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ABSTRACT

The objectives of the project were to generate basic data on the microbes associated with forest insects in the Kerala part of the Western Ghats and to test the potential of some of some these microbes as biocontrol agents. The study also envisaged *ex-situ* conservation of the microbes and to share them with other scientific institutions for further studies and practical use.

During the survey in the moist deciduous forests and teak plantations in the Western Ghats over a period of 3 years, 750 dead insect specimens infected with microbes were collected. Of these over 70 per cent belonged to fungi indicating the dominance of the group. Out of these, over 30 species were identified and the remaining is pending identification to species level. The available data indicate presence of new species of fungi under the genera, *Akanthomyces* and *Paraisaria*. Some fungi are apparently new records for the country.

In general, death of insects due to microbial infection was more during the wet period of the year. Compared to teak plantations, incidence of microbial death of insects was more in the moist deciduous forests. Insect death due to bacterial infection was considerably low and epizootics due to bacteria were not observed during the study period.

Insect death due to nucleopolyhedrovirus infection (HpNPV) was observed in teak plantations on *Hyblea puera* and epizootics of HpNPV were observed mostly during the peak outbreak period in many teak plantations from south to north.

The laboratory evaluation of the some selected fungal pathogens like *Beauveria brongniartti*, *Paecilomyces fumosoroseus*, and *Meatrhizium anisopliae* against the teak defoliator, *Hyblea puera* revealed their potential as biocontrol agents.

The study has generated valuable information on the microbes surviving on insects in a variety of habitats in the forests. The microbial pathogens collected are stored and periodically subcultured and conserved under *ex-situ* condition. A checklist of the microbial pathogens of forest insects has also been prepared.

INTRODUCTION

Kerala with its variety of ecosystems ranging from the high mountains supporting thick tropical evergreen forests, coastal plains, riverine and mangrove vegetation is known for its rich biodiversity. The Kerala part of the Western Ghats is considered to be the treasure house of floral and faunal diversity.

The microbial organisms constitute a major part of the biodiversity. Microbes are generally treated as harmful as many of them are known to cause disease in human beings and other living forms. However, when we critically analyze the positive functional role of microbes in many vital phenomena like biofertlization, biocontrol, biodegradation, nutrient cycling etc., we understand the importance of these organisms in our ecosystem. It may also be pointed out that both crop improvement and crop protection products are possible through bioprospecting of the microbes. The varied uses of several microorganisms in the fields of medicine, food, chemical industries etc., are well known and need to be further explored. Hence it is utmost important to collect and identify the microbes for further exploitation. Based on the Conservation of Biological Diversity (CBD) signed in 1992, there is an added responsibility to conserve these microbes for bioprospecting. The need for establishing microbial culture collection centers and gene banks in biodiversity areas in the tropics has been advocated (Hawksworth, 1992; Sly, 1998). In recent times, the utility of these culture collections in improving the strains through cloning and other biotechnological means are being attempted. Compared to other developed countries, in India the microbiologists and molecular biologists working in the fields of quality assurance, industry and human, animal and plant health are handicapped with the problem of access to culture collections.

The loss of biodiversity, especially in the tropics due to anthropogenic reasons, has also negative implications on microbial diversity. Overall loss of forests to the tune of 1.8 per cent in India calls for serious efforts to protect and conserve our microbial diversity with equal importance given to animal and plant species. The forest ecosystem varies widely

throughout the world and the unseen microbes are most important. However, the research attention is mostly focused on larger animals and trees in the forests and microbes are overlooked by the mainstream forestry.

There is considerable lacunae existing in our knowledge on the microbial wealth in our forests. In the past, Kerala Forest Research Institute have generated useful data on the potential of a number of useful microbes for the management of forest insect pests (Varma and Mohanan, 1984; Varma and Mohammad Ali, 1986; Sudheendrakumar et al., 1988; Sankaran et al, 1989; Mohammed Ali et al, 1991; Swaran and Varma, 2003; Sasidharan and Varma, 2005). However, no specific attempt has been made in the past to collect and conserve useful microbes from the natural habitats. Natural forests in India have never been explored to collect and identify the microbial pathogens associated with forest insects.

The present study was hence taken up to generate data on microbes associated with dead insects on a variety of habitats in the forests .It was also envisaged to maintain a collection of these microbes for future studies. The specific objectives were:

1. To generate information on the entomopathogens of forest insects through intensive surveys in the Kerala part of the Western Ghats

2. To isolate, identify and evaluate the potential of selected pathogens against some of the major pests of forest trees

3. To establish a documentation centre with reference collections of entomopathogens for *ex-situ* conservation and make them available for studies/ research.

REVIEW OF LITERATURE

Microbial diversity encompasses the spectrum of variability among all types of microorganisms including bacteria, fungi, viruses and many more in the natural habitat and also in the altered habitats due to human intervention. It is estimated that there are about 1.5 million fungi (Hawksworth, 1993; Hammond, 1992; Rossman, 1994) on earth. Almost 90 per cent of the micro biota on earth has yet to be described and compared to less than 3 and 0.5 per cent of plants and vertebrates, microbes constitute about 15 per cent of all species on earth (Hammond, 1992). Forest ecosystem is an abode of several microbes and many of them have a major role in ecosystem functioning. According to Hywel-Jones (1993), there are at least 13.5 million undescribed fungi that infect insects and also many are host specific.

Microbes as insect control agents

The naturally occurring biocontrol agents form an essential component of the forest ecosystem and presently constitute an effective alternative to chemical pesticides. Though in the agricultural sector, microbial agents are being used in the management of insect pests, this strategy has yet to pick up in forestry. Most of the serious pest problems in forestry are associated with forest plantations and native microbial control agents can have devastating effects on the collapse of pest populations. In the natural forests, pest problems are almost nil (Nair *et al.*, 1986). Prior to the introduction of the microbial control agents in a forest plantation, it is essential that an inventory of the natural enemies of the pest species is taken. Agrochemical companies search tropical forests to find out new entomopathogens because of the species richness and diversity of microbes (Martin and Travers, 1989; Jun *et al.*, 1991).

Major groups of microbial pathogens

Entomopathogenic organisms employed in the microbial pest control include bacteria, viruses, fungi, protozoa and nematodes. Of these, the first three are important and the review is focused only on these organisms. The advantages of using the biopesticides

based on the above three groups of organisms is that they are safe to use without harmful effect on the environment.

Fungal pathogens

Entomopathogenic fungi are widely distributed in nature and because of the fungal mycelial growth on the insect body, they are quite conspicuous. The effect of fungi ranges from occasional infection to spectacular epizootics. The largest group of insect pathogens are fungi, comprising more than 750 species representing about 100 genera, but only about 10 are being used for insect control (Hajek and Leger, 1994). Entomopathogenic fungi belong to all major subdivisions of fungi like Mastigomycotina, Zygomycotina, Ascomycotina, Basidiomycotina and Deuteromycotina. They have a wide host range which covers Lepidoptera, Coleoptera, Hemiptera, Diptera and Hymenoptera.

Many entomopathogenic fungi cause epizootics that often successfully regulate pest insect populations. Water has been recognized as essential for the germination of spores of most fungi and high atmospheric humidity is known to favour development of epizootic mycosis. Under field conditions poor control may be attributed to low temperature and humidity, which will prevent spore germination and infection.

Several species of fungi offer good potential for mass production on inexpensive artificial media and have better shelf lives. Commercial products based on *Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* (Metschnikoff) Sorokin, *Verticillium lecani* (Zinmermann) Viegas, *Paecilomyces fumosoroseus* (Wize) Brown and Smith and experimental isolates of *M. flavoviridae* Gams and Rozsypal, *Nomuraea rileyi* (Farlow) Samson and *Aschersonia aleyroids* Webber are currently in use or under development.

Auto dissemination and use of mycoinsecticide just like conventional insecticides are two approaches for control of insect pests using fungi. Several forest pests are reported to be controlled by fungal pathogens. *B. bassiana* is known to cause muscardine disease in many forest pests, *viz.*, *Malacosoma disstria* Hbn., *M. americanum* (F), *Hoplocerambyx spinicornis* (Nirmita and Joshi, 2004) *Lachnosterna consanguinea* Blanch., *Pissodes strobi* Peck, *Myllocerus viridanus* Fabr and *Calopepla laeyana* Letreille (Sankaran *et al.*,

1989), *Atteva fabriciella* Swed., *Sahyadrassus malabaricus* Moore. (Mohammed Ali *et al*., 1991), *Hyblaea puera* and *Eutectona machaeralis* (Agarwal *et al*., 1985). *Metarhizium anisopliae* is known to attack the larvae of *H. puera* and *E. machaeralis* from different parts of India (Agarwal *et al*., 1985). It was also reported to be a potential biocontrol agent against termites (Swaran and Varma, 2003). *Fusarium* sp. is reported to cause mortality in *H. puera* and *E. macheralis* (Agarwal *et al*., 1985; Nirmita and Joshi, 2004). *Paecilomyces farinosus* is reported to have potential as biocontrol agent against major pests of *Ailanthus* sp. (Varma and Mohanan, 1984). *P. farinosus* and *P. fumosoroseus* were found to be effective against *E. narcissus* (Mohammed Ali *et al*., 1991).

Bacterial pathogens

Because of the success of *Bacillus thuringensis*, which goes back to over half a century, commercial preparations of biopesticides became popular which were used just like chemical pesticides. Over 90 species and varieties of naturally occurring bacteria have been described from insect pests of agriculture and forestry importance. Many of them have also been reported to be effective as control agents against insect pests in the tropical forests (Martin and Travers, 1989). *B.thuringiensis* and its varieties have been successfully tested against more than 137 insect species belonging to different insect orders such as Lepidoptra, Hymenoptera, Diptera and Coleoptera. Within the genus *Bacillus*, many species like, *B.popillae, B.cereus, B.thuringiensis, B.sphaericus* etc., are pathogenic to insects.

B.thuringiensis when cultured under appropriate conditions sporulates and forms crystalline parasporal body which contains protenacious deltaendotoxins. The insecticidal activity is associated with these toxins and once sporulation is complete, the bacterial cells lyses releasing spores and crystals to the media. In nature, the ultra violet rays and variations in temperature interact with the spores. Epizootics of naturally occurring bacterial pathogens are rare.

The largest share of the biopesticide market currently goes to *B. thuringiensis*. About 200 *B. thuringiensis* based commercial products are registered in the United States alone. In forestry, use of commercial products of *B. thuringiensis* has increased compared to other

interventions including chemical pesticides (Evans, 1997). Other groups of bacteria are used only on a smaller scale for insect control. It may be mentioned that field application of *B. thuringiensis* has restrictions in areas where sericulture is practiced. Under natural conditions, *B. thuringiensis* in combination with NPV enhanced control of alfalfa caterpillar. Combinations of *B. thuringiensis* and the fungus, *B. bassiana* also provided effective control.

B. thuringiensis is reported as a potential biocontrol agent against *H. puera* which can cause 100 per cent mortality of the larvae in the laboratory within 72h, while it was only 70 and 60 per cent respectively in case of *Serratia marcescens* and *Enterobacter aerogens* (Mohammed Ali *et al.,* 1991). *B. thuringiensis* is also reported to be a potential biocontrol agent against forest pest like *Eligma narcissus,* a defoliator on *Ailanthus triphysa* (Varma *et al.,* 1988) and teak defoliator, *Hyblaea puera* (Senguttuvan *et al.,* 2000). Commercial products of *B. thuringiensis*, such as Bioasp and Biolep are also used against teak skeletoniser, *Eutectona machaeralis* under field conditions. *Bacillus firmus* isolated from *Atteva fabriciella,* a shoot webber on *Ailanthus triphysa* is reported to be effective against both *A.fabriciella* and *Eligma narcissus* (Varma and Mohammed Ali, 1986).

Viral pathogens

Baculoviruses have been credited to be potential biopesticide of future on account of their host specificity and eco-friendliness. Seven groups of pathogenic viruses have been isolated from insects and a large number of them offer effective control against insect pests (Payne, 1982). Among these the baculovirus (Baculoviridae) which include Nucleopolyhedroviruses (NPV) and Granulosis viruses (GV) are found to be the most successful groups of pathogens against insect pests. Baculoviruses have been isolated from more than 400 insect species, mainly from Lepidoptera and Hymenoptera.

Most baculoviruses infect only the larval stages of susceptible insects; exceptions are NPVs that infect Hymenoptera and non-occluded virus of *Oryctes*. After ingestion by the host, the occlusion bodies of NPVs and GVs are broken down in the alkaline mid gut fluids of susceptible larvae and infectious virus particles are released. The liberated virions enter the gut epithelial cells and replicate in the nuclei. Non-occluded virus

particles that buds from the gut cells into the haemocoel invade other tissues (fat body, tracheal matrix, hypodermis etc.), within the host. The host larvae normally rupture when dead and release large quantities of occlusion bodies into the environment that can persist for considerable periods of time.

Epizootics of baculovirus diseases are common in both Lepidoptera and sawflies, often resulting in population suppression (Hunter *et al.*, 1984). Various biotic and abiotic factors help in the dispersal of the virus inoculum. Forest plantations are good reservoirs for virus inoculum than annual plants. The stable environment of forest plantations and permanent pasture makes them better environments for vertical transmission of viruses than field crops that are harvested annually and fields in which crop rotation is practiced.

Regular use of viruses for the control of forest pests has been carried out in North America and Europe (Cunningham, 1995). Very little information is available on baculovirus infesting forest insect pests in India. Ahmed and Sen-Sarma (1983) reported the occurrence of NPV on the larvae of *Pygaera fulgurita* infesting Poplar in Uttar Pradesh. Investigations on the occurrence of natural epizootic of *Hyblaea purea* in the teak plantations of Kerala, led to the identification of HpNPV of the teak defoliator (Sudheendrakumar *et al.*, 1988). Ahmed (1992) reported NPV infection in natural populations of *Taragama siva*, the babul defoliator. Efficacy, specificity and production of secondary inoculum make baculoviruses attractive and ideal as one of the component of IPM systems (Cunningham, 1995). Some of the drawbacks of the use of entomopathogenic viruses are their relatively slow action compared to that of chemical insecticides, sensitivity to UV light, availability of living systems for production and timing of application.

Mohamed Ali *et al.* (1991) reported the potential of nucleopolyhedrovirus of the teak defoliator as a biocontrol agent. Nair *et al.* (1998) made detailed studies on HpNPV, including field trials which showed that one time foliar application of a crude preparation of HpNPV at the rate of 10^5 POBs per ml of the spray fluid, gave 70-76 per cent protection of foliage. Cross infectivity studies by Rabindra *et al.* (1997) revealed that HpNPV is highly host specific and did not affect other forest insects and/or agricultural pests. Nair *et al.* (1998) made further attempts to standardize methods to mass produce

HpNPV from laboratory reared *H. puera* larvae. A method of application of HpNPV virus under the control window concept was also standardized (Sudheendrakumar *et al.*, 2001).

Bioprospecting and conservation

Many microbes offer immense potential as a source of medicine, food or as bioproducts and hence of great value for bioprospecting. However, these resources have been poorly tapped in terms of bioprospecting and also there is a need to conserve them to understand their values. We are unable to predict which microorganism will provide us with a new drug or food. Conservation allows us to have a wider range of recourses to meet the challenges of a fast changing world. There is also a need to enhance the networking of national/state institutions to share the responsibility of conservation and bioprospecting in a mutually beneficial manner.

MATERIALS AND METHODS

Study sites and disease survey

Survey for collecting insect cadavers infected with microbial pathogens was carried out in Northern, Central, and Southern Forest Circles in the Kerala part of the Western Ghats (Fig. 1). Collections were made during both wet (May-September) and dry seasons (October-April) of the year. Data on temperature and humidity at different localities, identity of host insect, host plant from which dead insects were obtained, severity of infection and disease incidence etc., were also recorded from the various study sites.

Moist Deciduous Forests: Periodic monthly collections were made from the three permanent representative plots of size 25 m × 25 m at Nilambur (Northern), Vazhachal (Central) and Konni (Southern), covering the three major forest circles. Collections were also made from several other localities like Kottappara, Peechi, Silent Valley, Parambikkulam, Attappady, Vazhani, Wynad, Nelliampathy and Munnar.

The localities mentioned above represented various forest types such as evergreen, semi evergreen and moist deciduous forests. After a preliminary reconnaissance survey among the various forest types observation was confined to moist deciduous forests because of increased incidence of microbial death of insects there. From the permanent plots data were collected to understand the diversity, abundance, richness and evenness of microbial pathogens.

Teak plantations: Survey on insect cadavers was also extended to forest plantations, because the pest incidence was more in plantations compared to natural forests. Preliminary data on selected forest plantations like teak, eucalypts, bamboo and *Ailanthus* showed that pest incidence was more in teak plantations and therefore observations were confined to teak plantations. Three permanent plots of size 20 m × 20 m were laid out in the teak plantations at Nilambur, Vazhachal, and Konni and observations taken.

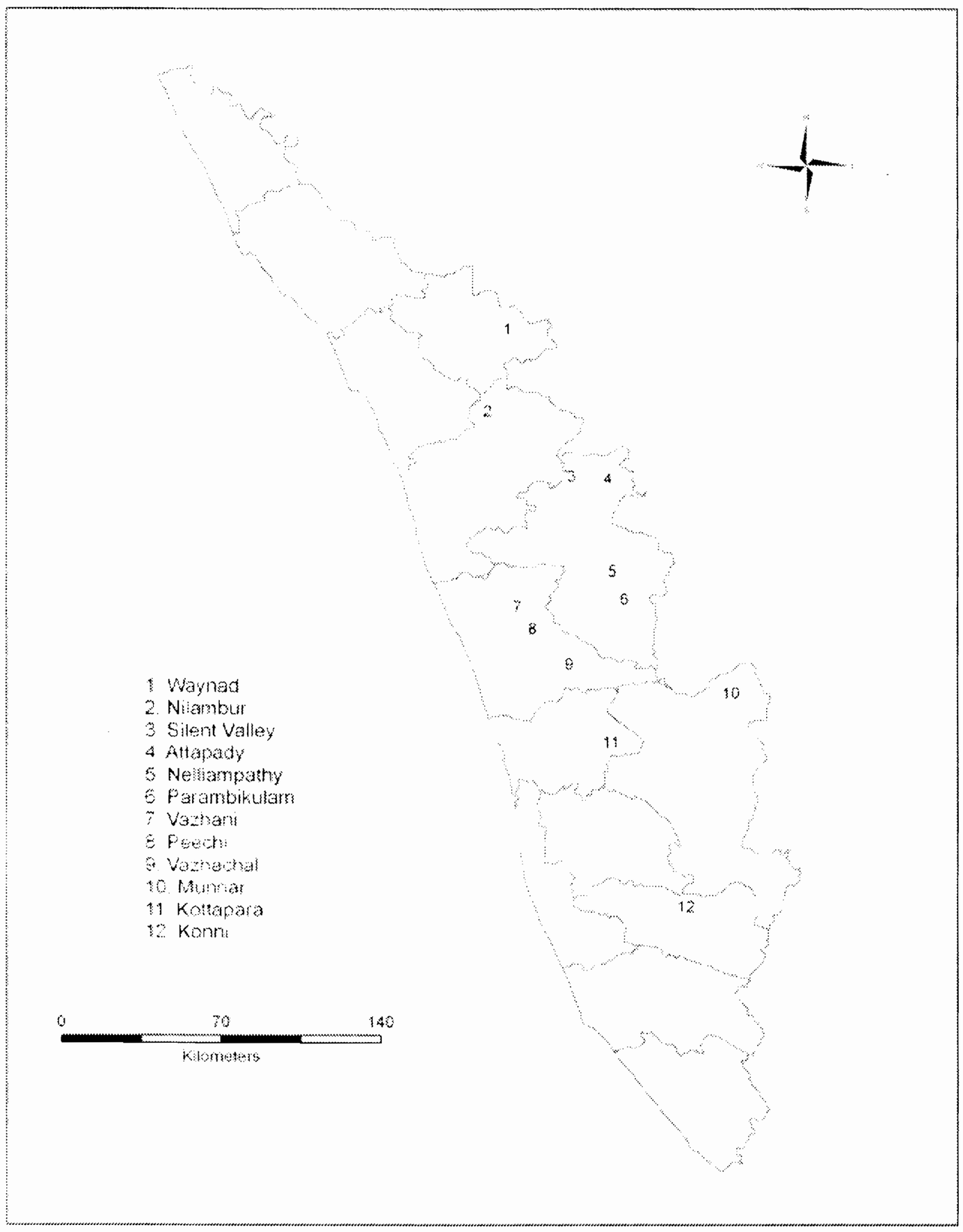

Fig. 1. Map of Kerala showing survey localities

Collection of infected insects: Dead and/or moribund insects fallen onto the ground or remained attached to stem or leaf of trees were collected. The Insects sampled were kept in sterile plastic vials with screw caps, paper bags or envelops. Live insects, which appear to be infected with disease were also collected and observed for the appearance of disease symptoms in the laboratory. All the collected specimens were labeled, showing collection number, date, location etc. The infected specimens were also photographed.

Storage of specimens: While collecting the fungal specimens, the containers were kept open for three to four days so that the cadavers may dry out. These air dried specimens were stored at 4°C in a refrigerator. Bacterial and viral infected specimens obtained from the field were stored at 4°C as soon as they are brought to the laboratory.

Isolation

***Fungi*:** In the case of insects with fresh external sporulation, fungal spores from insect cadavers were taken with a fine, sterile needle. Freshly dead insects with no external mycelial growth were incubated for several days in a moist chamber to induce external sporulation. Insects which were not freshly killed were surface sterilized in 0.1 per cent mercuric chloride for 40 seconds to remove saprophytes, followed by rinsing in three changes of sterile, distilled water. The internal dissected tissues or the external spores were streaked onto several different agar media in Petri-dishes with antibiotics like strepto-penicillin.

The media used included tap water agar, basal medium like Potato Dextrose Agar (PDA) and complex media like Potato Dextrose Agar- Yeast extract (PDA-Y), Sabouraud Dextrose Agar (SDA) and Sabouraud Maltose Agar (SMA). Sabouraud Dextrose Agar supplemented with egg yolk and milk were also used for isolation of fungi with exacting nutritional requirements.

The cultures were incubated at 20^0 C to 28^0 C and examined daily under a stereoscopic microscope for growth and sporulation. The fungal mycelia were subcultured on agar slopes and the fungus was allowed to grow until sporulation and stored in a refrigerator at 5^o C to 10^o C .

***Bacteria*:** The bacteria from the cadavers were isolated by dissecting the insect and the body contents were streaked using a wire loop onto nutrient agar and incubated at 37^0C for 24 hours.

***Virus*:** Virus was isolated by placing the dead insects in a culture tube with distilled water and after several days, the inclusion bodies settle as a white layer at the bottom of the

tube. The same has been centrifuged to remove insect debris or bacterial cells. The partially purified virus was stored in vials for further studies.

Identification

***Fungi*:** Preliminary identification of the field collected specimens was carried out under a stereoscope microscope for external morphology. The microscopic slides were prepared using fungal fructification obtained from the Petri-dish after 10 days of growth. Slides were stained using lactoglycerol cotton blue and observed under light microscope with ×100 and ×40 magnification. Identification of the fungi was attempted using relevant monographs and literature.

***Bacteria*:** Bacterial identification was carried out using stained smears and biochemical tests.

***Virus*:** Virus was identified for white shining inclusion bodies stained using Giemsa stain, and observed under ×40 objective of the bright field microscope.

Bioassay

The effectiveness of selected fungal isolates such as *Beauveria brongniartii, Paecilomyces fumoso-roseus* and *Metarhizium anisopliae* were tested against forest insect pests like *Hyblaea puera* (teak defoliator).

In order to maintain the virulence of the fungi and also for the selection of appropriate dose, a trial experiment was conducted using conidial suspension. The reisolated conidia from the pest were subcultured on PDA-Y media at 25 ± 1^{0} C. The conidial suspension was prepared from 14 day-old cultures using sterilized distilled water containing 0.05 per cent Tween 20. The conidia was counted using Hemocytometer and five different concentrations, 2×10^{6}, 4×10^{6}, 6×10^{6}, 8×10^{6} & 10^{7} conidia/ml were prepared from the stock.

Pathogenicity studies were carried out using both direct and indirect application methods. Five different concentrations of the fungal inoculum were used in both the methods. Three replicates for each dose and ten larvae were used in each replicate. A control was also kept for each dose. In direct application, each dose was sprayed onto larvae placed

in sterile Petri-dish with a standardized atomizer, whereas in the indirect application each dose was applied onto teak leaf disc of size 5mm× 5mm. After inoculation, larvae were transferred into artificial diet and maintained at <80 per cent RH. Mortality of the test larvae was counted at 24h interval until complete death or pupation.

Mortality data from direct and indirect application were pooled and subjected to probit analysis and LC_{50} / LD_{50} and $LT_{10,}$ $LT_{50,}$ LT_{90} were determined. Software POLO PC [LeOra software, 1987], based on Finney (1972) was used for replication wise data.

RESULTS AND DISCUSSION

Over 750 dead insect specimens (cadavers) were collected during November 2003 to March 2006 from both moist deciduous forests and teak forest plantations. Of them, about 70 per cent belonged to fungi indicating the dominance of the group followed by bacteria

Overall fungal records

Fungal infection was recorded on insects belonging to Coleoptera, Dictyoptera, Diptera, Epimeroptera, Hemiptera, Hymenoptera, Isoptera, Lepidoptera, Odonata, and Orthoptera. Insects belonging to 61 families were found to be infected. The highest fungal isolations were obtained from the family, Formicidae under Hymenoptera followed by Noctuidae under Lepidoptera.

A total of 401 sporulating fungi were isolated from diseased insect cadavers collected during the study period, of which 316 fungi were collected from Moist Deciduous Forests (MDF) and 85 from teak plantations. Compared to the dry period, the number of insects infected with fungi was more during the wet period.

Entomopathogenic fungi in moist deciduous forests

In the Central Circle of forest, frequent occurrence of *Beauveria bassiana* on *Gargara mixta* feeding on leaves of *Helicteres isora* and *Terminalia catappa* was observed both during dry and wet period at Vazhachal and Peechi, Epizootics of *Stilbella* sp. on ants were observed during the wet period at Vazhachal. Fungi were collected from 27 host families (Table 1). *B. bassiana*, which causes muscardine disease, has been reported from a number of insects including forest pests (Nirmita and Joshi, 2004; Sankaran *et al.*, 1989; Mohammed Ali *et al.*, 1991; Agarwal *et al.*, 1985),.

In the Northern Circle, occurrence of *Nomuraea* sp. on cicada was observed frequently during the wet period at Nilambur. Fungi were collected from 41 host families (Table 2).

In the Southern Circle, frequent occurrence of the fungal infected specimens was not observed and the collected fungi confined only to 23 host families (Table 3).

Table 1. Entomopathogenic fungi associated with different host insects in Moist deciduous forests and teak plantations (Central circle)

Host order	Host Family	Fungal Species	MDF (Isolates)	Teak (Isolates)	Total
Coleptera	Anthribidae	*Paecilomyces amoenoroseus*		1	1
	Bruchidae	*Beauveria bassiana*		1	1
	Cerambycidae	*Acremonium* sp.		1	1
		Aspergillus janus		1	1
		Beauveria bassiana	1		1
	Chrysomelidae	*Acremonium* sp.	1		1
		Aspergillus niger	1		1
		Aspergillus sulphureus		1	1
		Beauveria brongniartii		17	17
		Beauveria bassiana	3		3
		Cladosporium cladosporioides		1	1
		Paecilomyces amoenoroseus		1	1
		Verticillium lecanii		1	1
	Coccinellidae	*Acremonium* sp.	1		1
	Curculionidae	*Acremonium* sp.		1	1
		Alternaria sp.3	1		1
		Aspergillus flavus		1	1
		Aspergillus fumigatus	1		1
		Beauveria bassiana	1		1
		Fusarium camptoceras	1		1
		Pencilliuni terrestre series		1	1
	Scarabaeidae	*Alternaria alternata*		1	1
		Aspergillus janus	1		1
		Beauveria bassiana	2		2
		Pencillium duclauxi		1	1

	Scolytidae	*Paecilomyces amoeneroseus*	1		1
Dictyoptera	Blattidae	*Aspergillus sulphureus*		1	1
		Paecilomyces javanicus	1		1
		Verticillium lecanii	1		1
Hemiptera	Cercopidae	*Aspergillus flavus*		1	1
		Fusarium oxysporum	1	2	3
	Cicadidae	*Nonuraea* sp.1	1		1
	Coccidae	*Acremonium* sp.	1		1
		Alternaria sp.2	1		1
	Membracidae	*Acremonium* sp.	2		2
		Aspergillus flavus		1	1
		Aspergillus sulphureus	1		1
		Beauveria bassiana	11		11
		Fusarium moniliforme	1		1
		Pencillium tardum	1		1
	Pentatomidae	*Alternaria* sp.1	1		1
		Aspergillus niger	1		1
		Fusarium oxysporum		1	1
	Pseudococcidae	*Fusarium oxysporum*		1	1
	Pyrrhocoridae	*Fusarium oxysporum*		1	1
	Reduvidae	*Aspergillus janus*	1		1
		Fusarium lateritium	1		1

Hymenoptera	Apidae	*Aspergillus sclerotiorum*	1		1
		Metarhizium anisopliae	1		1
	Chalcididae	*Aspergillus janus*		1	1
	Formicidae	*Akanthomyces* sp.1	1		1
		Aspergillus niger	2		2
		Aspergillus sclerotiorum	1		1
		Aspergillus sulphureus	1		1
		Aspergillus versicolor group	1		1
		Beauveria bassiana	1		1
		Cladosporium cladosporioides	3		3
		Cordyceps sp.1	2		2
		Fusarium lateritium	1		1
		GlioclaFdium roseum	1		1
		Massospora sp.1	1		1
		Metarhizium anisopliae	1		1
		Paraisaria sp1	1		1
		Pencillium tardum	1		1
		Stilbella sp.1	13		13
		Stilbella sp.2	1		1
	Vespidae	*Beauveria bassiana*	1		1
		Paecilomyces fumosoroseus	1		1
Isoptera	Termittidae	*Aspergillus flavus*	1		1
		Beauveria bassiana	2		2
		Fusarium solani	1		1
		Verticillium lecanii	1		1
Lepidoptera	Arctidae	*Acremonium* sp.	1		1
		Aspergillus niger	1		1
		Beauveria bassiana	1		1
		Fusarium camptoceras		1	1
		Pencillium sp.1		1	1
		Verticillium lecanii	1		1

Lepidoptera	Gelechidae	*Fusarium lateritium*	1		1
		Paecilomyces amoeoeroseus	1		1
	Geometridae	*Aspergillus janus*	1		1
		Fusarium lateritium	1		1
		Mucor sp.1	1		1
		Pleurodesmospora sp.1	1		1
	Hespiridae	*Fusarium lateritium*		1	1
	Lymantridae	*Aspergillus niger*	1		1
		Aspergillus ochraceus		1	1
	Noctuidae	*Alternaria* sp.3	1		1
		Aspergillus flavus	2		2
		Aspergillus janus		2	2
		Aspergillus sclerotiorum	1	1	2
		Cladosporium cladosporioides	2	1	3
		Fusarium lateritium	4		4
		Fusarium solani	4		4
		Mucor sp.1	3		3
		Pencillium sp.1	2		2
		Pencillium tardum	1		1
	Pieridae	*Aspergillus flavus*	1		1
		Aspergillus niger	1		1
		Pencillium sp.1	1		1
	Psychidae	*Pencillium tardum*	2		2
		Pleurodesmospora sp.1	3		3
	Pyralidae	*Aspergillus janus*	2		2
		Pencillium tardum		1	1
	Sphingidae	*Aspergillus janus*	1		1
	Syntomidae	*Fusarium moniliforme*		1	1
		Fusarium solani	1		1
			125	49	174

Table 2. Entomopathogenic fungi associated with different host insects in moist deciduous forests and teak plantations (Northern circle)

Host order	Host Family	Fungal Species	MDF (Isolates)	Teak (Isolates)	Total
Coloptera	Buprestidae	*Pencillium tardum*	1		1
	Cerambycidae	*Aspergillus flavus*	1		1
	Chrysomelidae	*Aspergillus flavus*	1		1
		Aspergillus fumigatus		1	1
		Aspergillus sclerotiorum	1		1
		Beauveria brongniartii	1		1
	Coccinellidae	*Alternaria* sp.1	1		1
		Fusarium lateritium	1		1
	Curculionidae	*Ascomycete* sp.1		1	1
		Aspergillus sclerotiorum		1	1
		Mucor sp.1		2	2
	Dyticidae	*Paecilomyces fumosoroseus*	1		1
	Elateridae	*Gliocladium roseum*	1		1
		Pencillium sp.1	1		1
	Meloidae	*Aspergillus niger*		1	1
		Beauveria bassiana	1		1
	Hispidae	*Fusarium moniliforme*	1		1
	Scarabaeidae	*Aspergillus niger*	1		1
		Chaetomium sp.1	1		1
		Fusarium moniliforme	1		1
		Paecilomyces fumosoroseus	1		1
		Beauveria bassiana	1		1
	Scolytidae	*Beauveria bassiana*	1		1
	Tenebrionidae	*Aspergillus sclerotiorum*	1		1
Dictyoptera	Blattidae	*Aspergillus niger*	1		1
Diptera	Bombylidae	*Aspergillus niger*	1		1

Diptera	Calliphoridae	*Erynia* sp.1		1	1
		Mucor sp.1		1	1
Ephemeroptera	Ephemeridae	*Paecilomyces amoeneroseus*	1		1
Hemiptera	Belostomatidae	*Aspergillus parasiticus*	1		1
	Cicadidae	*Aspergillus fumigatus*	1		1
		Nonuraea sp.1	3		3
		Paecilomyces amoeneroseus	1		1
		Pencillium sp.1	1		1
		Pencillium thomii series	1		1
	Euribrachyidae	*Acremonium sp*	1		1
		Aspergillus niger	1		1
	Membracidae	*Beauveria bassiana*	1		1
	Pentatomidae	*Aspergillus flavus*	1		1
		Aspergillus sclerotiorum	1		1
		Paecilomyces amoeneroseus	1		1
		Pencillium tardum	1		1
	Pseudococcidae	*Acremonium* sp.	1	1	2
		Paecilomyces fumosoroseus	1		1
	Pyrrhocoridae	*Aspergillus janus*	1		1
		Aspergillus niger	1		1
		Pencillium tardum	1		1
Hymenoptera	Apidae	*Acremonium sp*	1		1
		Aspergillus flavus	1		1
		Aspergillus niger	1		1
		Alternaria sp.3	1		1
		Cladosporium cladosporioides	1		1
	Formicidae	*Acremonium* sp.1	1		1
		Alternaria sp.1	1		1
		Aspergillus flavus	4	1	5

Hymenoptera	Formicidae	*Aspergillus fumigatus*		1	1
		Aspergillus niger	4		4
		Cladosporium cladosporioides		1	1
		Cordyceps sp.1	13		13
		Paecilomyces amoeneroseus	1		1
		Paraisaria sp.2	1		1
		Pencillium lanosocoeruleum	1		1
		Pencillium sclerotiorum	1		1
		Pencillium sp.1	1		1
		Pencillium terrestre series		1	1
	Ichneumonidae	*Aspergillus niger*	1		1
		Beauveria bassiana		1	1
	Vespidae	*Aspergillus flavus*	1		1
		Cladosporium cladosporioides	1		1
		Gliocladium roseum	1		1
		Pencillium camemberti	1		1
Isoptera	Termittidae	*Aspergillus flavus*	1		1
		Pencillium funiculosum	1		1
Lepidptera	Arctidae	*Acremonium* sp.	1		1
		Aspergillus niger	1	1	2
		Aspergillus sulphureus	1		1
	Danaidae	*Aspergillus niger*	1		1
		Aspergillus ochraceus	1		1
		Curvularia lunata	1		1

Lepidptera	Geometridae	*Aspergillus parasiticus*	1		1
	Hespiridae	*Acremonium* sp.	1		1
		Aspergillus sclerotiorum		1	1
		Beauveria bassiana	1		1
	Lymantridae	*Aspergillus niger*	1		1
		Aspergillus parasiticus	1		1
		Chaetomium sp.1	1		1
		Fusarium lateritium	1		1
	Noctuidae	*Alternaria* sp.3	1		1
		Aspergillus flavus	1		1
		Aspergillus janus	1		1
		Aspergillus sclerotiorum		1	1
		Aspergillus sulphureus	2		2
		Cladosporium cladosporioides	1		1
		Fusarium lateritium		1	1
		Fusarium moniliforme	1		1
		Paecilomyces fumosoroseus	1		1
	Nymphalidae	*Alternaria* sp.1	1		1
		Aspergillus sclerotiorum	1		1
		Aspergillus sulphureus	1		1
		Curvularia lunata	1		1
		Mucor sp.1	1		1
	Papilionidae	*Aspergillus sclerotiorum*	1		1
		Paecilomyces amoeneroseus	1		1

Lepidptera	Pieridae	*Aspergillus niger*	1		1
		Aspergillus sulphureus	1		1
		Paecilomyces amoenoroseus	1		1
	Pyralidae	*Paecilomyces fumosoroseus*	1		1
	Pyraustidae	*Verticillium lecanii*	1		1
	Yponomeutidae	*Aspergillus janus*	1		1
		Beauveria brongniartii	1		1
		Fusarium lateritium	1		1
Odonata	Aeshnidae	*Aspergillus niger*		1	1
Orthoptera	Acrididae	*Aspergillus ochraceus*	1		1
		Beauveria bassiana	1		1
		Pencillium tardum	1		1
	Grillidae	*Aspergillus flavus*	1		1
		Metarhizium anisopliae	1		1
		Paecilomyces fumosoroseus	1		1
	Tettigonidae	*Fusarium* sp.1	1		1
			125	19	144

Table 3. Entomopathogenic fungi associated with different host insects in moist deciduous forests and teak plantations (Southern circle)

Host order	Host Family	Fungal Species	MDF (Isolates)	Teak (Isolates)	Total
Coleoptera	Anthribidae	*Aspergillus niger*	1		1
		Fusarium solani	1		1
	Bostrychidae	*Aspergillus flavus*	1		1
	Chrysomelidae	*Alternaria* sp.1	1		1
		Alternaria sp.2	1		1
		Aspergillus fumigatus	1		1
		Gliocladium roseum	1		1
	Elateridae	*Colletotrichum gloeosporioides*	1		1
	Scarabaeidae	*Aspergillus janus*	1		1
		Aspergillus ochraceus	1		1
	Scolytidae	*Aspergillus sulphureus*	1		1
Diptera	Culicidae	*Aspergillus sclerotiorum*	1		1
	Tabanidae	*Pencillium tardum*	1		1
Hemiptera	Coccidae	*Aspergillus janus*		1	1
		Cladosporium	1		1
		cladosporioides			
		Paecilomyces fumosoroseus	1		1
	Coreidae	*Aspergillus niger*	1		1
		Paecilomyces fumosoroseus	1		1
	Membracidae	*Beauveria bassiana*	2		2
	Pentatomidae	*Acremonium* sp.	1		1
		Aspergillus ochraceus	1		1
		Metarhizium anisopliae		1	1
	Pseudococcidae	*Aspergillus janus*	1		1
	Pyrrhocoridae	*Aspergillus sclerotiorum*	1		1
Hymenoptera	Apidae	*Acremonium* sp.		1	1
		Aspergillus janus	2		2
		Aspergillus wentii	1		1

Hymenoptera		*Cladosporium cladosporioides*	1		1
		Pencillium brevicompactum	1		1
		Pencillium tardum	1		1
	Formicidae	*Akanthomyces* sp.2		1	1
		Alternaria sp.1	1		1
		Alternaria sp.2	1		1
		Aspergillus flavus	1	1	2
		Aspergillus janus	1	4	5
		Aspergillus niger	2		2
		Aspergillus sclerotiorum	1		1
		Aspergillus versicolor group	1		1
		Gliocladium roseum	1		1
		Metarhizium anisopliae		1	1
		Paecilomyces amoenoroseus	1		1
		Paecilomyces fumosoroseus		1	1
Lepidoptera	Arctidae	*Acremonium* sp.	1		1
		Alternaria sp.1	1		1
		Aspergillus niger	1		1
		Aspergillus parasiticus	1		1
	Ascilidae	*Metarhizium anisopliae*		1	1
	Geometridae	*Aspergillus niger*	1		1
		Pencillium tardum	1		1
	Hyblaeidae	*Acremonium* sp.		1	1
	Lymantridae	*Gliocladium roseum*		1	1
	Noctuidae	*Alternaria* sp.1	1		1
		Aspergillus flavus	1		1
		Aspergillus janus	4		4
		Aspergillus niger		1	1
		Aspergillus sulphureus	1		1
	Noctuidae	*Fusarium lateritium*		1	1
		Gliocladium roseum		1	1
		Paecilomyces fumosoroseus	2		2
		Pencillium sp.1	1		1

Lepidoptera	Nymphalidae	*Aspergillus oryzae*	1		1
		Aspergillus sclerotiorum	1		1
	Psychidae	*Fusarium lateritium*	1		1
	Pyralidae	*Aspergillus janus*	1		1
		Pencillium citrinum	1		1
	Saturnidae	*Metarhizium anisopliae*	1		1
Orthoptera	Tettigonidae	*Acremonium* sp.	1		1
		Aspergillus flavus	1		1
		Aspergillus janus	1		1
		Aspergillus ochraceus	1		1
		Pencillium sp.1	1		1
			66	17	83

Table 4. Entomopathogenic fungi collected from moist deciduous forests of different Forest Circles.

Indices	Central Circle	Northern Circle	Southern Circle
Total number of fungi collected	125	125	66
Number of fungal species	38	36	27

The number of fungi collected was comparable both in Central (125) and Northern circles (125) whereas it was low in Southern circle (66) (Table 4).

There was a significant increase in the number of fungi obtained with increase in host diversity in the moist deciduous forests (MDF). In MDF, maximum number of occurrence was shown by *Beauveria bassiana* followed by *Aspergillus niger*. *A. niger* covered maximum number of host families (20 insect families) followed by *B. bassiana* (15 insect families). *Cordyceps* sp.1 collected from Northern and Central circles were isolated from ants. Of the total fungi collected largest host range was covered by *A. niger* (7 insect orders) followed by *B. bassiana* (6 insect orders).

Entomopathogenic fungi in teak plantations

In the Central circle, *B. brongniartii* epzootic was observed in beetles (Coleoptera: Chrysomelidae) feeding on teak leaves during the dry period in Vazhani teak plantation. The fungal species collected from 19 host families are listed in Table 1.

In the Northern Circle *Erynia sp.* was recorded only from Nilambur during wet period. The Fungi recorded from 11 host families are listed in Table 2.

In the Southern circle fungi were collected only from 8 host families (Table 3).

The number of fungi collected was high in the Central circle (49) and was low in Southern circle (17). Total number of species collected was also high in Central circle (20) and less in Southern circle (9) (Table 5).

Table 5. Total number of entomopathogenic fungi and its species collected from Teak plantations of different Forest Circles

Indices	Central Circle	Northern Circle	Southern Circle
Total number of fungi collected	49	19	17
Number of species	20	12	9

In the teak plantation, *B. brongniartii* was the most frequently recorded entomopathogenic fungi followed by *A. janus.* While *Acremonium* sp. and *Aspergillus janus were* recorded from 5 insect families, *Fusarium oxysporum* infection was observed in 4 insect families. Largest host range was shown by *Acremonium* sp. and *A. janus* (5 insect orders each).

Other sites

The number of fungi collected from other forest types and plantations was very low compared to permanent sites. Some fungal species like *Pleurodesmospora* sp. and *Massospora* sp. were collected only from Peechi. *Cordyceps* sp was more prevalent in Silent Valley. No fungal infection was observed on insect samples from Waynad and Munnar.

A few fungal isolates were obtained from forest pests, *viz.*, *Acremonium* sp. from *Hyblaea puera* and *Aspergillus flavus, Beauvaria bassiana, Fusarium solani, Pencillium funiculosum and Verticillium lecani* from subterranean termites.

Fungal pathogens offer good scope for pest suppression. One advantage of looking for wild type isolates of fungal strains from different hosts would be to isolate new virulent strains. Also with modern biotechnological approaches it should be possible to combine complex traits associated with pathogenicity and improved strains can be obtained.

Entomopathogenic bacteria and viruses

Bacteria infected specimens were collected from a wide range of hosts both from the moist deciduous forests and teak plantations. Bacterial collections were obtained mainly from Lepidoptera, Coleoptera, Diptera, Hymenoptera, Homoptera, Odonata and Orthopteran groups of insects. Infected specimens were collected more during the wet period (Tables. 6, 7). Epizootics of bacterial infection was rare in forest insects and as a group, they probably have little impact compared to viruses and fungi. Lacey *et al.* (2001) reported similar observations based on studies carried out in agriculture and forest insect pests.

Table 6. Entomopathogenic virus and bacteria recorded on insects in moist deciduous forests

Location	Wet period				Dry period			
	Host	Virus isolations	Host*	Bacteria isolations	Host	Virus isolations	Host*	Bacteria isolations
Vazhachal	Lep.	4	Lep. Hym. Orth.	16 2 1	Lepi.	1	Odo Lep. Homo.	1 9 2
Vazhani	Lep.	6	Col.	1	-	-	Col. Hym.	1 2
Peechi	Lep.	1	Lep. Hym.	15 1	Lepi.	1	Col. Lep.	1 2
Nilambur	Lep.	4	Lep. Col. Dip.	11 2 1	Lepi.	2	Lep. Hym.	3 6
Konni	Lep.	2	Lep. Col.	2 1	Lepi.	1	Lep.	2

*Col- Coleoptera; Dip- Diptera; Hom- Homoptera; Hym- Hymenoptera; Lep-Lepidoptera; Odo- Odonata; Orth- Orthoptera

Virus infected specimens were collected from Lepidoptera and Homopteran insects (Table 6, 7). Epizootics of Nuclear Polyhedrosis Virus (NPV) causing death of the teak defoliator (*H. puera*) were observed during the months ranging from May to October.

Table 7. Entomopathogenic virus and bacteria recorded on insects in teak plantations

Location	Wet period				Dry period			
	Host*	Virus isolations	Host	Bacteria isolations	Host*	Virus isolations	Host	Bacteria isolations
Vazhachal	Lep.	3	Lep. Hym.	7 1	Lep.	2	Lep.	2
Vazhani	Lep.	3	Lep.	2	Lep.	1	Col. Lep.	1 2
Peechi	Lep.	2	Lep.	2	Lep. Homo.	2 2	Lep.	2
Nilambur	Lep.	4	Lep. Orth.	4 2	Lep.	2	Lep.	1
Konni	Lep.	3	Lep. Col. Homo. Orth.	6 1 1 2	Lep	4	Lep. Orth. Homo. Col.	2 1 1 2

*Col- Coleoptera; Hom- Homoptera; Hym-Hymenoptera; Lep-Lepidoptera; Orth- Orthoptera

Compared to entomopathogenic fungi, the bacterium, *Bacillus thuringiencis* (*B.t*) and baculoviruses, particularly nucleopolyhedroviruse (NPV) are potential microbial control agents. It has been documented that *B.t* strains could be transferred by vectoring genetic materials by protoplast transformation, transduction and conjugation. Thus it is possible to modify the host spectrum of certain *B.t* strains. But what is needed is to record new *B.t* strains from different hosts and evaluate their potential. In forestry, bacterial insecticides are widely used on account of its safety to environment. The baculovirus, both nuclear polyhedrosis viruses (NPV) and Granulosis Virus (GV) are host specific and are safe for use in both agriculture and forestry pest scenario. Studies on various aspects of HpNPV to manage the teak defoliator were carried out in KFRI (Nair *et al.* 1998; Sudheendrakumar *et al.*, 2001).

A large number of microbes were collected as a part of this study. However, it is possible to record many more, if surveys are intensified. Thus the documentation of occurrence and distributional pattern of various microbes are to be continued. Identification and species description have to be accelerated to know more about the microbial wealth of our country. Lack of taxonomical expertise to identify microbes is an impediment to understand diversity of microbes in our forests and there is a real need to strengthen the field of microbial taxonomy.

Isolation and Identification

All the 750 insects specimens collected were processed in the laboratory. Out of this, 73.33 per cent of cadavers were infected with fungi followed by bacteria (18.33 per cent), viruse (7.67 per cent) and nematodes (0.67 per cent) (Fig. 1).

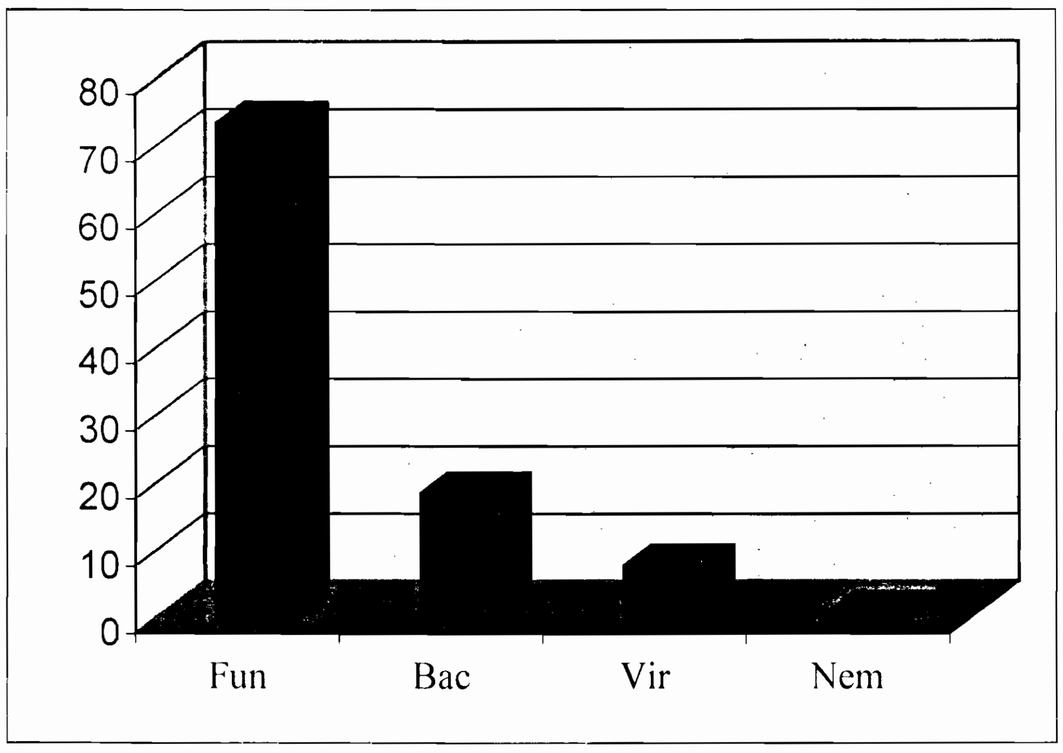

Fig. 1. Percentage of insect cadavers infected with microbial pathogens

Some fungi like *Erynia* sp. was isolated using special media- Sabouraud Dextrose Agar (SDA) supplemented with egg yolk and milk. *Massospora* sp. collected was could not be cultured. *Cordyceps* sp. was isolated on water agar by forceful dispersion of spores under light.

A total of 58 species of fungi were identified. Most of them belonged to the class Deuteromycota (18 genera). Under the class Ascomycota, the fungi collected belonged to

2 identified genera and one unidentified genus. The class Zygomycota was represented by 3 genera (Table 8).

Table 8. List of identified fungal pathogens

Class	SI. No	Fungal Species
Deuteromycota	1.	*Acremonium* sp.
	2.	*Akanthomyces* sp.1
	3.	*Akanthomyces* sp.2
	4.	*Alternaria alternata*
	5.	*Alternaria* sp.1
	6.	*Alternaria* sp.2
	7.	*Alternaria* sp.3
	8.	*Aspergillus flavus*
	9.	*Aspergillus fumigatus*
	10.	*Aspergillus janus*
	11.	*Aspergillus niger*
	12	*Aspergillus ochraceus*
	13	*Aspergillus oryzae*
	14	*Aspergillus parasiticus*
	15	*Aspergillus sclerotiorum*
	16	*Aspergillus sulphureus*
	17	*Aspergillus versicolor group*
	18	*Aspergillus wentii*
	19	*Beauveria brongniartii*
	20	*Beauveria bassiana*
	21	*Cladosporium cladosporioides*
	22	*Colletotrichum gloeosporioides*
	23	*Curvularia lunata*
	24	*Fusarium camptoceras*
	25	*Fusarium lateritium*
	26	*Fusarium moniliforme*
	27	*Fusarium oxysporum*
	28	*Fusarium solani*
	29	*Fusarium* sp.1
	30	*Gliocladium roseum*
	31	*Metarhizium anisopliae*
	32	*Nomuraea* sp.1
	33	*Paecilomyces amoeneroseus*
	34	*Paecilomyces fumosoroseus*
	35	*Paecilomyces javanicus*
	36	*Paraisaria* sp.1
	37	*Paraisaria* sp.2

	38	*Pencillium brevicompactum*
	39	*Pencillium camemberti*
	40	*Pencillium citrinum*
	41	*Pencillium duclauxi*
	42	*Pencillium funiculosum*
	43	*Pencillium lanosocoeruleum*
	44	*Pencillium sclerotiorum*
	45	*Pencillium* sp.1
	46	*Pencillium tardum*
	47	*Pencillium terrestre* series
	48	*Pencillium thomii* series
	49	*Pleurodesmospora* sp.
	50	*Stilbella* sp.1
	51	*Stilbella* sp.2
	52	*Verticillium lecanii*
Ascomycota	53	*Chaetomium* sp.
	54	*Cordyceps* sp.
	55	Unidentified
Zygomycota	56	*Mucor* sp.
	57	*Erynia* sp.
	58	*Massospora* sp.

Several non-sporulating fungi and nematode trapping fungi were also isolated. Only a few isolates were obtained from forest pests like *Acremonium* sp. from *Hyblaea puera* and Aspergillus flavus, *Beauveria bassiana, Fusarium solani, Pencillium funiculosum* and *Verticillium lecani* from termites. Species like *Akanthomyces* sp. 2 and *Paraisaria* sp.2 recorded from ants are apparently new to science.

Bacteria belonging to four genera namely, *Bacillus*, *Pseudomonas*, *Serratia* and *Steptococcus* were identified. Two viruses namely *Hyblaea puera* nucleopolyhedro virus on the teak defoliator, *H. puera* and a granulosis virus on an unidentified lepidopteran larva were identified.

Bioassay

The efficacy of selected fungi like *Beauveria brongniartii*, *Paecilomyces fumosoroseus* and *Metarhizium anisopliae* were evaluated against the teak defoliator, *Hyblaea puera*.

In direct application using *B. brongniartii,* mortality of *H.puera* larvae ranged from 53.33 to 100 per cent at concentrations ranging from 2×10^6 to 10^7conidia/ml, whereas in indirect application, mortality ranged from 30 to 70 per cent in concentrations ranging from 2×10^6 to 10^7conidia/ larva (Fig. 2).

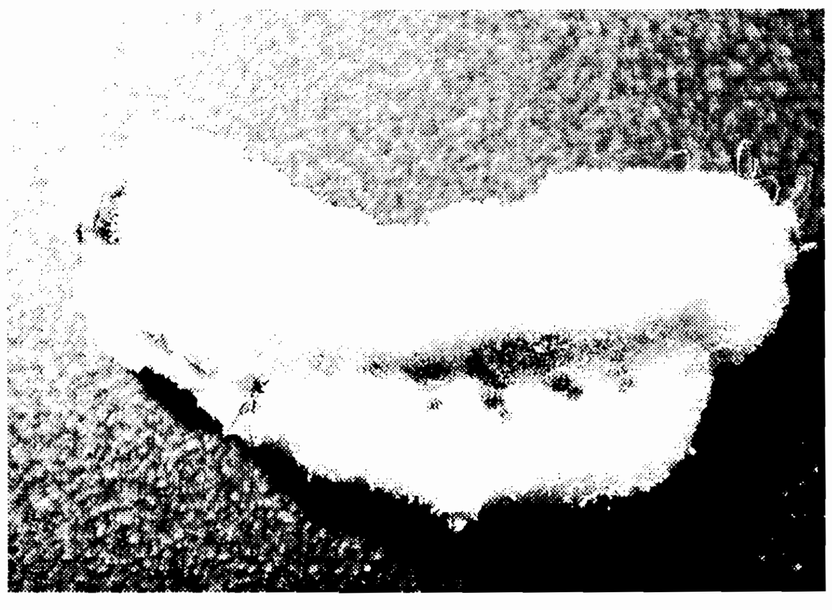

Fig.2. *H. puera* larva infected with *Beauveria brongniartii*

In both the application methods, death started from second day and reached 100 per cent in direct application by ninth day whereas in indirect application 50 per cent larval mortality occurred by nineth day for the dose 10^7 conidia/ ml (Fig. 3).

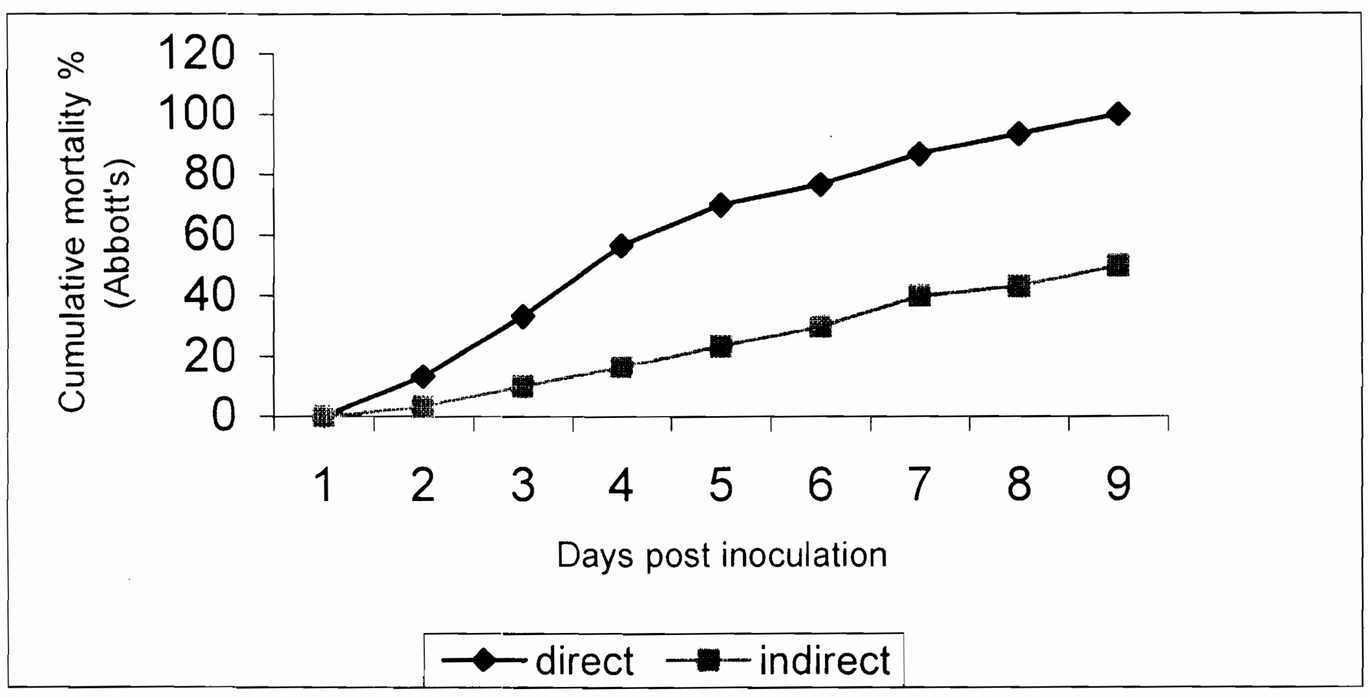

Fig. 3. Cumulative mortality of *H. puera* infected by *B. brongniartii* (10^7 conidia/ml)

In the case of *P. fumosoroseus* mortality of *H. puera* (Fig. 4) in direct application ranged from 50 to 100 per cent at concentrations ranging from 2×10^6 to 10^7 conidia/ml, whereas in indirect application, mortality ranged from 30 to 63 per cent. In both the methods of application, larval death started from third day and reached 93 per cent mortality in direct

application and 48 per cent in indirect application by the nineth day for the dose 10^7 conidia/ml (Fig, 5).

Fig. 4. *H. puera* larva infected with *P. fumoso-roseus*

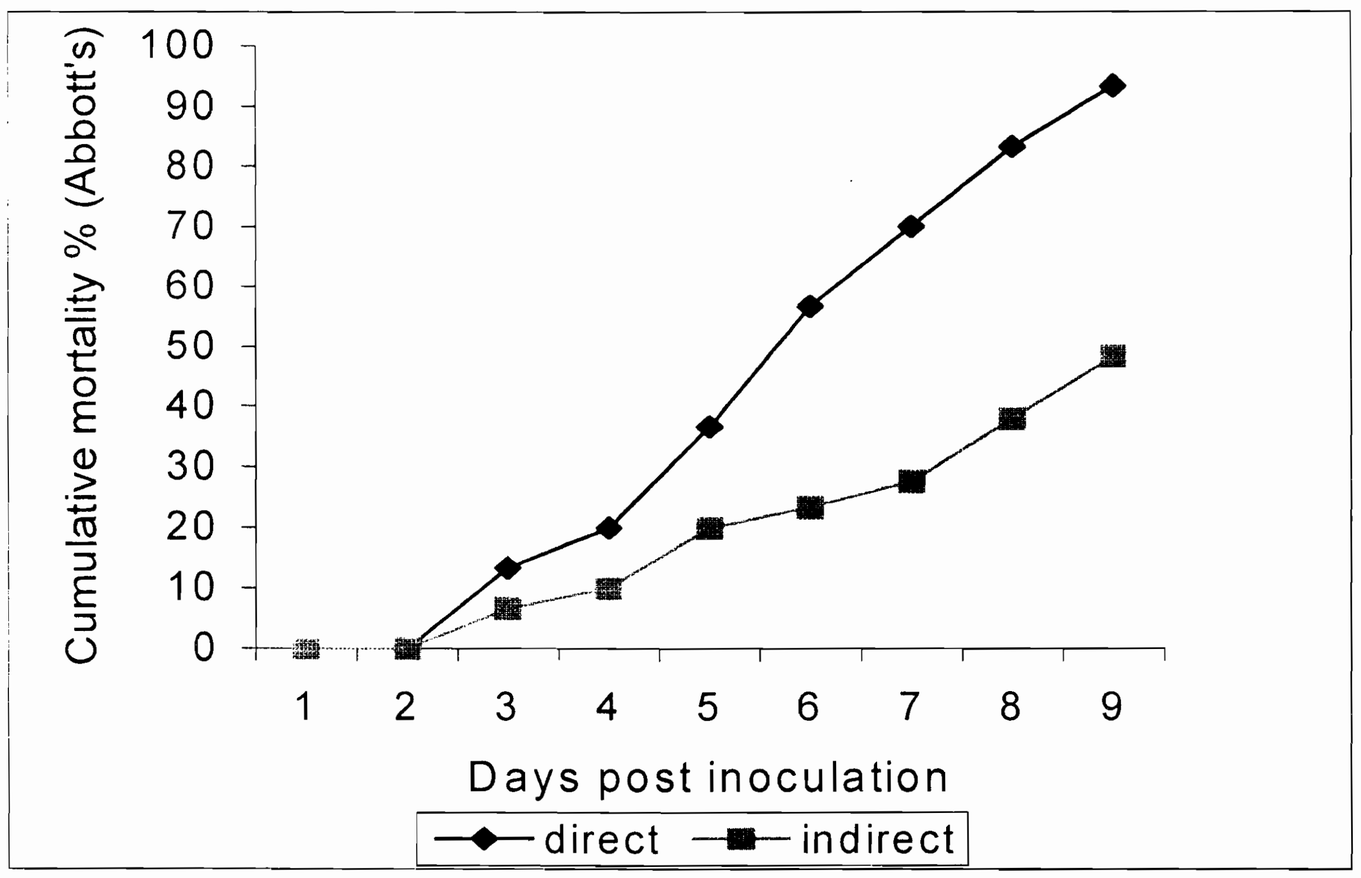

Fig. 5. Cumulative mortality of *H. puera* due to application of *P. fumoso-roseus* (10^7 conidia/ml)

Direct application of *M. anisopliae* caused 53 to 80 per cent mortality of the larvae at concentrations ranging from 2×10^6 to 10^7 conidia/ml, whereas mortality ranged from 20 to 56.6 per cent in indirect application (Fig. 6),. In direct application method death started from 2^{nd} day and reached 65.52 per cent whereas in indirect application death started only from 3^{rd} day and reached 43 per cent mortality by nineth day (Fig. 7).

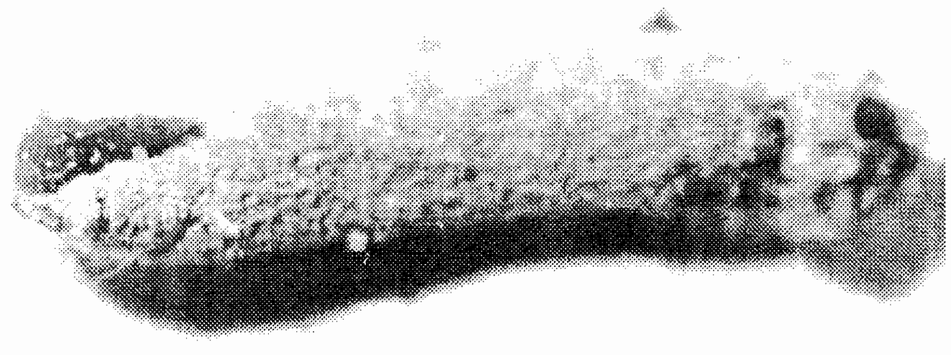

Fig. 6. *H. puera* larva infected with *M. anisoplia*

In both application methods, infection was noticed in the larvae with characteristic symptoms of fungal death, but appearance of external mycelial growth was noticeable early in direct application compared to indirect application. Re-isolation of the pathogens was carried out from the dead larvae by random selection.

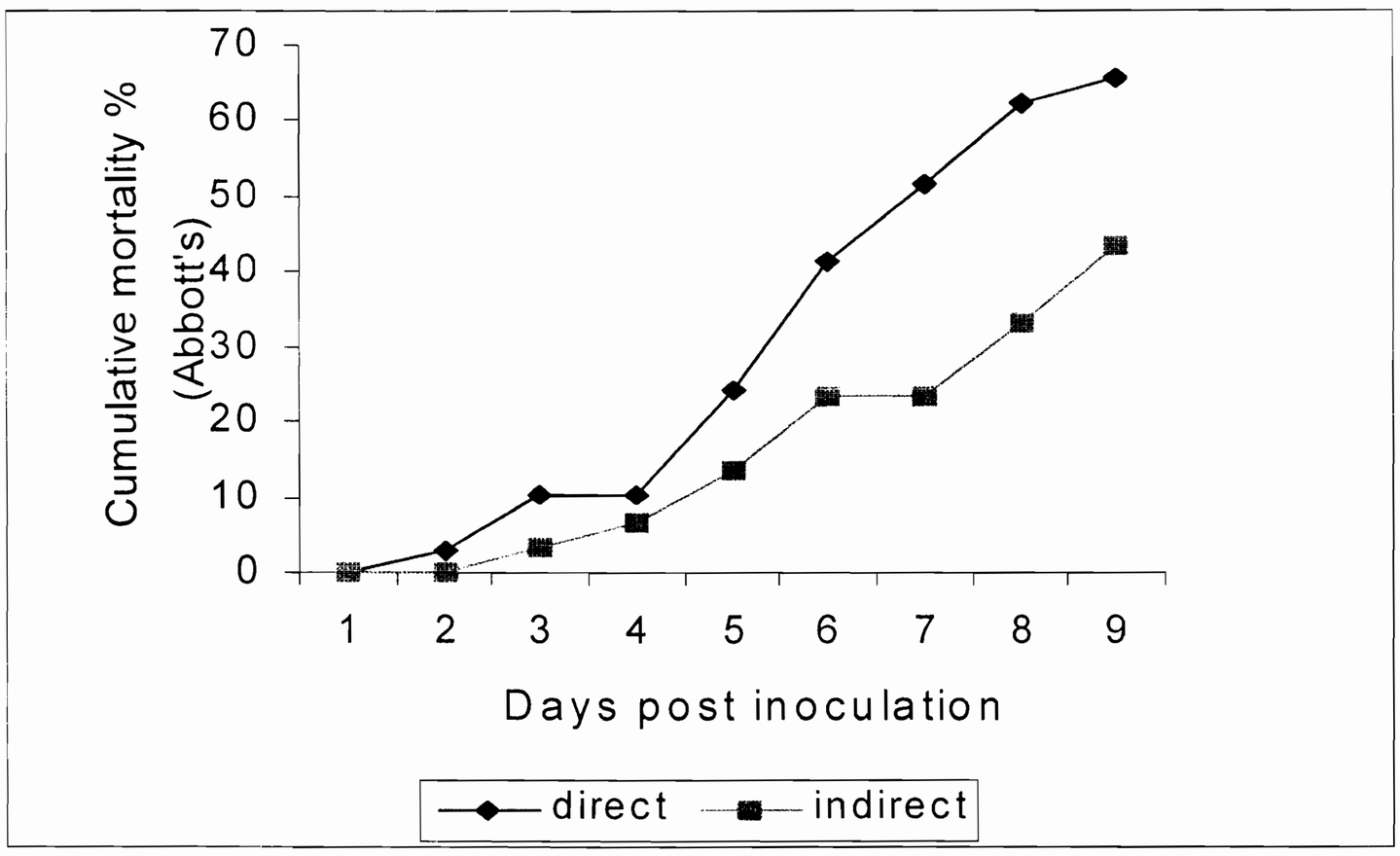

Fig. 7. Cumulative mortality of *H. puera* due to application of *M. anisopliae* (10^7 conidia/ml)

For all the three strains tested, LC_{50} in direct application was low compared to LD_{50} value in indirect application. In direct application least LC_{50} value was obtained for *B. brongniartii,* 1.89×10^6 conidia/ml (Table 10), followed by *P. fumoso-roseus*, 2.03×10^6 conidia/ml (Table 11) and *M. anisopliae,* 2.33×10^6 conidia/ml (Table 12). In indirect application also least LD_{50} was obtained for *B. brongniartii*, 6.50×10^6 conidia/ml

followed by *P. fumoso-roseus*, 7.03×10^6 conidia/ml and *M. anisopliae,* 7.84×10^6 conidia/ml.

Table 10. LC_{50} and LD_{50} values of *B. brongniartii* against 3rd instar larvae of *H. puera*

Mode of application	LC50/LD50 (Conidia/ml)	95 % CI		Slope	χ^2	df
		Lower	Upper			
Direct (LC_{50})	1.89×10^6	1.55×10^6	2.19×10^6	1.804±.148	5.8943	11
Indirect (LD_{50})	6.50×10^6	5.88×10^6	7.24×10^6	1.726±.168	4.6857	11

Table 11. LC_{50} and LD_{50} values of *P. fumosoroseus*. against third instar larvae of *H. puera*

Mode of application	LC50/LD50 (Conidia/ml)	95 % CI		Slope	χ^2	df
		Lower	Upper			
Direct (LC_{50})	2.03×10^6	1.69×10^6	2.34×10^6	1.832±.147	4.7870	11
Indirect (LD_{50})	7.03×10^6	6.27×10^6	8.05×10^6	1.510±.168	3.7940	11

Table 12. LC_{50} and LD_{50} values of *M. anisopliae* against 3rd instar larvae of *H. Puera*

Mode of application	LC50/LD50 (Conidia/ml)	95 % CI		Slope	χ^2	df
		Lower	Upper			
Direct (LC_{50})	2.33×10^6	1.67×10^6	2.89×10^6	1.093±.149	4.8659	11
Indirect (LD_{50})	7.84×10^6	6.99×10^6	9.04×10^6	1.436±.142	4.4203	11

LT_{10}, LT_{50} and LT_{90} values for the three strains in direct application was low compared to indirect application. In direct application, least LT_{50} value was obtained for *B. brongniartii,* 3.75 days (Table 13), followed by *P. fumoso-roseus*, 5.46 days (Table 14) and *M. anisopliae*, 6.81 days (Table 15). However, highest LT_{10} value was obtained for *P. fumoso-roseus* (3.22 days). In indirect application also least LT_{50} value was obtained for *B. brongniartii*, 8.51days, followed by *P. fumoso-roseus*, 9.32 days and *M.anisopliae,* 10.04 days. However, least LT_{90} value was obtained for *P. fumoso-roseus*.

Table 13. LT values of *B. brongniartii* against third instar larvae of *H.puera* (10^7 spores per larva)

Mode of application	LT values		95 per cent CI		Slope	χ^2	df
			Lower	Upper			
Direct	LT_{10}	1.95	1.52	2.31	4.505±.493	4.0118	23
	LT_{50}	3.75	3.34	4.14			
	LT_{90}	7.22	6.38	8.56			
Indirect	LT_{10}	3.23	2.38	3.39	3.037±418	3.6754	29
	LT_{50}	8.51	7.52	10.02			
	LT_{90}	22.49	17	36.06			

Table 14. LT values of *P. fumosoroseus* against third instar larvae of *H. puera* (10^7 spores per larva)

Mode of application	LT values		95 per cent CI		Slope	χ^2	df
			Lower	Upper			
Direct	LT_{10}	3.22	2.67	3.66	5.606±.657	3.91	23
	LT_{50}	5.46	5.03	5.91			
	LT_{90}	9.24	8.21	10.89			
Indirect	LT_{10}	3.97	3.07	4.66	3.461±.497	3.7478	29
	LT_{50}	9.32	8.28	10.99			
	LT_{90}	21.86	16.75	34.63			

Table 15. LT values of *M. anisopliae* against third instar larvae of *H. puera* (10^7 spores per larva)

Mode of application	LT values		95 per cent CI		Slope	χ^2	df
			Lower	Upper			
Direct	LT_{10}	3.07	2.33	3.63	3.764±.539	3.9687	23
	LT_{50}	6.81	6.08	7.85			
	LT_{90}	15.10	11.94	22.57			
Indirect	LT_{10}	4.56	3.60	5.24	3.733±.562	3.9558	29
	LT_{50}	10.04	8.92	12			
	LT_{90}	22.14	16.95	35.68			

LC_{50} and LT_{50} values of the above three strains tested indicate that direct application was better compared to indirect application. A virulent strain is one, which kills the pest within limited time (LT_{50}) and relatively at low doses (LC_{50}). Since the LC_{50} and LT_{50} was lower for *B. brongniartii*, it can be considered to be the most virulent fungal pathogen for use in the biological control of the teak dafliator. There is also much scope to evaluate this pathogen against other lepidopteran crop pests.

CONCLUSIONS

Microbial infection in insects was more prevalent in insects during the wet period compared to dry period.

Among the three major microbial pathogens isolated, fungi were the dominant followed by bacteria and viruses.

Abundance and diversity of the entomopathogenic fungi were significantly higher in moist deciduous forests compared to teak plantations. There was no significant difference on the diversity of entomopathogenic fungi associated with forest insects in the moist deciduous forests under the three forest circles. Diversity of fungi in teak plantations of southern circle was low compared to the central circle.

Epizootics of entomopathogenic fungi were frequently observed in the moist deciduousl forests in small pockets in Northern and Central circles, whereas epizootics of fungi were observed only in the teak plantation of Central circle.

The study recorded a number of pathogens which could be identified only upto generic level. Some of them are expected to be new species or new records.

The bioassay using three fungal strains, *Beauveria brongniartti*, *Paecilomyces fumoseroseus*, and *Meatrhizium anisopiae* against the teak defoliator, *Hyblea puera* proved their effectiveness as biocontrol agents.

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