Y (7)
Aspergillus (16)	IN (13), ML (11), SP (1), WS (1)	GA (12), KA (1)	Y (26)
Beltrania (1)	IN (1)	GA (1)	Y (1)
Chaetomella (1)	SP (1)	GA	Y (1)
Cladosporium (5)	IN (8), ML (1)	GA (7), KL (2)	Y (9)
Conidiobolus (1)	IN (1)	GA (1)	Y (1)
Curvularia (2)	IN (2), ML (1)	GA (3)	Y (3)
Cylindrocladium (1)	IN (1), ML (1), WS (1)	GA (3)	Y (3)
Fusarium (5)	IN (12), ML (4), SP (1)	GA (10), KL (1)	Y (17)
Gibellula (1)	SP (1)	GA (1)	N
Gliocladiopsis (1)	IN (2)	GA (2)	Y (2)
Gliocladium (3)	IN (13), SP (1)	GA (7), KL (1)	Y (14)
Hirsutella (2)	IN (5)	GA (5)	Y (5)
Hypocrella (1)	IN (1)	GA (3)	N
Mucor (6)	IN (2), ML (1), SP (3)	GA (6)	Y (6)
Paecilomyces (7)	IN (21), ML (1), MT (1), SP (2)	GA (10), KA (1)	Y (25)
Penicillium (4)	IN (11), ML (11)	GA (11), KA (1), KL (1)	Y (22)
Pestilotiopsis (1)	IN (1)	GA (1)	Y (1)
Pleurothecium (1)	IN (1)	GA (1)	Y (1)
Podonectria (1)	IN (1)	GA (1)	N
Syncephalastrum (1)	ML (1)	GA (1)	Y (1)
Trichoderma (5)	IN (12), ML (9), MT (2), SP(1), WS (5)	GA (11), KL (1)	Y (29)
Undetermined sp. (Phialidic) (18)	IN (12), ML (4), MT (1), SP (1)	GA (9), KA (1)	Y (18)
Undetermined sp. (Thallic) (3)	IN (2), ML (1),	GA (3)	Y (3)
Undetermined sp. (Pycnidial) (7)	IN (3), ML (4),	GA (4), KA (1)	Y (7)
Non-sporulating (70)	IN (58), ML (7), MT (1), SP (4),	GA (18), KA (2), KL (1)	Y (70)

Note: ML = mosquito larvae; IN = insects; WS = water samples; SP = spiders; MT= mites; GA = Goa; KA = Karnataka, KL=Kerala.

Epilogue

Entomogenous fungi have been reported from all divisions of Eumycota and over 700 species belonging to 90 genera of fungi were recorded as pathogenic to insects and mites. Many of these are known to grow on and regulate insect population in nature, including mosquito larvae. The potential of entomogenous fungi as biocontrol agents in vector control programmes has been recognized and, although not used beyond a measurable scale, mass production of fungal spores aiming at control of insect pests has been reported. Though cursory, many earlier publications on taxonomy, biology and ecology of entomogenous fungi are known from India.

In the absence of any detailed investigation from this part of the country, an effort was made to study the taxonomic diversity and ecology of entomogenous fungi and their biocontrol potential against mosquito larvae. A large number of microfungi has been recovered in pure culture form, some hitherto unknown, from a variety of insect and arachnid hosts. These cultures are housed in 'Goa University Fungus Culture Collection', a unique facility established to conserve rare, interesting, unique and useful fungi of the region in live form and make them available for utilization. The entomogenous fungi isolated and described in this work are thus a value-addition not only to our knowledge but also wealth of our nation.

Fungi recovered in pure culture were screened for larvicidal activity of mosquito vectors of malaria (*Anopheles stephensi*), dengue (*Aedes aegypti*) and JE (*Culex quinquefasciatus*). A number of fungal isolates especially those belonging to genera such as *Acremonium*, *Gliocladium*, *Penicillium* and *Trichoderma* were found to be promising with a varying degree of mosquito larvae mortality rate which some time was up to 98%. Insatiable feeders they are, the mosquito larvae engulfed fungal spores to the

extent of chocking their guts. Besides, as understood from this work, these fungi are armoured with powerful enzymes, which have immense industrial application value. Field trial run of a promising isolate satisfied all queries of standard protocol and vouched beyond doubt that Western Ghat region in our country has not only promising but also patentable bioresources of invaluable utilization in primary health sector.

Species of *Aschersonia* and *Hirsutella* are associated with their specific insect hosts. Some of these go further for specific plant hosts. Then they are confined to riparian habitat. All these step-by-step adjustments and arrangements of 'fungi-insect-plant' associations took time beyond months. Intricate ecological and biological associations when investigated systematically and *in situ*, as done in this study, unravel a treasure of information, which could be used for advantage. Besides, life cycle studies of entomogenous fungi are a joyful experience to an ardent student of mycology. It is something like nature unfolding a secret before us.

Use of bio-control agents against mosquitoes has emerged as a thrust area of research in the recent years and is further gaining importance due to large scale and wide spread resistance in vectors of malaria, filariasis and Japanese encephalitis to synthetic insecticides especially in the tropics. Among various biological control agents of mosquitoes that exist in nature, larvivorous fishes and bacilli have already been deployed in large scale vector control programmes. Entomogenous fungi are those 'wait-in-line' tools available for similar operations.

Although basic, the results presented here are undoubtedly of high value. This thesis, a written document of an exhaustive work carried out in not-so-near located two laboratories, the Botany Department of Goa University and ICMR Malaria Research Centre, Goa Division, is a perfect proof for and example of how mutually recognized

institutes could join hands and work together in understanding the taxonomy, biology and application of entomogenous fungi and contribute in scientific development of our nation.

Chapter V:

SUMMARY

This thesis embodies results of an investigation carried out during March 1999 to June 2002 to gather information on taxonomic diversity and ecology of entomogenous fungi from some localities in central part of western India covering provinces of Goa, Karnataka, Kerala and Maharashtra and to elucidate biocontrol activity of these fungi on early developmental stages of mosquito vectors.

Results obtained from the study were dealt under several parts. From a large collection of entomogenous hosts and substrates, 286 isolates of fungi were recovered in pure culture. The fungus-host relationship was studied on pre-isolation incubation period. Microscopic observations revealed that haemocoel in the body of infected larvae turned opaque, some times fungal hyphae ramified the body, yet in other cases the gut got choked by ingested fungal propagules. Amongst the isolates, 26 were from immature stages of *Anopheles* sp., 27 from *Culex* sp., 1 from *Aedes* and 3 from exuviae of unidentified mosquito larvae. Adult mosquitoes did not exhibit signs of fungal infection.

Aphids, ants, scale and other insects and arachnids from plant foliage and litter were scanned for fungi. Amongst these, immature stages of homoptera yielded 19.36%, scale insects 14.4 %, unidentified insects 12.6 % and aphids 11.7 % isolates of fungi. Maximum number of insect-infected fungi were recovered from insects found on plant hosts such as *Hopea ponga, Chromolaena odorata* and *Holarrhena antidysenterica* accounting for over 27% of all fungi recovered in culture from non-mosquito insects and arachnids. Seven fungal isolates were obtained by challenging 2nd instar larvae of *Culex quinquefasciatus* in water samples collected from 17 habitats containing decaying vegetation.

Entomogenous fungi were sourced from 34 localities. Amongst these, Molem was the most visited and yielded a highest number (16.43 %) of isolates followed by

Tambdi Surla (8.74 %), Taleigao (5.94 %) and Kollur (5.94 %) in descending order of density. Of the fungi obtained in culture, only 216 sporulated well. In all, 188 isolates were assignable to 20 genera of fungi. They belonged to Zygomycotina (8), and Deuteromycotina (168). Sporulating fungi belonged to genera *Acremonium* (14 isolates), *Aschersonia* (7), *Aspergillus* (26), *Beltrania* (1), *Chaetomella* (1), *Cladosporium* (9), *Conidiobolus* (1), *Curvularia* (3), *Cylindrocladium* (3), *Fusarium* (17), *Gliocladiopsis* (2), *Gliocladium* (14), *Hirsutella* (5), *Mucor* (6), *Paecilomyces* (25), *Penicillium* (22), *Pestilotiopsis* .(1), *Pleurothecium* .(1), *Syncephalastrum* (1) and *Trichoderma* (29). Amongst the 28 undetermined taxa, 7 were pycnidial (Coelomycetes), 3 thallic and 18 phialidic (Hyphomycetes) fungi. These fungi were described in detail with information on their taxonomy, cultural characters, morphology, specimens and/or cultures examined and distribution. Line drawings and photographic illustrations were provided to most fungi.

The isolates were screened for their mosquito larvicidal potential. They exhibited different degrees of mortality rate. Of the 57 fungi isolated from dead or inactive mosquito developmental stages, 35.08 % showed more than 50% mortality, whereas those obtained from non-mosquito sources, 8.11 % were found active against mosquito larvae. Isolates belonging to genera such as *Acremonium* Link (1), *Aspergillus* Link (3), *Chaetomella* Fuckel (1), *Gliocladium* Corda (8), *Penicillium* Fries (13), and *Trichoderma* Persoon (11) showed promise of utility. From the promising isolates, one each of *Acremonium* (E29), *Gliocladium* (E16), *Penicillium* (E9) and *Trichoderma* (C54) were tested further for mosquito-larvicidal activity by standard bioassay in 250 ml of water.

Special culture-container facilities were established to cultivate the fungi for production of conidia in bulk. Interesting results were obtained. In bioassays conducted

with 1500 ml of water, test fungi caused 0-100% mortality in *Culex quinquefasciatus, Anopheles stephensi* and *Aedes aegypti* based on variation in spore concentration and duration of treatment. Fungi yielded positive results at different concentration of conidia. Cell-free extracts of 20-day old cultures resulted with varying percentage of larval mortality.

Although presumptive, tests were conducted to understand the activity of esterase, gelatinase and chitinase enzymes of all 286 isolates of fungi. While some were positive for esterase, others were positive for either gelatinase or chitinase. For esterase, 90 isolates were with +++ activity, 20 with ++ activity and 27 isolates with + activity. For gelatinase, 96 isolates were given +5 activity, 2 isolates with +4 activity, 4 with +3 activity, 9 isolates with +2 activity and 14 isolates with +1 activity. Of all the isolates tested, only 31 showed positive to chitin utilization activity.

Mortality of 53.8 ± 8.53 % and 66 ± 10.56 % were recorded at 24 and 48 h of treatment among *Anopheles stephensi* 3rd and 4th instar larvae when treated with a dose of 20000 spores/ml of *Penicillium* sp. (E9), on a preliminary field trial. Microscopic examination of dead larvae after treatment showed varied impacts such as choked gut, sluggishness, absence of faecal matter and fungal growth over the bristles, inter-segmental and caudal regions. Presence of several short conidiophores with conidial chains indicated recycling ability of the fungus on exposure to mosquito larvae in water. Voluminous data obtained on experiments conducted were statistically analyzed and presented in the thesis in the form of tables and graphs.

Aschersonia aleyrodis, A. badia, A. indica and *Hirsutella* sp., on homopteran insects, were examined in the field at regular intervals for a duration of over one year to understand the life cycle and distribution of these fungi. The fungi exhibited remarkable diversity in their biology, anamorph-teleomorph association, distribution and 'fungus-

insect-plant' association against time and space. This has been depicted in schematic cycles.

The microfungi recovered in pure culture from insect and arachnid hosts during the study were deposited in Goa University Fungus Culture Collection, a unique facility established to conserve rare, interesting, unique and useful fungi of the region in live form and make them available for utilization. Large number of entomogenous fungi isolated and described in this work and housed in the culture collection will add to our knowledge and wealth.

Use of bio-control agents against mosquitoes has emerged as a thrust area of research in the recent years and is further gaining importance due to large scale and wide spread resistance in vectors of malaria, filariasis and Japanese encephalitis to synthetic insecticides especially in the tropics. Among various biological control agents of mosquitoes that exist in nature, larvivorous fishes and bacilli have already been deployed in large scale vector control programmes. Entomogenous fungi are those 'wait-in-line' tools available for similar operations.

An exhaustive review of literature, details of materials used and methods followed and bibliography are provided in different chapters in the thesis. Results presented in this thesis were based on an effort carried out in two laboratories, the Botany Department of Goa University and ICMR Malaria Research Centre, Goa Division. A few research papers published during the course of this work are appended at the end of the thesis.

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APPENDICES

Appendix-I

Research publications

In reviewed journals

1. Keshava Prasad, T.S. and Bhat, D.J. 2002. *Speiropsis rogergoosensis* sp. nov. from India. *Mycotaxon* **82,** 127-132.

2. Keshava Prasad, T.S. and Bhat, D.J. 2002. A new species of *Phalangispora* from India. *Mycotaxon* **83,** 403-407.

3. Keshava Prasad, T.S. and Bhat, D.J. 2002. *Stellomyces kendrickii,* a new hyphomycete from India. *Mycotaxon* **84,** 61-63.

4. Keshava Prasad, T.S., Asha, L.G. and Bhat, D.J. 2003. A new species of *Memnoniella* from India. *Mycotaxon* **85,** 341-344.

In edited books

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MYCOTAXON

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SPEIROPSIS ROGERGOOSENSIS SP. NOV. FROM INDIA

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ABSTRACT

A new dematiaceous hyphomycete, *Speiropsis rogergoosensis*, producing unicellular conidia connected by narrow isthmi in profusely branched chains on polyblastic discrete conidiogenous cells, recovered from decaying leaves of *Artocarpus hirsutus* Lam. (Moraceae) is described from the forests of Western Ghats in southern India.

Key words: Biodiversity, Taxonomy, Hyphomycetes, Western Ghats

INTRODUCTION

During studies on the taxonomy and diversity of microfungi of the forests of Western Ghats in southern India, an interesting dematiaceous hyphomycete producing hyaline, smooth, unicellular conidia connected by narrow isthmi in profusely branched chains on polyblastic discrete conidiogenous cells and long, thick-walled, dark brown, septate, conidiophores arising singly or in fascicles was isolated from fallen and decaying leaves of *Artocarpus hirsutus* Lam. (Moraceae) from the forests of Western Ghats in Karnataka State, India. The fungus is described here as a new species of the genus *Speiropsis* Tubaki.

Fallen, dead leaves of *Artocarpus hirsutus*, when incubated in sterile moist chamber in the laboratory, produced fascicles of conidiophores with white masses of conidia appearing on the leaf surface after two weeks.

TAXONOMIC PART

Speiropsis rogergoosensis Kesh. Prasad et Bhat **sp. nov.** (Fig.1 & 2)
[Etym.: In honour of Prof. Roger D. Goos, a distinguished mycologist, who contributed immensely to the study of biodiversity of hyphomycetous fungi]

Coloniae effusae, olivaceae vel atrobrunneae. *Mycelium* partim immersum, ex hyphis septatis, ramosis, hyalinis vel pallide brunneis, 2.5-3.0 µm lat. compositum. *Conidiophora* mononematosa, erecta, recta vel flexuosa, singularis vel fasciculata ex 2-6, enata fuscus stroma, 2-3-septata, crassitunicata, atro-brunnea, ad apicem pallidora, laevia, 40-65 µm longa, 3-4.5 µm lat. et proferens ad ramosa in supra, hyalina, 15-30 µm longa, 2-4.5 µm lat. *Cellulae conidiogenae* polyblasticae, discretae, terminalis, hyalina, denticulatae ad apicem, 6-9 µm longae, supra 2.5-4 µm lat., infra 2-3 µm lat.

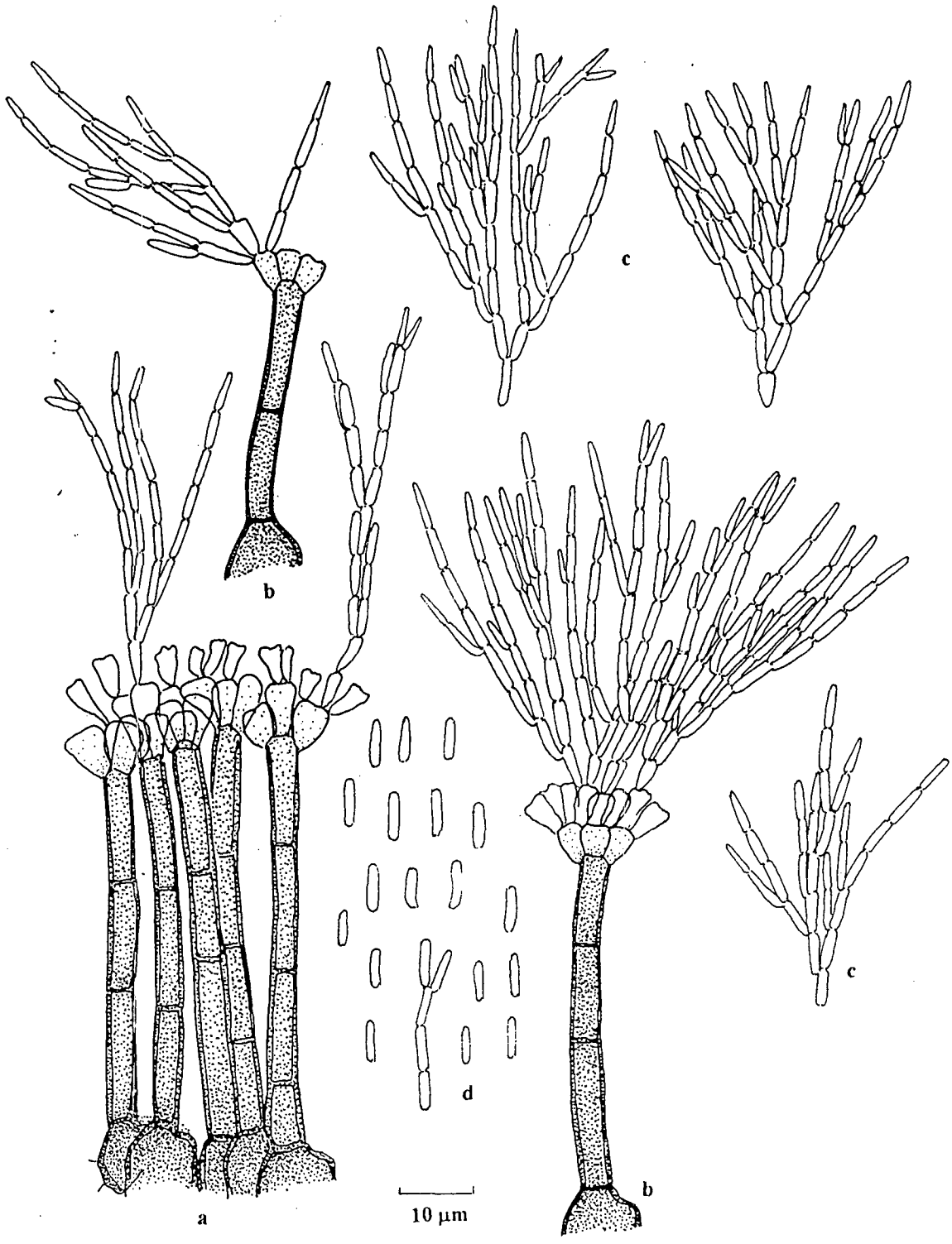

Fig. 1. *Speiropsis rogergoosensis:* **a**. Fasciculate conidiomata, **b.** Single conidiophore with fertile lateral branches, **c.** Conidia in branched chains, **d.** Conidia.

Conidiae catenata, hyalina, cylindricalis, interdum curvata, utrinque truncata, laevia, aseptata, 4-6 x 1-2 µm, plerumque in multiramosa, infra uniseriata et supra bi- ad heptaseriata, 40-65 µm longa; cellulae apicale et basalem, conicale vel obclavatae, 3-7 x 1-1.5µm.

HOLOTYPUS, In foliis putrescentibus *Artocarpus hirsutus*, Kumara Parvatha, Subrahmanya, Karnataka, India, 11 Sept. 2001, Keshava Prasad, Herb. No. IMI 387092.

Terrestrial litter hyphomycete. *Colonies* effuse, olivaceous brown, velvety. *Mycelium* partly immersed, composed of septate, and branched, colourless to pale brown hyphae 2.5-3 µm wide. *Conidiophores* mononematous, erect, straight or flexuous, arising singly or in fascicles of 2-6 from a dark brown stroma, 2-3 septate, thick-walled, smooth, dark brown, paler towards the tip, 40-65 µm long, 3-4.5 µm wide, with an apical cluster of 3-5 conidiogenous cells. *Conidiogenous cells* polyblastic, discrete, terminal, hyaline, wider above, smooth, thin-walled, with denticulate projections at the truncate apex, 6-9 µm long, 2.5-4 µm wide above, 2-3 µm wide below, sometimes in two tiers. *Conidia* catenate, hyaline, cylindrical, sometimes curved, truncate at both ends, smooth, aseptate, 4-6 x 1-2 µm, connected by narrow isthmi, in mass whitish, developing in branched chains of 40-65 µm long, uniseriate below, bi- to hepta-seriate above, with branches arising from basal up to penultimate terminal cell of the axis; apical and basal conidia conical to obclavate, 3-7 x 1-1.5 µm.

So far 8 species have been described in the genus *Speiropsis* Tubaki (1958), typified by *S. pedatospora* Tubaki. The genus is characterised by simple conidia connected by narrow isthmi developing in unbranched or branched chains on mononematous conidiophores with discrete, polyblastic conidiogenous cells. In *Speiropsis aquatica* Aramb., Cabello & Megascini (Arambari & al., 1987), *Speiropsis belauensis* Matsush. (Matsushima, 1985), *S. ixorae* Subram. & Sudha (Subramarian & Sudha,1986), *S. scopiformis* Kuthub. & Nawawi (Kuthubutheen & Nawawi, 1987) and *S. simplex* Matsush. (Matsushima, 1971) conidia are produced in unbranched chains whereas in *S. hyalospora* Subram. & Lodha (Subramarian & Lodha,1964), *S. irregularis* R.H. Petersen (1963) and *S. pedatospora* conidia are in branched chains. The new species, *Speiropsis rogergoosensis*, has catenate conidia developed in divergent branched chains.

In *S. irregularis*, the conidia are subspherical, pale to mid-brown, 4.4-7.0 x 5.5-12.5 µm and the branching is irregular and divergent. In *S. pedatospora*, the conidia are cylindrical to cuneiform, pale to mid-brown, 10-14 x 4-7 µm and branching is more or less regular and divergent. In *S. hyalospora*, conidia are hyaline, 8-10 x 3.5-5 µm, developing in triseriate chains with first and second branches originating from distal end of basal and epibasal cells of the main axis. In *S. rogergoosensis*, the cylindrical hyaline conidia are straight or curved and developed in profusely and dichotomously branched chains. The branching is visible even in the terminal cell of the chain. The conidia are 4-6 x 1-2 µm, the smallest of all so far known species in the genus.

The key to the species of *Speiropsis* proposed by Kuthubutheen & Nawawi (1987) is updated below incorporating the later described taxa.

1. Conidia in branched chains	2
1. Conidia in unbranched chains	5

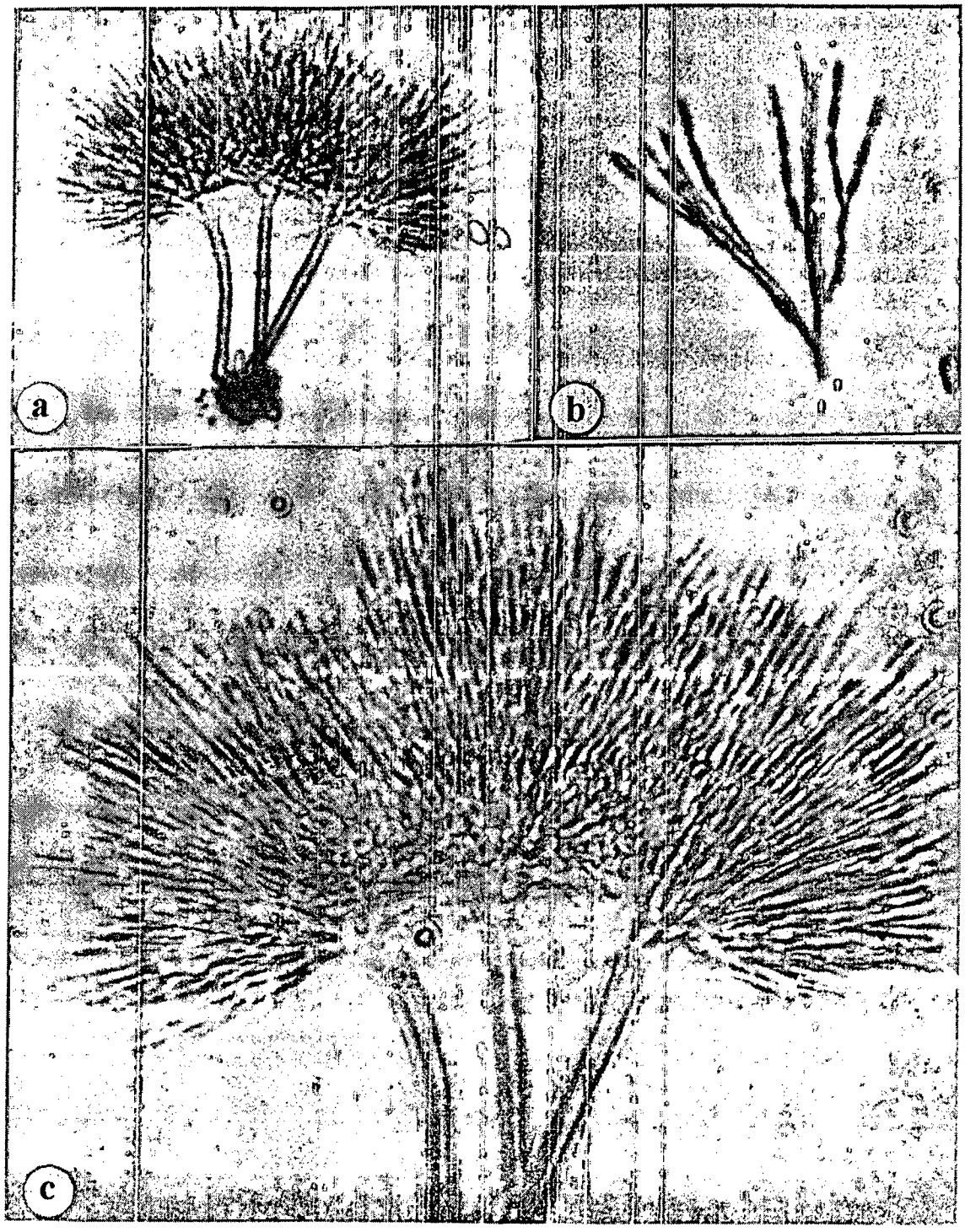

Fig. 2. *Speiropsis rogergoosensis:* **a.** Fasciculate conidiomata, **b.** Conidia in branched chains, **c.** Fertile portions of conidiophores.

2. Branching regular 3
2. Branching irregular, 3-7divergent branches; conidia subspherical, 4.4-7.0 x 5.5- 2.5 µm *S. irregularis*
3. Conidia pale to mid brown, 10-14 x 4-7 µm *S. pedatospora*
3. Conidia hyaline 4
4. Conidial chains tri-seriate, conidia 8-10 x 3.5-5 µm *S. hyalospora*
4. Conidial chains with many divergent branches; conidia narrow cylindrical, 4-6 x 1-2 µm *S. rogergoosensis*
5. Conidiophores branched, mostly aggregated in fascicles or appearing synnematous to sporodochial 6
5. Conidiophores unbranched and solitary 7
6. Conidia 7-9 cells, conidial chains 80-110 x 4-5 µm *S. simplex*
6. Conidia 5-7 cells, conidial chains 40-60 x 4-5 µm *S. belauensis*
7. Conidiophores 75-110 x 4-5 µm; conidial chains 5-7 cells, 40-65 x 2-3µm *S. scopiformis*
7. Conidiophores 45-52.5 x 5-5.5µm; conidial chains 5-6 cells, 33.5-46.5 x 2.8-3.3µm *S. ixorae*
7. Conidiophores 75-120 x 3-5µm; conidial chains 6-7 cells, 40-50 x 2.6-3µm *S. aquatica*

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MYCOTAXON

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A NEW SPECIES OF PHALANGISPORA FROM INDIA

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ABSTRACT A new dematiaceous setose, sporodochial hyphomycete, *Phalangispora bharathensis*, producing unicellular conidia connected by narrow isthmi in branched chains on polyblastic discrete conidiogenous cells, isolated from decaying leaves of *Holigarna arnotiana* (Wt. & Arn.) Hook.f. (F. Anacardiaceae) is described from the forests of Western Ghats in southern India.
KEY WORDS: Hyphomycetes, taxonomy

INTRODUCTION

During studies on the taxonomy and diversity of microfungi of the forests of Western Ghats in southern India, an interesting setose, sporodochial hyphomycete producing hyaline, smooth, unicellular conidia connected by narrow isthmi in branched chains on polyblastic discrete conidiogenous cells and short, thin-walled, hyaline, septate, conidiophores was isolated from fallen and decaying leaves of *Holigarna arnotiana* (Wt. & Arn.) Hook.f. (F. Anacardiaceae) collected from Cotigao Wildlife Sanctuary in Goa State, India and incubated in sterile moist chamber in the laboratory for about two weeks. The fungus is described here as a new species of the genus *Phalangispora* Nawawi & Webster (1982).

TAXONOMIC PART

Phalangispora bharathensis Keshava Prasad et Bhat **sp. nov.** (Fig. 1, a-d)
[Etym: Bharath = India]

Coloniae effusae, olivaceae ad atrobrunneae. *Mycelium* partim superficiale, partim immersum, ex hyphis septatis, ramosis, hyalinis vel pallide brunneis 2.5-3.5 µm lat. compositum. *Conidiomata* sporodochia, solitaria, pulvinata, 8-16 setas basim ferentia. *Setae* subulatae, apice acutae, septatae, crassitunicatae, atrobrunneae, laeves, ex strato conidiophorum protrudentes, 300-400 x 7-10µm. *Conidiophora* mononematosa, erecta, dwarfa, septata, ramosa in supra, hyalina, 15-30µm longa, 2-4.5 µm lat. *Cellulae conidiogenae* polyblasticae, discretae, terminalis ad ramosis fertilis; denticulatae ad apicem spherical. *Conidia* hyalina, laevia, aseptata, catenata, plerumque

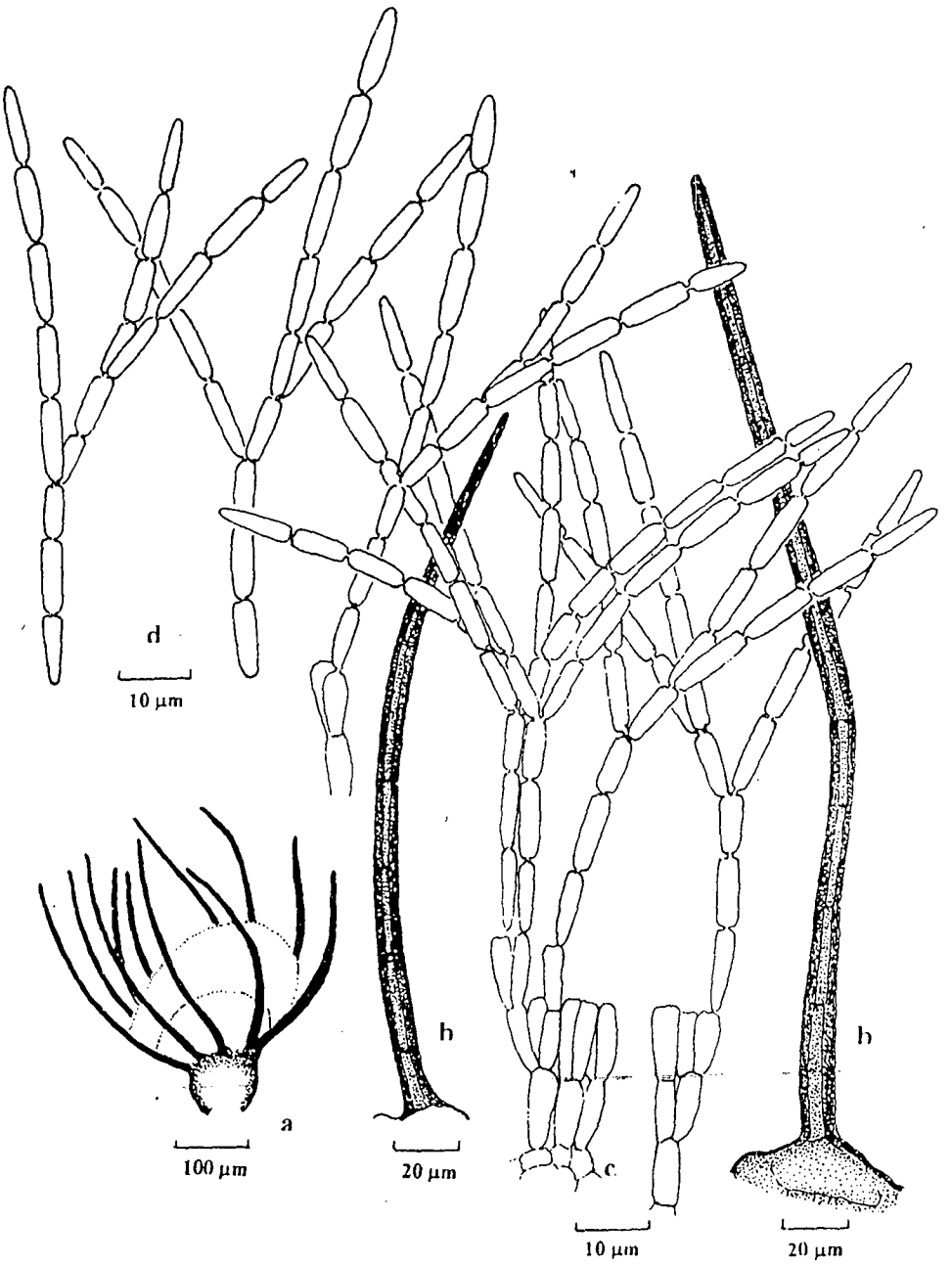

Fig. 1. *Phalangispora bharathensis:* **a.** Entire sporodochium, **b.** Setae, **c.** Conidiogenous cells with attached conidial chains, **d.** Conidia.

in 2-3-ramosa, infra uniseriata vel supra bi- ad triseriata, 75-85 µm longa, 2.5-4 µm lat.; cellulae apicale ad basale conicale vel obclavatae, 7-9 x 2.5-3.5 µm; cellulae

intermediatae cylindricale, terminale truncatae, 8-10 x 2.5-4 µm; in massis hyalina vel atrobrunneae.

HOLOTYPUS, In foliis putrescentibus *Holigarna arnotiana* (Wt. & Arn.) Hook.f., Cotigao Wildlife Sanctuary, Goa, India, 11 March 1999, Keshava Prasad, Herb. No. IMI 387091.

Terrestrial litter hyphomycete. *Colonies* effuse, olivaceous brown to dark brown. *Mycelium* partly superficial, partly immersed, composed of septate, branched, colourless to pale brown hyphae 2.5-3.5 µm wide. *Conidiomata* sporodochial, solitary, pulvinate, slightly elevated, with 8-16 setae arising from the margin of the base. *Setae* subulate, acute at the apex, septate, thick-walled, dark brown, smooth, protruding beyond the level of conidiophores and conidial mass, 300-400 x 7-10µm. *Conidiophores* mononematous, erect, short, arising in groups, septate, 1-2 times branched, thin-walled, colourless, smooth, 15-30µm long, 2-4.5 µm wide. *Conidiogenous cells* polyblastic, discrete, terminal, hyaline, smooth, with denticulate scars at the rounded apex. *Conidia* hyaline, smooth, aseptate, in 2-3-branched chains of 75-85 µm long, 2.5-4 µm wide, connected by narrow isthmi, uniseriate below, bi- to triseriate above, with branches arising from the third and forth cells of the main axis, of two types: apical and basal cells conical to obclavate, 7-9 x 2.5-3.5 µm; intermediate cells cylindrical with truncate ends, 8-10 x 2.5-4 µm; in mass initially whitish, later becoming pale brown.

Dematiaceous genera of Hyphomycetes producing unicellular conidia connected by narrow isthmi can be grouped into two catagories based on the branching of conidial chains. The genus *Wiesneriomyces* Koorders (1907), typified by *W. laurinus* (Tassi) P.M. Kirk (1984), producing unicellular conidia in unbranched chains in setose sporodochia has been known on terrestrial litter from many parts of the world (Subramanian, 1956; Manotis & Strain, 1968; Ellis, 1971, 1976; Matsushima, 1971, 1975; Kirk, 1983; Shaw & Sutton, 1985; Kuthubutheen & Nawawi, 1988). The genus *Isthmolongispora* Matsushima (1971, 1975), typified by *I. intermedia* Matsushima, is mononematous with conidia developing in unbranched chains. The genus *Speiropsis* Tubaki (1958), with *S. pedatospora* Tubaki as type species, is mononematous but has conidia in branched chains. The conidia of these litter inhabiting fungi are often recovered from freshwater stream habitat.

The genus *Phalangispora* Nawawi & Webster (1982), typified by *P. constricta* Nawawi & Webster, produces pale brown conidia in branched chains from setose sporodochia. The fungus has been reported from freshwater streams. The second species in the genus, *P. nawawi* Kuthubutheen (1987), differs from the type by its smaller-sized conidia and conidial chains and presence of fewer cells in the main axis of the conidial chain. In *P. constricta*, the conidia are 11-20 x 2.5-4 µm whereas in *P. nawawi* these are 10-12 x 2 µm. The conidial chains are 120-140 x 3-4 µm in *P. constricta* and 65-90 x 2 µm in *P. nawawi*. The branches in *P. constricta* and *P. nawawi* arise generally from the second and third cells of the main axis of conidial chain.

Unlike *P. constricta* and *P. nawawi*, in *P. bharathensis*, the branches arise from the third and forth cells of the main axis of the conidial chain. Although the overall dimension of the conidial chains is similar to that of *P. nawawi*, in *P. bharathensis* the intermediate conidial cells are 8-10 x 2.5-4 µm. Further, the number of cells in the main axis of the conidial chain in *P. bharathensis* is 8-11 (mostly 9) whereas in *P. nawawi*, it is always 6-

8. Individual conidia are wider in *P. bharathensis* (2.5-4 µm) than those of *P. nawawi* (up to 2 µm).

ACKNOWLEDGEMENT

This work is supported by a research grant to Dr. D. J. Bhat from the Ministry of Environment & Forests, Government of India, New Delhi. We are indebted to Prof. Roger D. Goos, University of Rhode Islands, for kindly reviewing the manuscript.

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MYCOTAXON

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STELLOMYCES KENDRICKII, A NEW HYPHOMYCETE FROM INDIA

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ABSTRACT

A new species of hyphomycete, *Stellomyces kendrickii*, is described. It produces single, cuneiform or triangular to kite-shaped, unicellular, smooth, colourless, blastic conidia at the tip of each of several very long denticles which radiate from small apical (subsequently intercalary) vesicles on a sympodially extending conidiophore. It was isolated from decaying twigs of *Hopea ponga* (Dennst.) Mabb. (F. Dipterocarpaceae) in the forests of Western Ghats in southern India.

Key words: Biodiversity, tropical fungi, taxonomy.

INTRODUCTION

During studies on the taxonomy and diversity of microfungi, an apparently undescribed hyphomycete was isolated from fallen and decaying twigs of *Hopea ponga* (Dennst.) Mabb. (F. Dipterocarpaceae) from forests of Western Ghats in Goa State, India. The fungus is described as a new species of the genus *Stellomyces* Morgan-Jones, Sinclair & Eicker.

Decaying twigs of *Hopea ponga* gathered from the forests were thoroughly washed in deionized tap water and incubated in a sterile moist chamber in the laboratory. Conidiophores with haloes of conidia appeared on the surface of the twigs after two weeks.

TAXONOMIC PART

Stellomyces kendrickii Keshavaprasad et Bhat anam.-sp. nov. **(Fig.1)**
[Etym: Specific epithet: Named in honour of Dr. Bryce Kendrick, a distinguished mycologist from Canada]

Ad fungos conidiales pertinens. *Coloniae* effusae, subhyalinae vel olivaceae, velutinae. *Mycelium* partim immersum, partim superficiale, ex hyphis septatis, ramosis, incoloris vel pallide olivaceis, 2-2.5 µm lat., compositum. *Conidiophora* erecta, recta vel flexuosa, non-ramosa, solitaria, angustata versus apicem, septata, tenuitunicata, laevia, hyalina, 20-100 x 1.5-2.5 µm, extendentia sympodialiter et ferentia fasciculos conidiorum apicales et intercalares. *Cellulae conidiogenae* polyblasticae, integratae,

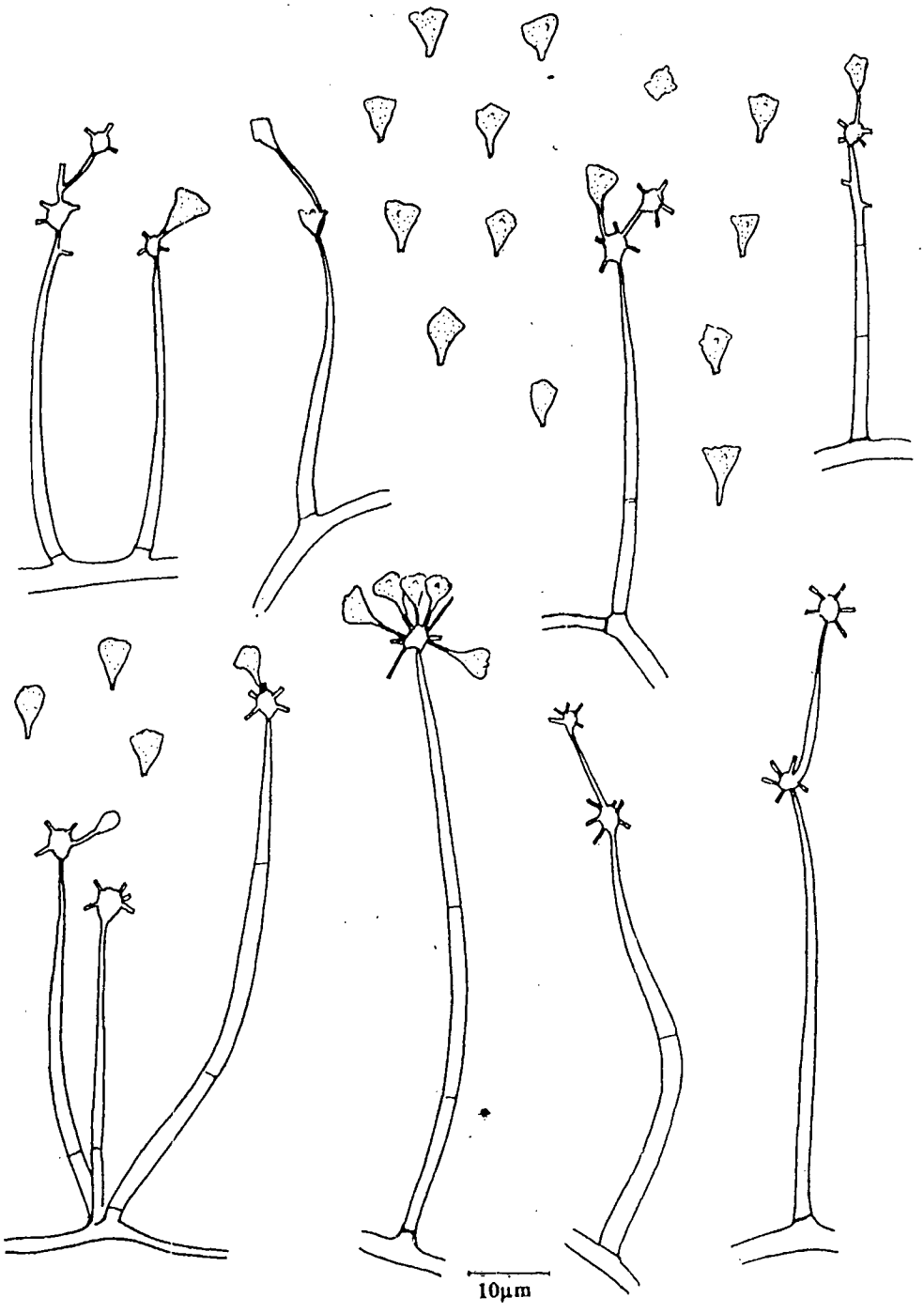

Fig. 1. *Stellomyces kendrickii* sp.nov.

terminalis vel intercalaris, hyalina vel olivacea, laevia, apicem instar stellae versus denticulis numerosis, 2-4 µm long. praeditae. *Conidia* solitaria, obovoidea vel obconica, cuneiforma vel triangularia, non-septata, crassitunicata, levia, hyalina vel olivacea, 4-5 x 3-4 µm, evolutum verticillatim.

HOLOTYPUS, In virga putrescentibus *Hopea ponga*, Sri Bhagwan Mahaveer Wildlife Sanctuary, Mollem, Goa , India, 17.10.2001, leg. Keshavaprasad, Herb. No. IMI 388263.

Terrestrial litter hyphomycete. *Colonies* spreading, almost colourless to olivaceous green, velvety. *Mycelium* partly immersed, partly superficial, composed of septate, branched, colourless hyphae 2-2.5 µm wide. *Conidiophores* arising from prostrate vegetative hyphae, single, erect, straight or flexuous, tapering above, septate, thin-walled, smooth, colourless to olivaceous, 20-100 x 1.5-2.5 µm, forming first crop of conidia on denticles arising from a swollen apical vesicle, with subsequent groups of conidia developing on new vesicles at higher levels, originally apical conidiogenous vesicles later becoming intercalary. *Conidiogenous cells* polyblastic, integrated, terminal or intercalary, colourless to pale olivaceous green, thin-walled, 3-5 µm diam., with denticulate fertile projections 2-4 µm long radiating from the swollen distal vesicle. *Conidia* solitary, obovoid to obconical, cuneiform or triangular, or kite-shaped, non-septate (amerosporous), thick-walled, smooth, colourless to olive green, with small projections at the corners, 4-5 x 3-4 µm, tapering below to form a narrow stalk, developing in whorls or halos.

The monotypic genus *Stellomyces* Morgan-Jones *et al.* (1987), with *S. suidafrikanus* Morgan-Jones, Sinclair & Eicker as type, was established for a hyphomycetous fungus producing gently curved, navicular, non-septate, colourless conidia on radially arranged long denticles arising from apically swollen conidiogenous cells and sympodially proliferating mononematous conidiophores. In its general morphology, *S. kendrickii* is clearly congeneric with *S. suidafrikanus*, but differs from the type species in the shape and size of its conidia, which are cuneiform and 4-5 x 3-4 µm, (the conidia in *S. suidafrikanus* are larger, 8-10 x 3-5 µm), and in its globose fertile vesicles and shorter denticles: the conidiiferous denticles in *S. kendrickii* are 2-4 µm long, while those of *S. suidafrikanus* are 4-7 µm long.

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This work is supported by a research grant to Dr. D. J. Bhat from the Ministry of Environment & Forests, Government of India, New Delhi. We are indebted to Prof. Bryce Kendrick, Sidney-by-the-Sea, BC, Canada, for kindly reviewing the manuscript.

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MYCOTAXON

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A NEW SPECIES OF MEMNONIELLA FROM INDIA

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ABSTRACT

A new species of Hyphomycetes, *Memnoniella indica*, isolated from decaying leaves of an unidentified dicot plant from the forests of Andaman Islands, India, is described and illustrated. The fungus produces catanulate, globose, verruculose, dark brown conidia on monophialidic, discrete, hyaline conidiogenous cells and short, partly hyaline, determinate, branched, septate, mononematous conidiophores. It is distinguished from the known species of *Memnoniella* on the basis of morphology and dimension of conidiophores and conidia. A key to the species of *Memnoniella* is given.

Key words: tropical fungi, hyphomycetes, Andaman Islands, biodiversity

INTRODUCTION

During studies on the taxonomy and diversity of microfungi of the forests of India, an interesting hyphomycete producing dark brown, verruculose, aseptate, spherical conidia with a hyaline minute basal papilla, developing on monophialidic, discrete, hyaline conidiogenous cells and short, partly hyaline, thick-walled, septate, branched, mononematous conidiophores was isolated in culture from fallen and decaying leaves of an unidentified dictot plant from the forests of Andaman Islands, India. The fungus is described here as a new species of the genus *Memnoniella* Hohnel.

Litter samples collected from the forests of Andaman Islands were air-lifted to Goa and immediately incubated in sterile moist chambers in the laboratory for about two weeks. Pure culture of the fungus was established by single spore isolation on to malt extract agar medium. The sporulating colonies of the fungus were mounted in lactophenol, examined under an Olympus bright field research microscope, and illustrated using a drawing tube fitted to the microscope unit.

TAXONOMIC PART

Memnoniella indica **sp. nov.** (Fig.1)

Ad fungos conidiales pertinens. *Coloniae* effusae, cum rotundus marginis, subhyalina ad brunneae, usque 3 cm in MEA in 7 dies. *Mycelium* partim superficiale, partim immersum, ex hyphis septatis, ramosis, hyalinis vel pallide brunneis 2.5-3.5 µm lat. compositum. *Conidiophora* mononematosa, erecta, septata, ramosa, crassitunicata, infra hyalina et levia ad supra pallide fusca et verruculosa, 35-75 µm longa, 5-5 µm lat. ad basim, 3-4 µm ad ramosis et usque 1 µm ad extremum distalis; *Cellulae conidiogenae*

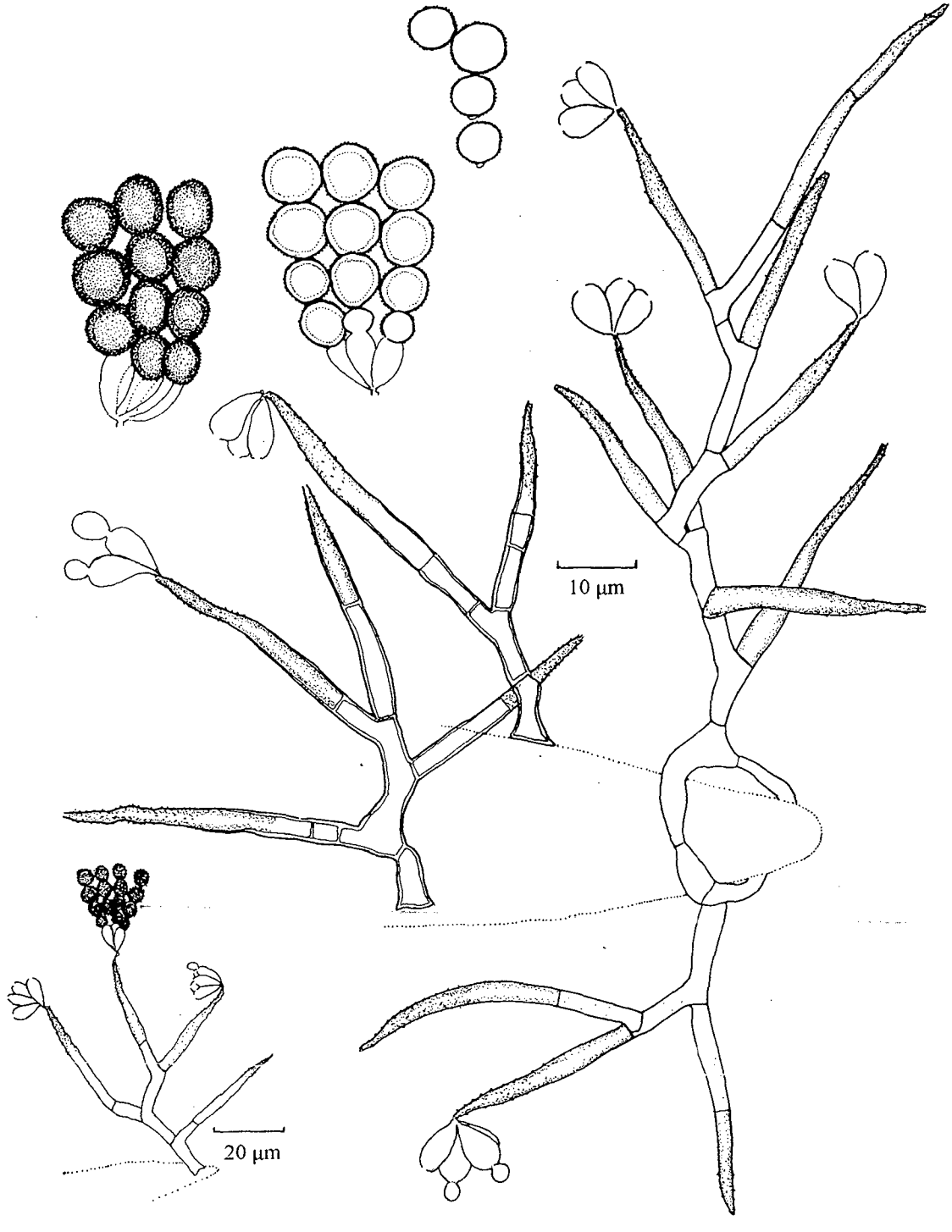

Fig. 1. *Memnoniella indica* **sp. nov.:** a. Branched conidiphores with conidia, b. Conidiogenous cells with attached conidial chains, c. Conidia.

monophialidicae, discretae, terminalis, ellipsoidea ad pyriforma, levis, hyalina ad atrobrunnea, 5-10 x 5-7 µm. *Conidia* catenata, globosa, aseptata, verrucosa, crassitunicata, atrobrunnea to nigra, 5-6 µm in diam.

HOLOTYPUS, In foliis putrescentibus dicota, Andaman Islands, India, 20th January 2001, leg. Rajiv Kumar, Herb. No. IMI 389316.

Terrestrial litter hyphomycete. *Colonies* effuse, flat, with a circular margin, subhyaline to brown, attaining a diam. of 3 cm on MEA after 7 days. *Mycelium* partly superficial, partly immersed, composed of septate, branched, colourless to pale brown hyphae 2.5-3.5 µm wide. *Conidiophores* mononematous, erect, septate, branched, thick-walled, colourless and smooth below and slightly pigmented and minutely verrucose in the above half, with the tip of the stipe tapering into a pointed and fragile end on which groups of 3-7 phialides arise, 35-75 µm long, 3.5-5 µm wide at the base and up to1 µm at the pointed end. *Conidiogenous cells* monophialidic, discrete, terminal, 3-7 on each conidiophore tip, ellipsoidal to pyriform, without collarette, smooth, hyaline, 5-10 x 5-7 µm. *Conidia* catenate, spherical, with a minute basal papilla, aseptate, verrucose, thick walled, dark brown to black, 5-6 µm in diam.

The genus *Memnoniella* Hohnel, typified by *M. echinata* (Riv.) Galloway, is characterized by production of catenate, simple, spherical to sub-spherical, grey to black conidia on discrete phialides, usually with a small opening and without a collarette, in groups of up to 10 at the apices of mononematous, unbranched and occasionally forked conidiophores which are sometimes inflated at the apex, grey to brown, smooth, minutely verruculose and often covered in part with dark granules (Ellis, 1971).

In addition to the type, 5 species of *Memnoniella* have been described. *M. stilboidea* (Munjal & Kapoor) M.B. Ellis and *M. leprosa* Castaneda possess scattered synnema whereas all the other species have mononematous conidiophores. While the former produces verruculose dark brown to blackish brown spherical conidia of 4-5.5 µm, the latter gives rise to 7-12 µm globose conidia with an upper dark brown thick-walled portion and lower pale to olivaceous brown comparatively thin-walled region (Ellis, 1976; Castaneda, 1986). Ellis (1971) maintained *M. echinata* and *M. aterrima* Hohnel & Mazzuchetti as synonyms. *M. zingiberis* Vasant Rao with verrucose dark conidia of 4-7 µm differs from *M. echinata* in having very short conidiophores and more phialides, besides being a pathogen on rhizomes of *Zingiber officinale* Rose (Rao, 1963). *M. subsimplex* (Cooke) Deighton produces spherical to sub-spherical, dark brown, verrucose (with widely spaced large warts), 6-9 µm diam. conidia. *M. levispora* Subram. produces smooth and 4-6 µm diam. conidia. *M. indica* produces catenate conidia on thin-walled phialides which often hang or swivel on thick-walled, branched condiophores with pointed tips.

A taxonomic key is proposed to delineate species of the genus ***Memnoniella.***

1. Conidiomata synnematous.....2
1. Conidiomata mononematous.....3

2. Conidia verruculose, 4-5.5 µm---------- ***M. stilboidea***
2. Conidia verrucose, leprose, 7-12 µm----------***M. leprosa***

3. Conidia 6-9 µm in diam.---------- ***M. subsimplex***
3. Conidia less than 6 µm in diam.....4

4. Condia smooth, 4-6 µm---------- ***M. levispora***
4. Condia verrucose.....5

5. Conidiophore tip tapered to a narrow point----------***M. indica***
5. Condiophore tip inflated.....6
6. Conidiophore 50-100 µm, Phialides in groups of 4-8,
7-9 x 3-5 µm ----------***M. echinata* (=*M. aterrima*)**
6. Conidiophore 37-50 µm, Phialides in groups of 8-14,
6.3-14.7 x 4.2-5.2 µm ----------***M. zingiberis***

ACKNOWLEDGEMENT

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Vermiculariopsiella Bender : Present Status of Species Diversity

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Genus *Vermiculariopsiella* Bender is briefly reviewed in this paper. Three new species, *V. elegans*, *V. parva* and *V. indica*, producing solitary, cylindrical, straight or slightly curved, smooth, aseptate, thick-walled, colourless, phialoconidia with rounded ends at the tip of conidiogenous cells arranged in palisade manner in setose sporodochium, recovered as litter and endophytic fungi from various plant species in India, are added. A taxonomic key is provided to the species in the genus.

Key Words: Biodiversity, tropical fungi, taxonomy.

Hohnel (1918) transferred *Excipula immersa* Desm. into *Vermiculariopsis* v. Hohnel, as *V. immersa* (Desm.) Hohnel. The new genus being a later homonym of *Vermiculariopsis* Torrend, Bender (1932) proposed *Vermiculariopsiella* Bender for *Excipula immersa* Desm. as *Vermiculariopsiella immersa* (Desm.)Bender. The original diagnosis of the genus included species with one-celled conidia. Considering the greater pertinence of this feature at the specific rather than generic level, Pasqualitti and Zucconi (1992) suggested inclusion of species also with septate conidia in the genus. This provided room for retension of *V. falcata* Nawawi *et al.* (1990) characterized by the presence of 3-septate conidia in the genus.

While adding two more new species, *V. falcata* Nawawi, Kuthubutheen and Sutton and *V. parvula* Nawawi Kuthubutheen and Sutton, Nawawi *et al.* (1990) revised the genus *Vermiculariopsiella* with three new combinations and provided a key for then recognised species. *V. immersa* var. *ramosa* Sutton was elevated to the species level as *V. ramosa* (Sutton) Nawawi Kuthubutheen and Sutton, *Oramasia hirsuta* Urries var. *cubensis* Castaneda and *Gyrothrix cornuta* V. Rao & de Hoog were recognized as *V. cubensis* (Castaneda) Nawawi Kuthubutheen and Sutton and *V. cornuta* (V. Rao & de Hoog) Nawawi Kuthubutheen and Sutton, respectively. Pasqualitti and Zucconi (1992) added *V. arcicula* Pasqualitti and Zucconi into the genus.

Till date, *Vermiculariopsiella* accommodates 7 species. Of these, 3 species,

viz., *V. cornuta, V. cubensis* and *V. ramosa* possess branched setae. The other 4 species produce unbranched setae and narrow conidia with less than 4 µm width. Of these, *V. falcata* produces falcate, 3-sepate hyaline conidia of 36-47 x 1.5-2 µm. *V. immersa* and *V. parvula* produce aseptate hyaline conidia of 13-23 x 1.5-2.5 µm and 8-13 x 2-2.5 µm, respectively with rounded base and pointed, slightly curved apex. The apex of conidiogenous cell is recurved in the former while it is narrow, long and straight in the latter. *V. arcicula* produces aseptate hyaline fusiform conidia of 15-19.5 x 2.5-4 µm.

During studies on the taxonomy and diversity of microfungi, three new hyphomycetous fungi belonging to *Vermiculariopsiella* were recovered as endophytes from *Saraca asoca* (Roxb.) De Wilde from forests of Western Ghats in Goa State and *Pimenta dioica* (L.) Merr. from a mixed plantation in north Malabar region of Kerala State, India. Two of these were also isolated from leaf litter of the respective plants. The fungi are described below.

Fresh leaves of *Saraca asoca* and *Pimenta dioica* were thoroughly washed and subjected to a 'three-step-isolation' process of Fisher and Petrini (1986) for isolation of endophytic fungi. Decaying leaves of the plants were thoroughly washed in deionized tap water and subjected to (i) 'moist chamber incubation' (Hawksworth, 1974) and (ii) 'particle plating technique' (Bills and Polishook, 1994) for recovery of litter fungi. Conidiophores with a crown of conidia appeared on the surface of the twigs from 4th day and on culture media after two weeks of incubation.

Taxonomic Part

Vermiculariopsiella elegans **sp.nov. (Fig.1)**

Coloniae irregularise, rhizoidacea ad marginis, nigra ad centrum et hyalinae ad ambitum, cum fructificatum mucosum, inverto incouloris vel pallide brunneus, 5.4 cm in diam. in 7 dies. *Mycelium* partim superficiale, ex hyphis laevis, tenuitunicatus, septatis, ramosis, pallide brunneus, 3.5 µm lat., compositum. *Sporodochia* disseminata, dissita, punctiformia, circumscripta, irregulariter circularia, nonnumquam coalescentia, conidis agglutinatis cremea, 80-130 µm. *Setae* numerosae, laeves, 1-3-septata, ereta, curvata vel modice flexuosa, simplex, 103-165 µm longa, 6-7 µm lat. ad basim, 5-5.5 µm ad supra et 3.5 ìm ad apicem, prope basim crassae, crassitunicatae, brunneae, gradatim tenuiores tunicatae et pallidiores ad angustiorem et leviter rotundatum apicem. *Conidiophora* laevia, septata, ramosa, atrobrunnea ad nigra, crassitunicata, 8-12 x 3-5 µm. *Cellulae conidiogenae* monophialidicae, intergrata vel discretae, 15-30 µm long, 4-5 µm lat. ad basim, 4.8-6.2 µm lat. ad supra et 1.3 µm lat. ad apicem, experse collarettis. *Conidia* solitaria, cylindricale, aseptata, levia, hyalina 20-27 x 6-8 µm.

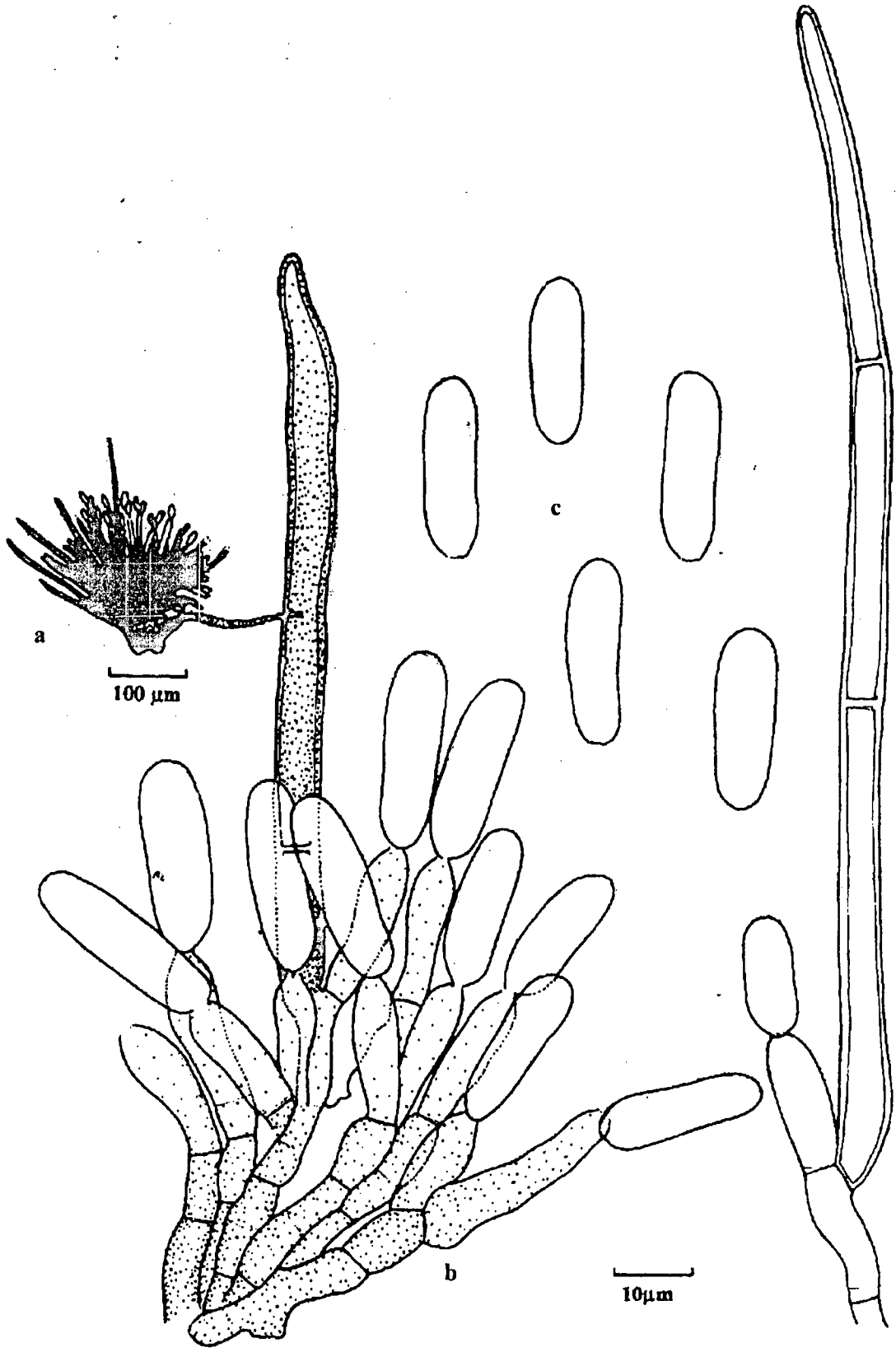

Fig.1. *V. elegans* sp.nov: a. Habit, b. A sporodochium with setae, c. Conidia.

HOLOTYPUS On fallen dead and decaying leaves of *Saraca asoca*, Bondla Wildlife Sanctuary, Goa, India, leg. Maria D'Souza, 25-10-1999, Herb. GUBH No.1496.

Terrestrial, conidial fungus, Hyphomycete. *Colonies* irregular, with rhizoidal margin, blackish in the centre and colourless towards the periphery, with distinct creamish fructification, fast growing, attaining a diam. of 5.4 cm in 7 days; reverse of the colony brown to white. *Mycelium* partly superficial, composed of smooth, septate, thin-walled, branched, pale brown, 3.5 µm wide hyphae. *Conidiomata* sporodochial, scattered, punctiform, round, sometimes coalescing to form irregular patches of pale cream mass of conidia, setose, stromatic at the base, 80-130 µm. *Setae* many, smooth, 1-3-septate, unbranched, dark brown at the base, paler towards the apex, 100-165 µm long, 6-7 µm wide at the base, 5-5.5 µm wide in the middle and 3.5 ìm wide at the apex, arising from basal thick-walled, rounded stromal cells. *Conidiophores* smooth, septate, branched, dark brown to black, thick-walled, 8-12 x 3-5 µm. *Conidiogenous cells* monophialidic, integrated or discrete, 15-30 µm long, 4-5 µm wide at the base, 4.8-6.2 µm wide in the middle and 1.5 µm wide at the tip, without a conspicuous collarette. *Conidia* solitary, cylindrical rounded at both ends, slightly narrower and truncate at the base, smooth, aseptate, hyaline, 20-27 x 6-8 µm.

Additional specimens examined: (i) On dead and decaying leaves of *Careya arborea*, Mollem Wildlife Sanctuary, Goa, India, leg. Maria D'Souza, 11-03-1999, Herb. GUBH 324. (ii) On fallen dead and decaying leaves of *Mangifera indica*, Taleigao, Goa, India, leg. Maria D'Souza, 2-7-1999, Herb. GUBH 1038. (iii) On fallen dead leaves of *Flacourtia montana*, Cotigao Wildlife Sanctuary, Goa, India, leg. Maria D'Souza, 11-04-1999, Herb. GUBH 344. (iv) On fresh leaves of *Sanseiviera zeylanica*, Bondla Wildlife Sanctuary, Goa, India, leg. Maria D'Souza, 15-09-1999, GUFCC No.1370; recovered by endophytic isolation method. (v) On fallen dead and decaying leaves of *Dendrocalamus strictus*,Bondla Wildlife Sanctuary, Goa, India, leg. Maria D'Souza, 15-08-1999, Herb. GUBH 1190. (vi) On fresh leaves of *Bambusa arundinaceae*, Bondla Wildlife Sanctuary, Goa, India, leg. Maria D'Souza, 15-08-1999, Herb. GUBH 1195.. (vii) On dead and decaying leaves of *Ficus benghalensis*, Baga, Goa, India, leg. Maria D' Souza, 11-10-1999, Herb. GUBH 1405. (viii) On fallen dead leaves of *Helictris ixora*y Alorna, Goa, India, leg. Maria D'Souza, 19-11-1999, Herb GUBH 1566.

***Vermiculariopsiella parva* sp. nov. (Fig.2)**

Coloniae irregularise, levia, subhyalina ad centrum et hyalina ad ambitum, cum fructificatum mucosum, aurantium, disseminatum, 6 cm diam. in MEA

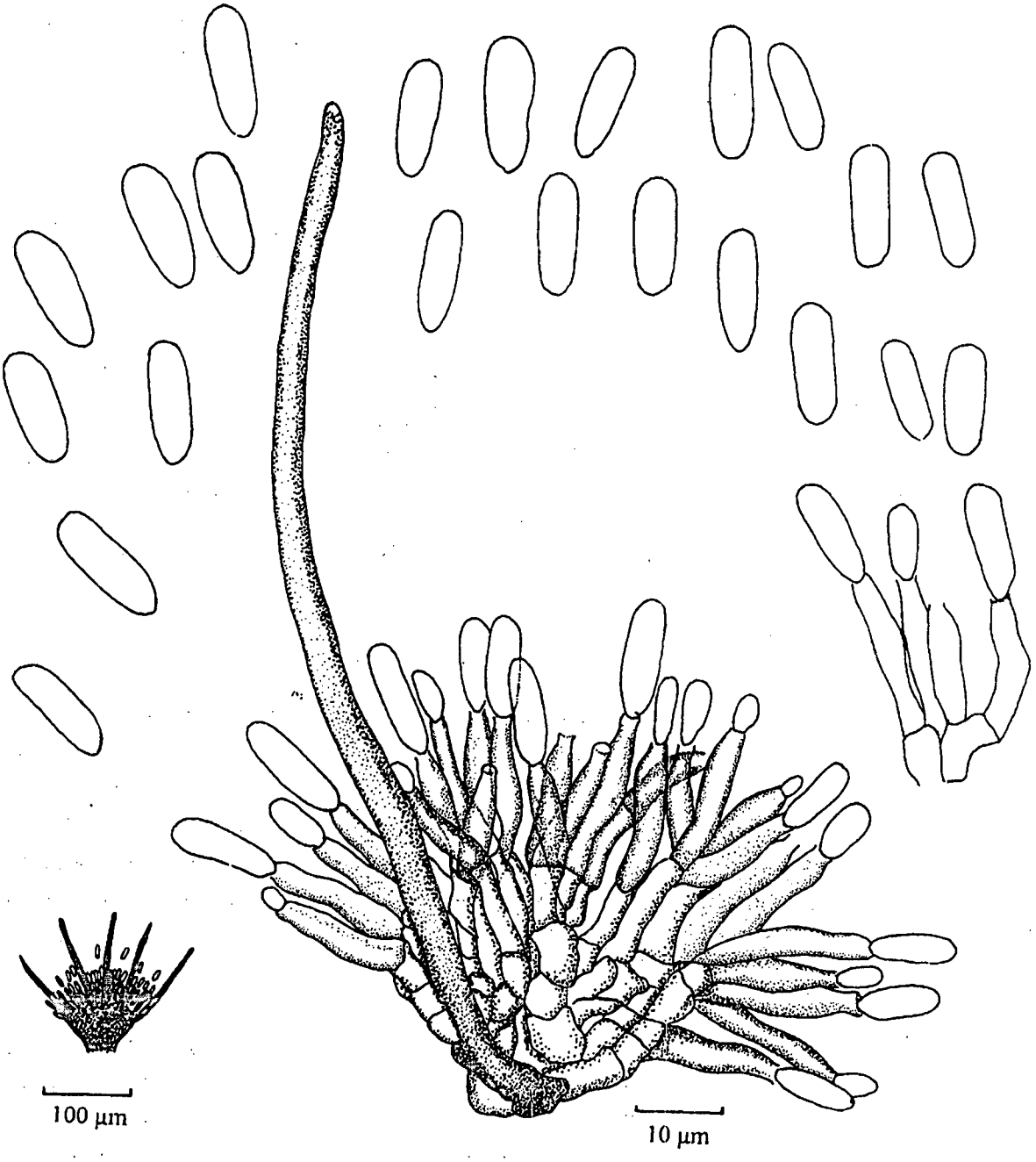

Fig.2. *V. parva* sp.nov: a. Habit, b. A sporodochium with a seta, c. Condiogenous cells with conidia, d. Conidia.

in 7 dies, inverto pallide brunneus vel. Incouloris. *Mycelium* partim superficiale, ex hyphis septatis, ramosis, crassitunicatus, incoloris vel pallide olivaceis, 2-6 µm lat. compositum. *Conidiomata* sporodochia, setosa, dissita, punctiformia, circumscripta, irregulariter circularia, nonnumquam coalescentia, conidis agglutinatis cremea, 25-125 x 55-75 µm. *Setae* numerosa, ereta, curva vel modice flexuosa, simplex, septata, laeves, 80-125 x 3-6 µm, prope basim crassa, crassitunicata, brunneae, gradatim tenuiores tunicatae et pallidiores ad

angustiorem et leviterrotundatum apicem. *Conidiophora* erecta, recta vel modice flexuosa, ramosa, laevia, septata, pallide ad atrobrunnea tenuitunicata, 40-80 x 3-4 µm, inter setas densum vallum formantia. *Cellulae conidiogenae* monophialidicae, discreta, determinata, leves, cylindrica, 12-23 x 3-5 µm. *Conidia* solitaria, cylindrica, levia, aseptata, hyalina 12-15 (10-19) x 4-6 µm.

HOLOTYPUS, Dried culture on MEA, isolated from fresh leaves of *Pimenta dioica*, 11-11-2001, Pookala, Kasaragod, Kerala, India, leg. Keshavaprasad, Herb. GUBH No. 5201

Colonies irregular, smooth, pale brown in the centre, colourless towards the periphery, wet, with distinct orange-coloured fructification scattered all around, fast growing, attaining a diam. of 6 cm in 7 days; reverse of the colony pale brown to colourless. *Mycelium* partly superficial, composed of smooth, branched, septate, thick-walled, pale to dark brown, 2-6 µm wide hyphae. *Conidiomata* sporodochial, setose, scattered, punctiform, sometimes coalescing to from irregular patches with pale cream mass of conidia, 25-125 x 55-75 µm; *setae* arising laterally from the basal cells of sporodochium, solitary, unbranched, swollen and thick-walled at the base, smooth with wavy surface, erect, slightly curved to flexuose, septate, dark brown, progressively paler and thin-walled towards slightly rounded and tapered apex, 80-125 x 3-6 µm. *Conidiophores* erect, straight or slightly flexuose, smooth, septate, branched, light to orange brown, progressively thin-walled, 40-80 x 3-4 µm. *Conidiogenous cells* monophialidic, discrete, determinate, narrow, cylindrical, 12-23 x 3-5 µm, without a distinct collarette. *Conidia* solitary, cylindrical, rounded at the tip, rounded and narrow at the base, smooth, aseptate, unbranched, hyaline,12-15 (10-19) x 4-6 µm.

***Vermiculariopsiella indica* sp. nov. (Fig.3)**

Coloniae effusae, irregularise, leviae, nigra cum fructificatum mucosum, 6-8 cm diam. in MEA in 7 dies, inverto pallide brunneus vel nigrum. *Mycelium* partim superficiale, ex hyphis septatis, ramosis, incoloris vel pallide olivaceis 2 ìm lat., et hyphis atrofucus, crassitunicatus, 3-6 µm lat., compositum. *Conidiomata* sporodochia, setosa, disseminata, punctiformia, circumscripta, irregulariter circularia, nonnumquam coalescentia, conidis agglutinatis cremea, 120-150 x 100-200 µm. *Setae* numerosa, erecta, curva vel modice flexuosa, simplex, septata, leves, 125-300 x 4-6 µm prope basim crassae, crassitunicata, brunnea, gradatim tenuiores tunicatae et pallidiores ad angustiorem et leviterrotundatum apicem. *Conidiophora* erecta, recta vel modice flexuosa, laevia, septata, ramosa, pallide brunnea, tenuitunicata, 60-100 x 6-10 µm longa. *Cellulae conidiogenae* monophialidicae, integrata vel discreta, determinata, cylindricales, laeves, tenuitunicata, hyalina, expers collerettis, 15-26 x

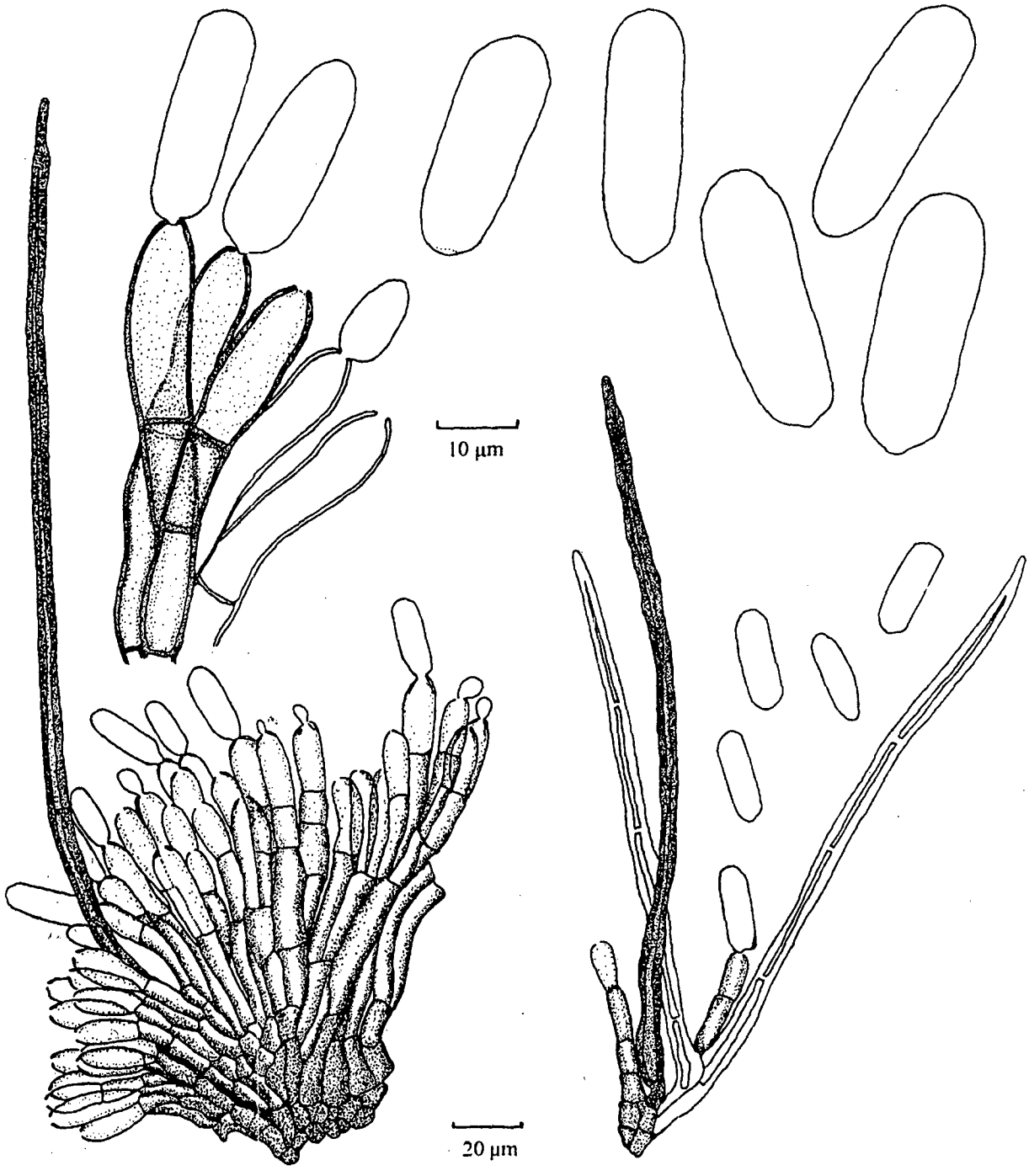

Fig.3. *V. indica* sp. nov: a. A sporodochium with a seta, b. Setae with condiophores, c. Condiogenous cells with conidia, d. Conidia.

6-10 µm. *Conidia* solitaria, cylindrica, levia, aseptata, tenuitunicata, hyalina 22-30 x 8-11 µm.

HOLOTYPUS, Dried cutlure on MEA, isolated from fresh leaves of *Saraca asoca*, Bondla Wildlife Sanctuary, Goa, India, leg. Maria D'Souza, 11-12-1999, GUFCC No.1502, GUBH No. 1502.

Colonies effuse, irregular, smooth, black, wet, with distinct black fruiting bodies scattered on the surface, fast growing, attaining a diam. of 6-8 cm in 7 days; reverse of the colony pale brown to black. *Mycelium* partly superficial, composed of smooth, septate, thick-walled, branched, pale to dark brown, 2-6 µm wide hyphae. *Conidiomata* sporodochial, setose, scattered, punctiform, sometimes coalescing to from irregular patches with cream mass, 120-150 x 100-200 µm; *setae* many, arising laterally from the basal cells of sporodochium, solitary, unbranched, smooth, erect, slightly curved to flexuose, septate, dark brown, swollen and thick-walled at the base, progressively paler and thin-walled and slightly rounded and tapered apex, 125-300 µm long, 15-25 µm wide at the base, 4-6 µm wide above. *Conidiophores* erect, straight, slightly flexuose, smooth, septate, branched, light brown, thin-walled, 60-100 x 6-10 ìm. *Conidiogenous cells* monophialidic, integrated to discrete, determinate, wide cylindrical with rounded apex, without distinct collarette, 15-26 x 6-10 µm. *Conidia* solitary, cylindrical, rounded at the tip, slightly rounded and truncate at the base, smooth, aseptate, unbranched, hyaline, 22-30 x 8-11 µm.

Discussion

The three new species described here differ from earlier known species in that they produce cylindrical, smooth, unicellular, hyaline conidia with rounded ends and wider than 4 µm. The conidia are sometimes slightly curved and truncate at the base. The sporodochia when mature coalesce into slimy mass. The setae mature at different stages of development and, therefore, number and size of the setae are discernible only in mature sporodochia.

Key to the species of *Vermiculariopsiella* proposed by Nawawi *et al.* (1990) is updated below incorporating the later described taxa.

Key to the species of *Vermiculariopsiella*

1. Setae unbranched ---------- 2
1. Setae branched ---------- 6
2. Conida cylindrical with rounded ends ---------- 3
2. Condia with pointed apex ---------- 4
3. Conidia 20-25 x 6-8 ìm ---------- *V. elegans* sp.nov.

3. Conidia 12-15 (10-19) x 4-6 ìm ---------- *V.parva* sp.nov.

3. Conidia 22-30 x 8-11 ìm ---------- *V. indica* sp. nov.

4. Conidia falcate, apex curved and pointed ---------- *V. falcata*

4. Conidia fusiform, slightly curved, 15-19.5 ìm long ---------- *V. arcicula*

4. Conidia cylindrical, straight, apex acute, base rounded ---------- 5

5. Conidia 14-25 ìm long; conidiogenous cell apex recurved - *V. immersa*

5. Conidia 8-13 ìm long; conidiogenous cell apex narrow, straight -------- ---------- *V. parvula*

6. Setae mostly once dichotomously branched ---------- *V. ramosa*

6. Setae thrice dichotomously branched ---------- *V. cornuta*

6. Setae with short primary and secondary branches, terminal cells with hair-like appendages ---------- *V. cubensis*

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Mosquito Larvicidal and Pathogenic Fungi from Goa

❖ *Keshava Prasad T S, Ashwani Kumar & Bhat D J*

Introduction

Mosquito-borne diseases such as malaria, filariasis, Japanese encephalitis, dengue fever, dengue haemorrhagic fever and yellow fever continue to afflict mankind heavily around the world, particularly in the tropics and subtropics, despite the serious efforts to control these diseases (Kumar *et al.*, 1994). The development of induced resistance in mosquito vectors, realisation of detrimental effects of insecticides especially on non-target organisms and environmental pollution and contamination of food-chains due to insecticides have necessitated search for other safer and sustainable alternatives (Kumar *et al.*, 1996). A number of biological agents such as fishes, bugs, mesocyclops, bacteria and fungi are known and attempts were made to use such organisms against mosquito developmental stages (Kumar *et al.*, 1994, 1996).

Entomopathogenic fungi have been reported from all Divisions of the Kingdom Mycota (Agarwal and Rajak, 1988; Kendrick, 1992). Species belonging to seven genera of fungi (*Coelomomyces* Keilin, *Culicinomyces* Couch, Romney & Rao, *Entomophthora* Fresen., *Lagenidium* Schenk., *Leptolegnia* de Bary, *Metarhizium* Sorokin and *Tolypocladium* W. Gams) were thought possible biocontrol agents against mosquito larvae (Lacey and Lacey, 1990; Rawlins, 1989). However, the success of utilisation in the field was not been

confirmed. Technical difficulties in formulation, application of biocontrol agents, storage and delivery facilities for live propagules have been reported (Lisansky and Hall, 1983). In view of their intricate life cycle coupled with an intermediate copepod host, the members of *Coelomomyces* were found to be untenable as larvicidal agents. The species of *Culicinomyces* and *Lagenidium* exhibited sensitivity to temperature (>32°C) and organic pollutants at low concentration and their use as biocontrol agents in the tropics was also found to be limited (Rawlins, 1989). Vijayan and Balaraman (1991) tested the activity of crude extracts of a number of fungal isolates against larvae of *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti* and have shown that several fungi are possibly larvicidal. This paper describes the survey of various localities in the State of Goa and to isolate mosquito larvicidal and pathogenic fungi.

Materials and Methods

Collection of samples was made from 11 localities in Goa *viz.*, Panaji, Porvorim, Vasco (coastal towns), Bicholim, Carambolim, Cuncolim, Curchorem, Pernem (sub-coastal centres) and Bondla, Cotigao and Mollem (forest hinterlands). The samples included the following:

1. Live mosquito larvae from breeding sites (well, pond, ditches, puddle, paddy field, slow flowing stream, curing water at the construction site, overhead tank, sump tank, septic tank, drain, abandoned container, barrel and tyre) and transient rain water pools on building terrace. The collection of samples was done following the method of Service (1976). The larvae at different developmental stages were gathered and transported in plastic containers and maintained in the laboratory until the adult mosquitoes emerged out. Some of the larvae found sluggish or completely dead during the period of incubation were used as source material of fungi.
2. Dead adult mosquitoes from their resting site.
3. Dead and live non-mosquito insect and arachnids (Hemipteran, Coleopteran, Orthopteran, Phasmida, Dipteran, mites and spiders) from the foliage of forests and scrub-jungle.

4. Healthy and later infected larval baits introduced in the laboratory in water samples collected from puddles, paddy fields and ponds that generally lacked mosquito breeding.
5. Healthy and later infected mosquito larval baits used in simulation float chambers maintained in ponds and slow running streams. Baits used in the latter two exercises were second instar larvae of *Culex quinquefasciatus* Say reared in the laboratory (Ansari *et al.*, 1978; Singh *et al.*, 1975).

The samples were thoroughly washed in sterile deionised water (SDW), placed at equidistant on malt extract agar (MEA) plates (malt extract, 10 g; agar agar, 20 g; distilled water, 1000 ml) incorporated with a mixture of antibiotics (20 ppm each of bacitracin, neomycin, penicillin, polymixin, streptomycin and tetracycline) and incubated at 23-25° C. The plates were observed on 2nd, 5th and 8th day for the emergence of fungi. Fungi developed on each sample were transferred into MEA slants. Part of the samples was incubated in separate moist chambers to encourage conidia generation of the fungi. The conidia developed were aseptically transferred to MEA plates.

The test fungi were grown on corn meal agar medium (corn meal, 17 g; agar agar, 20 g; distilled water, 1000 ml) to encourage mass production of conidia. The conidial suspension was prepared by harvesting spores from 14-days-old cultures in 10 ml deionised water. Forty ml of tap water was added to dilute the conidial suspension and final concentration was between 105 and 106 conidia/ml. Twenty healthy late 2nd instar larvae of *Culex quinquefasciatus* were introduced into the container and observation was made for features such as sluggishness, mortality and other associated symptoms for five days at a regular intervals of 24 hr. Mosquito larvae unable to swim to the surface were also treated as affected. Larval feed (dog biscuit/baker's yeast in 2:1 ratio) was provided during the bioassay. Control was maintained with 50 ml tap water. Experiment was done in three replicates with two repetitions. Fungi, which caused more than 50% mosquito mortality were considered as possible biocontrol agents.

When the control mortality was more than 20%, the bioassay was discarded and repeated in order to correlate and confirm the results obtained. The control mortality when ranged between 5-20%,

the 'corrected mortality' was calculated using Abbott's formula (Abbott, 1925):

$$\text{Corrected Mortality} = \frac{\text{Observed mortality (\%)} - \text{Control mortality (\%)}}{100 - \text{Control mortality (\%)}}$$

If the control mortality remained below 5%, corrected mortality was not calculated (Das and Kalyanasundaram, 1989; Plestina, 1984).

Results and Discussion

In all, 165 samples of mosquito developmental stages were collected from 16 different breeding sites. Each sample contained a number of developmental stages of mosquitoes. Every breeding site was scanned thrice during the experimental period which lasted for 8 months (March-November, 1999). From 389 dead and inactive mosquito larvae/pupae, 57 fungal isolates were recovered in pure culture. From 453 moribund and dead non-mosquito insects and arachnids, 47 isolates were recovered in pure culture. Seven fungal isolates were obtained by challenging second instar larvae of *Culex quinquefasciatus* in water samples gathered from 17 habitats containing decaying vegetation. The remaining two sources, i.e., dead/moribund adult mosquito adults and larval baits used in simulation experiments did not yield any fungus. All the isolates obtained were maintained in MEA slants in pure and viable condition. Of the 57 fungi isolated from dead or inactive mosquito developmental stages, 20 showed more than 50% mortality (Table 1), whereas of the 47 isolates obtained from non-mosquito sources, 17 were found active against mosquito larvae (Table 2). Two of the seven isolates from water samples showed more than 50% bioactivity. The active fungal isolates belonged to *Acremonium* Link (1), *Aspergillus* Link (3), *Chaetomella* Fuckel (1), *Gliocladium* Corda (6), *Penicillium* Fries (13), and *Trichoderma* Persoon (11).

The gut of larvae contained abundant conidia in case of *Penicillium* (E2). It is presumed that the non-selective filter-feeding habit of mosquito larvae resulted in the packing of the gut with spores within one hr of exposure to dilute spore suspension. The conidia-fed larvae were found unable to surge to the surface. In case of *Gliocladium* (E16), *Acremonium* (E29) and a few *Trichoderma* isolates, the challenged mosquito larvae showed melanisation which is said to be an indication of penetration of the fungal hyphae (Butt *et al.*,

1988). Melanisation and disruption of gut was observed in several larvae within 12-24 hr after exposure. The larvae placed in high concentration of conidial suspension of *Trichoderma* isolates were also found to contain conidia adhering to anal papillae, cervical collar and inter-segmental areas. The normal infection by *Culicinomyces clavisporus* in *Culex fatigans* is through the digestive tract (Sweeney, 1975, 1979) from where these further invade the haemocoel. Al-Aidroos and Roberts (1978) noted gut invasion of aquatic mosquito larvae *Aedes aegypti* by *Metarhizium anisopliae*, indicating that mosquitoes are generally vulnerable to this mode of attack. Reports also indicate insect mortality due to toxicity of ingested and ungerminated conidia in mosquito larval gut (Dillon and Charnley, 1991). Boucias *et al.* (1988) observed several entomopathogenic fungi with features, which aid in successful adhesion and germination on cuticle.

Table 1: Preliminary bioassay of promising fungi isolated from mosquito larvae

Fungus	*Code No.*	*Bioactivity (Mortality %)*
Aspergillus sp.	C15	60
Penicillium sp.	C2	100
Penicillium sp.	C4	94
Penicillium sp.	C5	85
Penicillium sp.	C20	100
Penicillium sp.	C26	100
Penicillium sp.	C29	100
Penicillium sp.	C42	100
Penicillium sp.	C44	100
Penicillium sp.	C51	100
Trichoderma sp.	C13	100
Trichoderma sp.	C36	70
Trichoderma sp.	C37	65
Trichoderma sp.	C48	60
Trichoderma sp.	C52	95
Trichoderma sp.	C53	80
Trichoderma sp.	C54	100
Unidentified	C7	100
Unidentified	C17	100
Unidentified	C27	100

Table 2: Preliminary bioassay for promising fungi isolated from non-mosquito insects, arachnids and stagnant water samples lacking mosquito breeding (Note that W5 and W6 were isolated from the water)

Fungus	*Code No.*	*Bioactivity (Mortality %)*
Acremonium sp.	E29	100
Aspergillus sp.	E21	50
Aspergillus sp.	W5	100
Chaetomella sp.	E35	94
Gliocladium sp.	E6	100
Gliocladium sp.	E13	100
Gliocladium sp.	E15	100
Gliocladium sp.	E16	100
Gliocladium sp.	E19	100
Gliocladium sp.	E23	95
Penicillium sp.	E2	100
Penicillium sp.	E9	100
Penicillium sp.	E11	100
Penicillium sp.	E26	100
Trichoderma sp.	E37	100
Trichoderma sp.	E39	92.1
Trichoderma sp.	E42	90
Trichoderma sp.	W6	60
Unidentified	E20	100

Screening of fungi for mosquito larvicidal/pathogenic activity by preliminary bioassay is a relatively simple procedure but concentration of spores in suspension should range between 10^5-10^6 spores/ml depending upon the amount of spores produced by each fungus. All the sporulating fungi were tested by preliminary bioassay method described in this paper. The fungi isolated from mosquito larvae and non-mosquito insects and arachnids opens up new avenues in the search of possible biocontrol agents against vector mosquitoes.

Summary

Fungi isolated from different insect and mosquito-inhabiting

substrates and habitats in Goa, were tested for their mosquito-larvicidal potential through preliminary bioassay. Potnetial isolates recovered belong to the genera: *Acremonium, Aspergillus, Chaetomella, Gliocladium, Penicillium* and *Trichoderma*. These are new records as mosquito larvicidal fungi.

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