1.1 Pest problems in forests

India’s forest cover was estimated to be about 67.701 million hectares or 22.8 percent of the country’s land area and other wooded lands comprise of 4.11 million ha (UNEP Report, 2000). The dense forests in almost all the major states has been reduced and forest degradation is a matter of serious concern now. Principal plantation species such as *Acacia* spp., *Eucalyptus* spp. and *Tectona grandis* Linn. are the main species occupying greater area in planted forests. The other species such as *Eucalyptus globules* Labill., *E. grandis* W.Hill ex Maiden, *E. tereticornis* Sm., *Acacia auriculiformis* A. Cunn. ex Benth., *A. catechu* (L.) Wild. Oliv., *Albizia* spp., *Azadirachta indica* (L.) Adelb., *Casuarina equisetifolia* L., *Dalbergia sissoo* Roxb., *Gmelina arborea* Roxb., *Populus* spp. *Prosopis* spp., *Shorea robusta* Roth, *Terminalia* spp., *Cedrus deodara* (Roxb.) G. Don and *Pinus roxburghii* Sargent are also planted for various commercial utilization purposes.

Growth and productivity of forests are adversely affected by frequent outbreaks of pests and diseases. Several groups of insects belonging to orders, Coleoptera, Hymenoptera, Lepidoptera and Isoptera are the major pests that cause high economic loss. The damage to nursery stock by forest insects is often considerable at times. The most important among the nursery pests are the cutworms, termites and cockchafers besides some defoliators, sapsuckers and shoot borers as major pests. Natural and plantation forest trees in India suffer serious seasonal insect outbreaks, which lead to considerable economic loss in the timber productivity.
1.2 Economic importance of xylophagous insects

While the folivorous pests reduce the leaf biomass and lead to impaired growth of trees, the wood feeding pests cause malformation of stem and final timber structure and also reduce the timber yield. The borer, *Xystrocera festiva* Pasc. (Nair, 2001; Michale et al., 2002) and *Zeuzera coffeae* Nietner affect the agro-forestry plantations and saplings of *A. mangium* Willd. and *Eucalyptus* spp. which decreases the quantity as well as the quality of timber (Manon et al., 2004). Two to three year old trees of *Paraserianthes falcatoria* (L.) Nielsen were also infested by *X. festiva*, where the larvae bore inside the stem and damage the plant completely. The borer, *X. festiva* has several other hosts including *Pithecellobium* sp., *Albizia saman* F. Muell. and *Enterolobium* sp. The estimated yield loss in young trees (4 year old) was about 12% and about 74% in mature trees (Nair, 2001).

The tusam pitch moth, *Dioryctria rubella* Hampson bores in the young shoots (up to 30 cm) of *Pinus merkusii* Jungh. & de Vriese (Tusam) and causes dieback of the shoots and stem (Yulvizar, 2009). The ambrosia beetle, *Xyleborus destruens* Bldf. and beehole borer, *Xyleutes ceramica* Walker attack the trunk of teak trees and make branching tunnels that extend into the heartwood. The three years old trees of *T. grandis* were infested by wood-dwelling termite, *Neotermes tectonae* Damm. where the portion of stem and branches were hollowed (Nair, 2001).

The larvae of *Indarbela quadrinotata* Walker bores into the shoot of *Santalum album* L. and form a shelter tunnel downwards in the wood from which it emerge to feed upon the outer surface of bark at night. *Aristobia octofasciculata* Aurivillus is also
reported to bore into the heart wood of this tree (Beeson, 1941; Remadevi and Raja Muthukrishnan, 1998; 2007). The volume of wood lost due to the attack of insects and microbes in two most important trees, *S. album* and *T. grandis* has been estimated (Remadevi and Veeranna, 2005). *Toona sureni* (Blume) is mostly planted as individual in private lands, often mixed with other plant species and it is a native host of *Hypsipyla robusta* Moore. The insect attacks flowers and young fruits of *T. sureni*, and mahogany. The growing shoot is mostly preferred and is the most serious pest of mahogany now.

**1.3 Mahogany shoot borer and its impact**

The tropical timber species of the subfamily Swietenioideae (the true mahoganies) of the Meliaceae family include some of the finest cabinet woods in the world, based on generally shared characteristics such as dimensional stability and workability. The wood of these species is prized for cabinetry, veneers, interiors, and artisan uses. The Swietenioideae includes *Cedrela* and *Swietenia* in the Neotropics, *Entandrophragma*, *Khaya* and *Lovoia* in Africa, and *Chukrasia* and *Toona* in Australia. High-value Meliaceae timber are traded in international markets since the Spanish began exploiting *Swietenia* in the New World in the 1500s (Lamb, 1966). Commercial exploitation has intensified in recent decades for all high-value members of the subfamily Swietenioideae.

**1.3.1 Current status of Mahogany Shoot borer**

Mahogany (*Swietenia mahagoni* Jacq.) is a large deciduous high timber value tree used for making furniture, paneling, railway sleepers, industrial and domestic wood wares, traditional medicine etc. (Wylie, 2001; Lopes et al., 2008). Continuous supply of this species is often constrained by low natural regeneration and difficulty in
establishment mainly due to the attack by the shoot borer, *Hypsipyla robusta* Moore and *Hypsipyla grandella* Zeller in the tropical and sub tropical parts of the world (Cunningham *et al*., 2005; Ofori *et al*., 2007).

In India, *Hypsipyla robusta* Moore is a serious pest of meliaceous forest trees such as the exotics, *Swietenia macrophylla* King, *S. mahagoni* Jacq. and native *Toona ciliata* M. Roem. Although *Swietenia* spp. is grown in plantations in many states, the establishment is difficult because of shoot borer attack during the sapling stage (Varma, 2001). There are five generations of *H. robusta* in temperate and sub tropical regions with different generations feeding on flowers, fruits and shoots (Beeson, 1941). The most important host response associated with *Hypsipyla* attack is the sprouting of multiple shoots on infested plants which affects the growth and economic value of the timber (Bygrave and Bygrave, 2001). Despite ready germination of its seeds, the transition from seedlings to saplings is hindered by the pest in natural forests (Lauma, 2003). The borer is reported to cause 29% loss in potential biomass production by attacking about 40% of the saplings in a plantation (Hossain *et al*., 2004a; 2004b).

Plants in the age group of 3-6 years and height class 3-5 m were reported to be more susceptible to the pest attack revealing up to 90% infestation, showing the relationship between infestation and age of tree (Cipiao *et al*., 2009). Repeated attacks in the early years of plantation eventually lead to death of trees (Lim *et al*., 2008).

### 1.3.2 Existing management methods for mahogany shoot borer

Different silvicultural practices such as mixed or enrichment plantings, wider spacing, varying tree density (Guimaraes *et al*., 2004; Perez and Esquivel, 2008)
provision of shade, promoting vigorous tree growth, encouraging natural enemies (Newton et al., 1999; Lopes et al., 2008) and leaving weed rows between young plantations are generally adopted for the management of Hypsipyla species. The attack of H. robusta varies with the clones and genetic variation for resistance is sometimes exploited as an element for integrated management of mahogany shoot borers (Cornelius and Watt, 2003).

A few systemic insecticides like carbofuran (Mayhew and Newton, 1998) and deltamethrin (Goulet et al., 2005) are occasionally used in the management of shoot borers. These insecticides have limited prospects in today’s pest management practices due to environmental concerns. Eco-friendly approaches such as biological control including microbial control assumes greater importance in this context.

1.4 Termites and their impact in forestry

Termites are an important part of the community of decomposers. They are able to decompose cellulose, the main component of the wood. They are abundant in tropical and subtropical environments. These termites become economically important pests when they start destroying wood and wooden products of human homes, building materials, forests and other commercial products (Meyer, 2005; Monica et al., 2009). There are over 2800 described species of termites with about 185 considered as pests (Lewis, 1997). Termites that belong to the families Hodotermitidae (Anacanthotermes and Hodotermes), Kalotermitidae (Neotermes), Rhinotermitidae (Coptotermes, Heterotermes and Psammotermes) and Termitidae (Amitermes, Ancistrotermes, Cornitermes, Macrotermes, Microtermes, Microcerotermes, Odontotermes, Procornitermes and
Syntermes) cause great loss in natural and plantation forestry (UNEP Report, 2000). In India they are responsible for 15-25% of crop loss which amounts to huge losses annually (Joshi et al., 2005).

The major mound building termite species in India are Odontotermes obesus Rambur, O. redemanni Wasmann and O. wallonesis Wasmann. The subterranean termite species, Heterotermes indicola Wasmann, Coptotermes kishori Roonwal & Chhotani, C. heimi Wasmann, O. horni Wasmann, Microtermes obesi Holmgren, Trinervitermes biformis Wasmann and Microcerotermes beesoni Snyder attack the bark and heart wood of the standing trees (Rjagopal, 2002) such as A. leucophloea, Butea monosperma (Lam.) Taub., Dipterocarpus indicus Bedd., Eucalyptus sp., Pterocarpus marsurpium Roxburgh, Santalum album, Shorea robusta, Terminalia bellirica (Gaertn.) Roxb. Swietenia macrophylla, Dalbergia sissoo, Pinus wallichiana A. B. Jacks., T. grandis, Toona ciliata, Haldina cordifolia (Roxb.) Ridsdale etc. Odontotermes spp. are the major arboreal termites damaging the bark and stems of many species of trees including sandalwood and teak and chemical methods of management have been tested (Remadevi et al., 1998)

1.4.1 Existing management methods for wood destroying termites

Although termites are excellent decomposers of dead wood and other sources of cellulose, they become a serious problem when they attack standing trees, logs, and crops. Therefore, effective control methods have to be extensively studied and exploited. Physical methods are a very popular method of preventing subterranean termite attack on wooden structures. Toxic physical barriers (Chlorfenapyr) and non-toxic physical barriers (sand or gravel aggregates, metal mesh, or sheeting) have been used as physical
termite barriers by Rust and Saran (2006) and Monica et al. (2009). Other physical methods including heat (45 °C for 30 min), freezing (liquid nitrogen -20 F), electricity (90,000 V) and microwaves were effectively used in the studies by Myles (2005). Sundararaj et al. (2003) also studied the effectiveness chemical insecticides on the arboreal termites in sandal tree orchard. Chemical treatment measures are one among the various techniques used to reduce the infestation of termites. Several termiticides containing active ingredients: bifenthrin, chlorfenapyr, cypermethrin, fipronil, imadaclorprid and permethrin are registered for termite control around the world under various brand names (Ahmed et al., 2006; Monica et al., 2009).

Although chemical control is a proven means of protection from termites, its excessive use is harmful for the environment. New methods of termite control are always being developed by researchers. Plant derived natural products (Park and Shin, 2005; Mao et al., 2006; Cheng et al., 2007), entomopathogenic fungi (Mo et al., 2006; Monica et al., 2009), nematodes (Weeks and Baker, 2004) and bacteria (Devi et al., 2006) are some of the alternative methods being developed against termites.

1.5 Biological control using fungal entomopathogens

Unlike other biological control strategies, conservation biological control does not require the introduction or augmentation of natural enemies. Instead, it relies on modification of the environment or management practices to protect and encourage natural enemies that are already present within the system. This improves their ability to control pest populations in a reliable way and is only possible if the biology, behavior and ecology of both the pests and their natural enemies are understood (Eilenberg et al., 2001;
Pimentel, 2008). Unfortunately, for most entomopathogenic fungi, our understanding of their ecology and epizootiology is incomplete. The majority of examples of conservation biological control to date have been for arthropod natural enemies (Gurr et al., 2004; Jonsson et al., 2008). However, similar approaches are relevant to entomopathogenic fungi where fungi are principal enemies of the target pest and where their ecology and epizootiology are understood (Pell, 2007; Tscharntke et al., 2008).

The entomopathogenic fungi are a diverse assemblage of fungi with one thing in common: they infect and cause disease in insects and other arthropods. Most are found within two main groups: the order Hypocreales within the phylum Ascomycota (subkingdom Dikarya) and the order Entomophthorales. Although our understanding of the ecology and epizootiology of entomopathogenic fungi is often incomplete (Vega et al., 2009), a conservation biological control approach could have significant potential if we identify and fill the gaps in our ecological knowledge. Pell (2007) stated that by understanding the factors that promote or inhibit epizootic development, strategies can be identified that ensure favorable conditions for proliferation of entomopathogenic fungi, and consequently reliable epizootics.

1.5.1 Fungal entomopathogens

Fungal entomopathogens are diverse and globally ubiquitous natural enemies of arthropods. There has been considerable research focus on their potential as microbial control agents as stated by Eilenberg et al. (2001) and Hajek et al. (2005). There are over 700 species of fungal entomopathogens and these are broadly found within two main groups: Entomophthorales, Hypocreales and Laboulbeniales. The Hypocreales have both
sexual (teleomorph) and asexual (anamorph) forms although most of the research have been focused on the anamorphs. Anamorphic hypocrealean fungi are considered to be generalist pathogens with broad host ranges and even switching between pathogenic and saprophytic lifestyles. The Laboulbeniales (Ascomycota: Laboulbeniaceae) are a group of obligate ectoparasitic fungi that are mainly associated with Coleoptera and do not cause death of their hosts (Hajek et al., 2007).

1.5.2 *Metarhizium anisopliae* (Metsc.) Sorokin.

The mitosporic ascomycete (hypocreales) fungus, *Metarhizium anisopliae* (Metsc.) Sorokin has gained significant attention as a biocontrol agent due to its wide geographic distribution, high virulence and vast spectrum of infectivity to a wide range of insect pests (Ypsilos and Magan, 2005). *M. anisopliae* usually infect by conidia and they enter into insect body mainly through the integument by adhesion, penetration into haemoceol and development of fungal infection. Nahar et al. (2004) stated that the process of penetration through the insect integument by a hyphal germination from a spore involves chemical (enzymatic) and physical forces. In vitro studies indicated that the digestion of the integument follow a sequential lipase-protease-chitinase process of digestion.

1.6 Focus of the study

Much work has been carried out on different aspects of pest management in forestry, and still mahogany cannot be grown successfully due to the classic low-density pest, *Hypsipyla robusta* Moore. Even one or two attacks on young trees may render their future timber production uneconomic. Similarly, the effectiveness of termite control
using entomopathogens in forest pest management, particularly in plantation forestry is
yet to be explored and evaluated. With this background, focused studies were conducted
to examine the complex relationship between pathogenicity, insecticidal activity,
enzymatic activity and field potency of *M. anisopliae* isolates against these pests. The
phylogenetic relationship of the different isolates was also explored in a detailed way.
The main objectives of the study were,

- Screening the pathogenicity of *M. anisopliae* isolates against *H. robusta* and
  *Odontotermes* spp.
- Study the field efficacy of highly virulent *M. anisopliae* strains.
- Characterization of virulent isolates of *M. anisopliae*