PATHOGENICITY AND EFFECT OF ENTOMOPATHOGENIC FUNGI ON NATURAL ENEMIES OF TOMATO INSECT PESTS

INTRODUCTION

Tomato (*Lycopersicon esculentum*) is one of the most popular and widely grown vegetable crops in both field condition and home garden. Tomato is a very versatile vegetable for culinary purposes. Ripen tomato fruits are consumed fresh and utilized in the manufacture for a range of processed products such as puree, paste, powder, ketchup, sauce, soup, and canned whole fruits. The unripen green fruits are used for pickles and are consumed after cooking (Salunkhe and Desai, 1984) There is high potential for tomato plantation since more widespread cultivation of tomatoes could generate rural employment, stimulate urban employment, expand exports, improve nutrition of the people, and increase the income of farmers (Villareal, 1980)

The insect pests of tomatoes consist of lepidopteran fruit and stemborers and a variety of plant sucking herbivores which include fruitworm, hornworm, cutworm, flea beetles, cabbage loopers, aphids, leaf miner, whitefly, and caterpillars. (Salunkhe and Kadam, 1998). Polyphagous species like the fruit borers, *Helicoverpa* (*Heliothis*) *armigera* and *Phthorimea operculella*, as well as *Spodoptera* spp. are the main lepidopteran pests of tomatoes in Southeast Asia (Deang, 1969; Gomaa *et al*., 1978; Kakar *et al*., 1990). Field-grown tomatoes, as well as those under protected cultivation, are attacked by aphids (*Myzus persicae, Aphis gossypii*), whiteflies (*Trialeurodes vaporariorum, Bemisia tabaci* (Homoptera: Aleyrodidae)) and thrips (*Thrips tabaci, T. flavus, T. palmi, Frankliniella occidentalis* (Berlinger *et al*., 1993)). In Thailand, thrips *Ceratothripoides claratris* (Thysanoptera: Thripidae) is found to caused serious damage on tomato (Rodmui, 2002). Thrips is a very small insect that can damage tomato plant as well as vectoring severe virus diseases such as various germiniviruses, which are recently being specifically identified (Polston and Anderson, 1997). Thrips is becoming increasing difficult to control even with a group of highly toxic insecticides (Benton and Jones, 1999).

With the increasing demand of fresh tomato with no chemical insecticide residues, tomato cultivated in protected condition for example greenhouse receives great attention. However, damage caused by some tiny insects is unavoidable even in well-protected greenhouse. There is a need to explore for safe, yet effective biological control agents alternative to chemical insecticides to be employed in greenhouse condition.

Fungi provide satisfactory microbial means of biological control of plant sucking insects such as aphids and whiteflies that are not susceptible to bacteria and viruses. The well-studied entomopathogenic fungi include *Beauveria bassiana* and *Paecilomyces fumosoroseus* for whitefly control and *Verticillium lecanii* for the control of greenhouse whitefly, thrips and aphids (Boucias and Pendland, 1998; Kim *et al*., 2001). The fungi *B. bassiana*, *V. lecanii*, *P. fumosoroseus*, and *Hirsutella* sp. were effective against thrips, *T. tabaci* (Lewis, 1997), while *V. lecanii*, *Aschersonia* sp. and *Hirsutella* sp. were effective against mealybug, *Pseudococcus cryptus* (Hemiptera: Pseudococcidae) (Boucias and Pendland, 1998). In December, 2002 the cooperative project between National Research Council of Thailand (NRCT) and National Research Council of German (DFG), entitled “Integrated Management of Tomato Pests under Protected Cultivation using Biological Products” had been launched in Thailand. The project results on the screening bioassay had found that the entomopathogenic fungi *B. bassiana* and *P. fumosoroseus* were effective against tomato thrips, *C. claratris*. The fungi *Metarhizium anisopliae* and *Hypocrella hypocreoidea* (Ascomycotina: Hypocreales) were effective while against mealybug *P. cryptus* and *P. fumosoroseus* were effective against whitefly, *B. tabaci*. All these effective strains of fungi are expected to be used as biological control agents for the targeted insect species of tomato cultivated in both greenhouse and field conditions.

Entomophagous insects which include predators and parasites are key factors suppressing insect populations in natural environment. Green lacewing, *Mallada basalis* (Neuroptera: Chrysopidae) is an important predaceous insect in various agroecosystem (Sirimachan, 2005). Anthocorid bug, *Wollastoniella rotunda* (Hemiptera: Anthocoridae) is important predator of thrips, *T. palmi* (Rattanaka, 2003). The parasite, *Encarsia formosa* (Hymenoptera: Aphelinidae) has played an important role in the control of the whitefly, *T. vaporariorum* in greenhouse (Feldhege and Schmutterer, 1993). Introducings these natural

enemies into certain areas where no effective indigenous species of entomophagous insects, are well–practiced. It is believed that, not only in field condition but also in glasshouse or screenhouse, beneficial insects can play a significant role as effective biological control agents. Entomopathogenic fungi and/or natural enemies often are introduced simultaneously or nearly simultaneously to control insect pests in greenhouse. These biological-control agents may act synergistically, additively or antagonistically (Ferguson and Stiling, 1996).

This study is aimed at examining the pathogenicity and adverse effects of entomopathogenic fungi toward some important predators and parasites of tomato insect pests. Results from this study will provide important informations for forming proper strategy on utilization of entomopathogenic fungi as effective and safe biological control agents against tomato insect pests. Moreover, the results can suggest the possibility of using these two types of beneficial biological agents together in the framework of IPM (Integrated Pest Management) program for the maximum control of several insect pests of tomato.

OBJECTIVES

1. To examine the pathogenicity and any adverse effect of entomopathogenic fungi toward some beneficial insects which serve as natural enemies of tomato insect pests.

2. To support the potential utilization of fungal pathogens as a biological control agents againstsome tomato insect pests.

LITERATURE REVIEW

Tomato insect pests

The vast majority of tomato pests are aphids, leafminer, leafhopper, red spider mite, springtails, thrips, tomato moth, whitefly, wire worm and woodlice (Morgan, 1979). However the most important insect species, particularly in greenhouse condition are whitefly, thrips and mealybugs. (Benton and Jone, 1999)

1. Thrips

Bernardo (1991) reported that thrips, *Ceratothripoides claratris* are polyphagous insects and serious pests of vegetable crops. It has been reported on tomato, pepper, eggplant, watermelon, muskmelon, cucumber, garlic and potato in low-elevation areas. The plant parts attacked are buds, flowers, leaves, young fruits occasionally and silk in case of baby corn. On the leaves, where the damage is usually more noticeable, the symptom is bronzing or silvery patches, becoming more extensive and turning brown, then the entire leaf dries up and dies. However, in Thailand the thrips fauna on tomatoes is not well documented. So far only *T. flavus* and *C. claratris* have been recorded on tomatoes (Bensiddhi and Poonchaisri, 1991; Murai *et al*., 2000; Rodmui, 2002)

Rodmui (2002) reported that eggs of *C. claratris* were bean-shaped or kidney-shaped and very small. The newly laid eggs were pale white in color. Female usually laid eggs singly, scattered and inserting them into tissue of leaves, flower or fruit by the saw-like ovipositor. There were two larval instars during the larval period. The first instar larvae were white or nearly transparent at first and turned yellow. The larvae body resembled the adult in general. There were no wing buds, lacked wing and smaller in size. Larvae feed in groups. The second instar larvae differ from the first instar in color, they were light yellow. Antennae shape differed from that of the previous stage. They moved to pupate under the leaf. There were two pupal periods. The first was called prepupa. The wing pads of the pupae were longer than that of the prepupae. Both pupal instars were nonfeeding stage. After the prepupal development was completed, the insect

moved into the pupal stage. The pupa had developed antennae that curve back over the head. Newly emerged adults were pale yellow and turned dark brown in color. The slender fringed wings were pale. There were four wings, narrow with a long fringe of setae along the margin. The hair or fringe on the anterior edge of the wing were considerably shorter than those on the posterior edge.

2. Mealybugs

Mealybugs, *Pseudococcus cryptus* are serious pests in some area such as Australia and South America (Hill, 1983). The most crops are seriously attacked by mealybugs are coffee, cocoa, citrus, cotton, jute, groundnut, beans, cassava, sugarcane, sweet potato, cashew, guava, tomato and many other plants. They feed on young shoots, berries and leaves. In many records refer to heavy attacks following periods of prolonged drought.

Mealybugs are the least scale-like of the group, mainly because they are soft-bodies, without the outer shell associated with insects in the other scale insect families. Instead, mealybugs are usually covered with a white waxy powder, and have filamentous projections around the perimeter. Hill (1983) described that female mealybug lays 300-400 eggs, the egg hatches in a few hours and the young nymph can move away quite rapidly. They were full grown in about six weeks. The female is a distinctive mealybug with a pair of conspicuous longitudinal sub-median dark strips, long glassy wax threads, a pronounced tail, and a powdery waxy secretion. The entire life cycle takes about 40 days.

Researchers in Florida, where the new pest was initially identified, isolated a highly virulent strain of *Paecilomyces fumosoroseus* from a naturally infected mealybug (Osborne and Landa, 1992).

3. Whitefly

The whitefly, *Bemisia tabaci* occurs throughout most tropical and subtropical regions of the world. It occurs naturally in a band around the equator. The key pest of tomato crop in

recent times has been the whitefly *B. tabaci* that causes direct damage to tomato due to removal of plant sap and deposition of honeydew that serves as a substrate for sooty mold. Even more damaging are the virus diseases vectored by this pest, particularly in tomato where it readily transmits the geminivirus tomato yellow leaf curl (TYLCV) (Stansly, 2005)

Gameel (1974) reported that aleyrodids were unusual (resembing complete metamorphosis), in that the nymphs appear 'scale-like' and the last nymphal instar was quiescent and 'pupa-like'. The four-winged adult was 1-3 mm. long and the entire insect was covered by a white-colored, waxy bloom. Adults could fly for only short distances, but may be dispersed over large areas by wind. Females laid their eggs on the lower surface of the leaf on which they emerged, but soon moved upwards to young leaves, generally on the same plant. Pear-shaped eggs, which hatched in about 7 days, are about 0.2 mm. long and were inserted vertically into the leaf tissue. They were anchored at the larger end by a stalk, which penetrates the leaf epidermis through a puncture made by the ovipositor, and passes into the spongy parenchyma. Water could pass from the plant tissue into the egg, and when there were high numbers of eggs, the plant may become water-stressed.

Early in the season, eggs were laid singly but later were laid in groups (Gerling *et al*, 1984). The average daily egg-laying capacity was 160 eggs per female (Gameel, 1974). Upon hatching, nymphs move only a very short distance before setting down to feed. Once feeding begins, nymphs do not move again. All nymphal instars were greenish-white in color, oval in outline, scale-like and somewhat shiny. The last instar was about 0.7 mm. long, and the red-colored eyes of the adult could often be seen through the larval integument. Nymphs completed three moults before pupation and emergence as adults.

Natural enermies of tomato insect pests

Conservation and enhancement of natural enemies of agriculture pests deal with indigenous and imported species. In general, beneficial arthropods suffer largely from inappropriate conditions during the plant growth and hibernation periods. By providing proper environments, beneficials may be preserved and enhanced. To achieve this, several measures can

be utilized. (Sengonca, 1998). Some beneficial insects had been substantial successful in controlling tomato insect pests, for example green lacewing for mealybug control (Sirimachan, 2005), anthocorid bug for thrips control (Rattanaka, 2003) and whitefly parasite (Rose *et al*., 1996) for whitefly control.

1. Green lacewing

Green lacewing, *Mallada basalis* is an important predator which was considered particularly effective at reducing several preys including of aphids, mites, thrips, whiteflies, eggs of leafhoppers, small caterpillars, scale and mealybugs (Chandish and Singh, 1999; Kabissa *et al*., 1996*)*. This predator was commercially released as biological control agent for control many serious insect pests both in the open-field and especially in greenhouses in many countries. (Sirimachan, 2005)

Sirimachan (2005) reported the biology of *M. basalis* as follows: the eggs of *M. basalis* were laid in single in loose groups with the stalk on plant leaves and stem. Adult was capable of laying 466-520 eggs. The egg stage was 2-3 days. The duration of developmental stages from first instar to third instar were 2-3, 2-3, 3-5 days respectively. The larva moulted three times and the total period of larval stage was 8-9 days. Growth increment of the larva based on width of the head capsule, assumed a geometric progression with a ratio being 2.417. The pupal stage was 9-10 days. The longevity of adult male and female were 29-42 days and 52-67 days respectively. The life cycle from egg to adult stage was 21-26 days.

The green lacewing, *M. basalis* has recently been used as biological control agent primarily against *Acari* spp. on several crops in Taiwan (Cheng and Chen, 1996). Change and Huang (1995) evaluated the effectiveness of the predator *M. basalis* for the control of tetranychid mites on strawberries and the results showed that 60 to 90% of *Tetranychus kanzawai* (Arachnida: Tetranychidae) population and 50 to 90% of the *Tetranychus urticae* Koch (Arachnida: Tetranychidae) population were suppressed by the green lacewing. This result was not only save the cost of the control up to more than US $233/hectare, but also increased fruit production by 15% and that of first class fruit by 7.7%. Broadley and Thomas (1995) reported the use of another

species of green lacewing, *Mallada signata* (Schneider) (Neuroptera: Chrysopidae) for controlling aphids, two spotted mites *T. urticae*, greenhouse whitefly *Trialeurodes vaporiorum*, moth eggs and small caterpillars. The releasing rates were 500-1000 lacewing larvae per hectare in field crop. For nurseries, a releasing rate of 1-5 lacewing larvae per plant was recommended. Riddway and Murphy (1984) reported that the release of second instar of chrysopid larvae on peppers to control the green peach aphid, the cowpea aphid, *Aphis craccivora* was highly successful. The result showed that the aphid numbers were reduced 94 to 98% in six days after colonization, and yields of peppers were increased by 13%.

2. Anthocorid bug

Anthocorid bug, *Wollastoniella rotunda* is an important predator of thrips. Biological studies by Rattanaka, (2003) indicated that eggs of *W. rotunda* were inserted singly in the upper or lower of eggplant leaves in the midrib or major veins. The newly laid eggs were creamy white and became red before hatching. Head was highly smaller than abdomen, red in color. Stylet length was long when compared with body length. It immediately became predator after emergence. It attacked prey by piercing stylet into the prey body. In its second nymphal instar, the body color turn to dark red and very active. The wing pads appeared on mesothorax in the third nymphal instar. In this instar, the body was round-oval, more dark red in color. The adult body was rounded with shining surface, punctate, uniformly, cover with silky hair, chestnut brown in color. In female, all four segments of the antennae were yellowish brown in color but in male, the fourth segment tinged with reddish in color. The hemelytra wing was covered with silky hair, chestnut brown in color. The female was slightly larger than the male in size and could be differentiated from male by the abdomen Male abdomen distinctly twisted in general shape. The copulation repeated usually several times during their life span, but one copulation was enough to give the female permanent fertility.

Among of natural enemies of thrips, the anthocorid predator *Bilia* sp. received considerable interest and played are important role for controlling *T. palmi*. Yasunaga and Miyamoto (1993) reported that *Bilia* sp. was described as a new species belonging to the genus *Wollstoniella*. After that, Yasunaga (1995) found a new species of *Wollastoniella* on the eggplant

gardens in Northern of Thailand. It was described as *Wollastoniella parvircuneis* Yasunaga. Shima and Hirose (2002) reported the possibility of using *W. rotunda* as a biological control agent against *T. palmi* in greenhouses.

3. Whitefly parasitoid

Whitefly parasitoid, *Encarsia formosa* is a parasitoid of eight whitefly species (Lopez-Avila, 1986), including the greenhouse whitefly, *Trialeurodes vaporariorum*, and the sweetpotato or cotton whitefly, *Bemisia tabaci*.

Hoddle and van Driesche (1998) reported that *E. formosa* is a solitary endoparasitoid that lay 8-10 eggs per day. Daily egg maturation and oviposition rates decline as wasps age. Adults obtain energy and nutrients by consuming honeydew and hemolymph of hosts that are pierced with the ovipositor, but in which no egg is deposited. Killing hosts for nutritional purposes is termed host feeding. *E. formosa* will host feed on all pre-imaginal stages of *T. vaporariorum* except the egg, but prefers second instar nymphs and pupae. To host feed, *E. formosa* wounds nymphs or pupae by probing with the ovipositor for up to six minutes and feeds from wounds which wasps may enlarge with their mandibles. This probing followed by feeding kills hosts. Nymphs that have been used for feeding are not used for oviposition, and previously parasitized whiteflies are not used for host feeding. Principal greenhouse crops in which *E. formosa* is used include tomato (*Lycopersicon lycopersicus*) and cucumber (*Cucumis sativus*). The parasitoid is also used, or is being tested, on eggplant (*Solanum melongena* var. *esculenta*) gerbera (*Gerbera jamesonii*), poinsettia (*Euphorbia pulcherrima*), marigolds (*Tagetes erecta),* and strawberry (*Fragaria ananassa*). Virtually nothing is known about the ecology of *E. formosa* in outdoor agricultural systems.

Entomopathogenic fungi

Approximately 750 species of fungi have been documented to infect insects (Hajek, 1997). The majority of these are from either the Entomophthorales (Zygomycotina) or Hyphomycetes (Ascomycota). Although aspects of their life-history attributes vary, both of these

groups of fungi produce conidia or other asexual spores and, in the case of some entomopthorales, zygospores. The conidium is the infective unit and is acquired by new hosts when they contact sporulating hosts or when dislodged or discharged conidia are encountered in the environment. Both fungal groups are capable of inducing natural epidermics or epizootics within susceptible host populations (Ullyett and Schonken, 1940; Smith *et al*., 1976; Velasco, 1983; Thorvilson and Pedigo, 1984; Pollew *et al*., 1986), although epizootics of entomophthoralean species are more commonly observed (Pell *et al*., 2001).

Attathom *et al*., (2003) reported that entomopathogenic fungi were found to be effective natural enemies of plant sucking insect species including thrips, whitefly, aphids and scale insect. In their study, 23 isolates of entomopathogenous fungi were tested for their efficacy against thrips, *C. claratris.* The preliminary bioassay revealed that the fungus, *P. fumosoroseus* was the most virulent strain followed by *B. bassiana*. The common entomopathogenic fungi, *M. anisopliae* and *V. lecanii* were less effective

1. *Metarhizium anisopliae* var.*anisopliae*

Tanada and Kaya (1993) revealed that the colony of *M. anisopliae* appears white when young, but as the conidia mature, the color turns to dark green. The conidiophores are branched, and the initial conidium is produced by simple abstriction at the distal end of the conidiophore. A chain of conidia is formed on each conidiophore with the youngest conidium being adjacent to the conidiophore. The mass spore chain becomes so dense and coheres with each other to produce prismatic masses of columns of spore chains. *M. anisopliae* caused tetanic paralysis when inoculated into larvae of *Galleria mellonella*. *M. anisopliae* are also produced in fungus-infected larvae and are important in the development of symptom. The rapid production of destruxins in the larvae causes death. The destruxins are toxic to insects only by ingestion and not through the integument.

McCoy *et al* (1988) described that there are two important entomopathogenic species of *Metarhizium, M.anisopliae* and *M. flavoviride.* It has been used to control rhinoceros beetles in coconut-growing regions of the South Pacific. *M. anisopliae* var. *anisopliae* has a much wider

host range than the longer-spored form and attacks insects from Coleoptera, Lepidoptera, Orthoptera, Hemiptera, and Hymenoptera as well as species of arachnid. In addition, some isolates have been found to be virulent to mosquito larvae (Diptera), termites (Isoptera), and spittlebugs (Homoptera). *M. anisopliae* can infected eggs of some hosts (leaf miner) but generally invades insects in larval, pupal, or adult stages of development. It is relatively easy to identify by its dry, catenulate conidia, which arise from host insects on densely packed conidiophores to from palisade-like masses. (Drion and Pendland, 1998)

Sosa-Gomez *et al*. (1997) reported that the infection pathway of *M. anisopliae* in host insects is much the same as for other Hyphomycetes. The infectious conidia have a three-layered wall. Conidia are uninucleate and contain large lipid inclusion, oil globules, and a few small mitochondria. These dry spores attach to host cuticle via hydrophobic interactions. In detailed experiments on stinkbug (*Nezara viridula*), it was found that conidia adhere to cuticular areas containing large numbers of setae rather than to heavily sclerotized head and thorax regions. This preferential binding dose not appear to be nearly a trapping mechanism but is due rather to unique surface chemistry of the setal regions.

Although *M. anisopliae* conidia generally invade host insects through the outer integument, they occasionally can initiate infection in other ways. *M. anisopliae* invaded weevils (*Hylobius pales*) via the buccal cavity as well as the outer integument (Schabel, 1976), and infection of mosquito larvae can take place at the siphon tip (Lacey *et al*., 1998). The fungus also has been reported to invade starved, axenic (germ-free) desert locusts, but not conventional ones (*Schistocerca gregaria*), through the gut. It was concluded that bacteria in the gut of conventional hosts produce antifungal phenols that inhibit conidial germination in this region (Dillon and Charnley, 1991).

2. *Beauveria bassiana*

The entomopathogenic fungus, *B. bassiana* has been shown to be an excellent potential biological control agent for many agricultural pests (Colorado potato beetle, European corn borer, codling moth and greenhouse pests) (Goettel *et al*., 1990; Lewis *et al*., 1996;

Poprawski *et al.*, 1997). Once its infective propagules come in contact with the host, they penetrate directly through the cuticle. This route of exposure is essential for the control of pests with sucking mouthparts, which are unlikely to ingest the microbe during feeding (Dunn and Mechalas, 1963). Moreover, *B. bassiana* is safe for pollinators (Keven et al., 2003) and other beneficial organisms under field conditions (Goettel *et al*., 1990)

Steinhaus (1949) described that the perfect stage of *B. bassiana* are unknown. *B. bassiana* grows well in culture as well as in nature with white mycelium. The optimum temperature for its growth is approximately about 28^{o}C on most of the artificial media used to cultivate fungi. It characteristically produces a flat, meaty, chalky, pulvirulent growth with spore formation taking in place from 3-7 days after inoculation. After germination of germtubes, produces a very short branch hypha.

Tanada and Kaya (1993) reported that the conidiospore is characterized by bearing hyaline conidia singly on zig-zag sterigma. The conidium is globosely and oval spore. The conidia of *B. bassiana* is dry conidia which have well-organized fascicles of rodlets in these conidia, the attachment is non-specific and is to the hydrophobility of the rodlets and insect cuticle. Generally, the germ tube produces an aspersorium for the fungal invasion into insect integument and produces the enzymes to digest the integument. After the germination, hypha has penetrated the insect's integument and wall-less protoplasts which may develop and disperse rapidly in the hemocoel. The color of conidia of *B. bassiana* is white in all period. The insect cadavers will generally be covered with mycelium and conidia of the fungus, then the cadavers become hard and dry like a mummified.

2. *Paecilomyces fumosoroseus*

The filamentous fungus *P. fumosoroseus* is a common insect pathogen and soilborne organism that has been isolated from a wide variety of insects from different orders located throughout the world (Humber, 1992). Because of its potential to cause epizootics naturally, *P. fumosoroseus* is considered to be a good candidate for microbial control of insect pests

(Jackson *et al*., 1997). The fungus *P. fumosoroseus* has been reported from whiteflies and over 40 other insects, but is not commonly reported from aphids (Lacey *et al*., 1996).

P. fumosoroseus causes rapid infection and death of all whitefly stages. Under optimal condition, hyphae are present in the haemocoel within 24 h of inoculation, death occurs between 24 and 48 h, hyphae emerges and conidiogenesis occurs on the surface of the cadaver within 72 h (Osborne *et al*., 1990). Optimal growth rates are between 20 and 30^{o}C, with optima related to the microclimate of the fungal isolate's biotype (Vidal *et al*., 1997). Highly virulent isolates of *P. fumosoroseus* with considerable control potential against whiteflies are widespread and numerous (Lacey *et al*., 1996; Vidal *et al*., 1990; Wraight *et al*., 1998). Wraight *et al* (2000) demonstrated that infection can take place at ambient relative humidities as low as 25%. Hyphal bodies are more virulent than conidia (Lacey *et al*., 1999) and can be rapidly produced in liquid culture, remaining viable and virulent following drying (Jackson *et al*., 1997).

P. fumosoroseus demonstrated limited lethal and sub-lethal effects on *Serangium parcesstosum*, an important coccinelid predator of whiteflies, suggesting that the integration of these two control agents in IPM may be possible (Poprawski *et al*., 1998). Moreover, laboratory and field studies revealed its potential for control of *Diuraphis noxia* (Homoptera: Aphididae) in combination with *Aphelinus asychis* (Hymenoptera: Aphelinidae) (Mesquita *et al*., 1996). However, under conditions of high humidity, *P. fumosoroseus* can have direct negative impact on *A. asychis* (Lacey *et al*., 1997).

Effect of entomopathogenic fungi to entomophagous insects

In both natural and agricultural ecosystems, complex multitrophic interactions involving herbivores, predators, parasitoids and pathogens contribute to arthropod community structure (Furlong and Pell, 2005). Studies of the individual relationships among insects and their predators, parasitoids and pathogenic microorganisms are many and varied and several represent landmarks in the theory and understanding of population ecology and biological control (Bellows and Hassell, 1999). It is common for a herbivore to be exploited by a number of different natural enemies that will interact directly and indirectly not only with each other but also with the

herbivore. However, until recently, studies examining the interrelationships between phylogenetically distinct organisms such as arthropod and pathogen natural enemies utilization the same insect resource were uncommon (Hochberg and Lawton, 1990; Rosenheim *et al*., 1995). Only a limited number of these studies have considered interactions between arthropod natural enemies and entomopathogenic fungi (Brooks, 1993; Roy and Pell, 2000).

1. Effect of entomopathogenic fungi to predators

Magalhaes *et al*. (1988) demonstrated that although two species, *Coleomegilla maculate* and *Eriopis connexa*, of predatory coccinelid were susceptible to infection by topically applied *B. bassiana* in the laboratory, infection rates were considerably reduced if the predators received the inoculum only when they walked over contaminated leaf surfaces (the most likely mode of inoculum acquisition in the field). Such studies provide useful information, as species that are not physiologically susceptible to infection under optimal laboratory conditions for the fungus, where environmental and behavioral constraints to infection have been removed, are unlikely to be ecologically susceptible to infection in the field. However, physiological susceptibility in the laboratory does not necessarily translate into field ecological susceptibility, where the impact of a given fungus on a physiologically susceptible predator or parasitoid may be minimal (Jaronski *et al*., 1998). Pell and Vandenberg (2002) showed that under optimal conditions for the fungus, stressed individuals of *Hippodamia convergens* (Coleopera: Coccinellidae) were killed by high dose of *P. fumosoroseus* after 72 h at 100% relative humidity in the laboratory, but they speculated that as these conditions are unlikely to be replicated in the field, this important aphid natural enemy would be unlikely to be affected by field application rates of the fungus. In similar studies, James and Lighthart (1994) showed that *H. convergens* was physiologically susceptible to *B. bassiana*, *M. anisopliae* and *P. fumosoroseus* in laboratory tests. However in field tests only *B. bassiana* caused significant mortality in *H. convergens* populations and only early in the season when environmental conditions were favorable. Applications later in the season, when temperatures were higher than the optimum for infection, had little impact on the insect (James *et al*., 1995).

Poprawski *et al*. (1998) found that feeding the predator *Serangium parcesetosum* (Coleoptera: Coccinellidae) with whitefly prey that had been contaminated with *B. bassiana* 24, 48, 72, or 96 h before significantly increased predator mortality and they speculated that, in addition to the possibility of direct infection by the pathogen, *S. parcesetosum* may have been adversely affected by toxins such as oosporein produced by the fungus within its whitefly hosts. Such detrimental interactions have previously been suggested as a likely consequence of intra-host fungus-parasitoid interaction (Brooks, 1993) but require further study within the context of predatory and scavenging arthropods that may consume infected prey.

However, arthropod predators can ingest entomopathogenic fungi by consuming infected host insects (Rosenheim *et al*., 1995). Roy *et al*. (1998) reported for example, in nonchoice tests, the beetle, *Pterostichus madidus* (Coleoptera: Carabidae) consumed more *P. neoaphidis*-infected and sporulating aphid cadavers than uninfected cadavers. However, the consumption of *P. neoaphidis*-infected aphids in the field is unlikely to affect the epidemiology of the disease because the beetle forages on the ground where it can only contact dislodged cadavers already removed from the environment of transmission on the plant. Both adults and larvae of *Coccinella septempunctata* (Coleoptera: Coccinelidae) can also feed on *P. neoaphidis*-infected sporulating cadavers, occasionally consuming them entirely. Although partial consumption of cadavers significantly reduced conidium production, it did not impact subsequent transmission rates in laboratory experiments, an indication of the limitation of a detrimental impact in this interaction. Other common arthropod predators of aphids in the United Kingdom, the hoverfly *Episyrphus balteatus* (Diptera: Syrphidae) and the lacewing *Chrysoperla carnea* (Neuroptera: Chrysopidae), never fed on *P. neoaphidis*-infected sporulating aphids cadavers. Predators foraging on *P. neoaphidis*-infected aphids never became infected by the pathogen; however, the potentially more subtle sub-lethal effects of consuming diseased prey were not examined.

Encounters with sporulating cadavers on the soil surface and the subsequent acquisition of *B. bassiana* conidia by *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) pre-pupa searching for pupation sites in the soil of the potato agroecosystem is essential to the secondary cycling of the disease in the pest population (Long *et al*., 2000). Hartke *et al* (1998) reported that *Harpalus rufipes* (Coleoptera: Carabidae), which commonly preys and caches on

seeds in the potato agroecosystem of northern Maine, was suspected of being responsible for the rapid removal of tethered mummified and sporulating *B. bassiana*-infected *L. decemlineata* cadavers from the soil surface and their subterranean storage. Such changes in the distribution of sporulating cadavers have the potential to seriously reduce the secondary transmission of the fungus to the pest population.

2. Effect of entomopathogenic fungi to parasitoids

These biological control agents may act synergistically, additively or antagonistically. Synergistic interaction between pathogens and insect natural enemies will enhance control efficacy, whereas antagonistic interactions will reduce total control efficacy (Roy and Pell, 2000). For example, adult females of *Aphelinus asychis*, a common parasitoid of Russian wheat aphid, *Diuraphis noxia*, exposed to *P. fumosoroseus* conidia at high humidity and at high pathogen dose significantly reduced host searching (walking time, speed, and distance) (Lacey *et al*., 1997). Accordingly, Lacey *et al*. (1997) suggested that interactions between specific entomopathogenic fungi and natural enemies should be examined before their use in wide-scale field or greenhouse pest-control systems, e.g., aphid control in greenhouses. Although a parasitoid may not die of direct infection of its host by a pathogen, the altered nutritional status and premature death of a fungus-infected aphid host may negatively affect parasitoid survival (Brooks, 1993).

Furlong and Pell, (1996) studied interactions between the fungal entomopathogen (*Zoophthora radicans*) and two hymenopteran parasitoids (*Diadegma semiclausum* and *Cotesia plutellae*) attacking the diamondback moth, *Plutella xylostella* L.. It was found that particular strain of *Z. radicans* (NW250R) is not pathogenic exclusively to *P. xylostella*; it also infects *D. semiclausum* and is unlikely to have a direct impact on the parasitoid in the field. Although *D. semiclausum* is not able to vector *Z. radicans* infection to *P. xylostella* larvae, the presence of foraging individuals increases the larval movement to such an extent that infection levels are enhanced. Thus, the presence of foraging *D. semiclausum* within populations of *P. xylostella* which support *Z. radicans* can potentially enhance fungal infection levels in the pest population and this may increase overall control levels in the field.

Furlong (2004) reported that detrimental effects of *Beauveria bassiana* on *D. semiclausum* cocoon production and adult parasitoid emergence increased with increasing pathogen concentration and some parasitoid larvae became infected by *B. bassiana* within hosts. The negative impact of *B. bassiana* on *D. semiclausum* cocoon production decreased as temporal separation between parasitism and pathogen exposure increased. Adult parasitoid emergence was significantly compromised by highest rates of *B. bassiana* tested even when exposure of host larvae to the pathogen was delayed until one day before predicted parasitoid cocoon formation. Parasitoid pupae were infected by the pathogen in all *B. bassiana* treatments which did not preclude their development.

Washburn *et al*. (2000) reported that data do not prove that parasitization stimulated fungal infection of either the host aphids or the larval parasitoid. Both direct fungal infection and malformed parasitoid larvae or pupae were observed on dissection of mummies from aphids treated with spores shortly after parasitoid exposure. The cuticle of mummified aphids sprayed with fungal spores 5- and 7- days after parasitoid exposure were not penetrated by parasites.

Fransen and van Lenteren (1994) studied the intra-host interactions among the greenhouse whitefly, *T. vaporariorum* (Homoptera: Aleyrodidae), its fungal pathogen *A. aleyrodis* (Ascomycota: Hypocreales), and the parasitoid *E. formosa* and found that increasing the time between parasitoid oviposition and fungal infection decreased the competitive advantage of the pathogen over the parasitoid. If parasitized hosts were treated with fungus 4 or more days after parasitoid oviposition, the parasitoid had absolute competitive advantage over the pathogen, and parasitoid development was completed with no detrimental effect on the reproduction potential of emerging adults.

The study of interactions among the Russian wheat aphid, *Diuraphis noxia* (Hymenoptera: Aphididae), its parasitoid *Aphelinus asychis* (Hymenoptera: Aphelinidae), and the pathogen *P. fumosoroseus*, Mesquita and Lacey (2001) showed that treatment with pathogen 24-96 h after parasitoid oviposition did not effect cocoon production, but adult parasitoid emergence from cocoons was depressed following pathogen application 24 h after parasitism. When aphid hosts were treated with the fungus before exposure to parasitoids, the number of cocoons

produced was significantly affected by the degree of temporal separation between fungal attack and parasitoid oviposition. When the fungus and the parasitoid attacked host aphids at the same time, the number of cocoons produced was not reduced, but if the delay in parasitoid attack increased by 24 h or more the number of cocoons produced declined, approaching zero when the delay was 72 h. In this system, due to the similar development time of the two natural enemies, the fungus did not have a complete competitive advantage over the parasitoid, providing a rare example of successful parasitoid development in hosts previously infected by a fungal pathogen.

MATERIALS AND METHODS

1. Stock culture of insect preys of entomophagous insects

Tomato insect pests, thrips *Ceratothripoides claratris* (Thysanoptera: Thripidae), aphid *Aphis gossypii* (Homoptera: Aphididae) and whitefly, *Bemisia tabaci* (Homoptera: Aleyrodidae) (Figure 1) were colonized in netted greenhouse. These three species of insects will be used as host for mass rearing the entomophagous insects employed in this study.

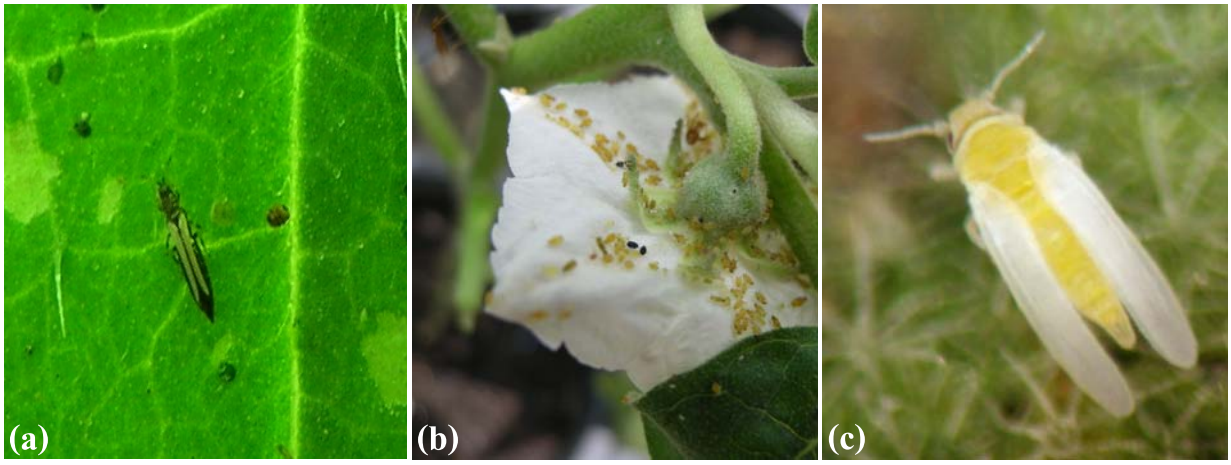

Figure 1 Insect pests of tomato employed in this study

(a) tomato thrips, *Ceratothripoides claratris* (13x)

(b) aphid, *Aphis gossypii* (1x)

(c) whitefly, *Bemisia tabaci* (26x)

1.1 Tomato thrips, *Ceratothripoides claratris*

Thrips, *C. claratris* was mass reared on tomato plant, *Lycopersicon esculentum*. Two-week old seedlings of tomato in celled-tray were transferred into plastic pots, three plants per pot and maintained in netted greenhouse (Figure 2) to serve as host plants for the entire experiment. To initiate thrips stock culture, the six-week old tomato plants were transferred into another netted greenhouse and a number of field-collected adults thrips, *C. claratris* were released onto the tomato plants which served as food and oviposition sites. Within 15 days, colonization of

C. claratris in the netted greenhouse was achieved. New tomato plants in plastic pots were moved into the greenhouse to replace the old ones when necessary for thrips nutrition.

Figure 2 Plastic-potted tomato plants, *Lycopersicon esculentum* maintained in the netted greenhouse as food supply for the colonization of tomato insect pests and entomophagous insects

1.2 Aphid, *Aphis gossypii*

Eggplant, *Solanum melongena* was used as host plant for mass rearing aphid, *A. gossypii*. Eggplants were cultivated in plastic pots, one plant per pot and maintained in netted greenhouse. The seven-week old plants were then moved into another netted greenhouse to initiate aphid stock culture. Field-collected populations of aphid, *A. gossypii* were released onto the plants and within 3 weeks, aphid populations were increased in sufficient quantity for the experiments. New and healthy eggplants were weekly supplied into the netted greenhouse.

1.3 Whitefly, *Bemisia tabaci*

Eggplants were prepared as host plants for whitefly, *B. tabaci* the same as for aphid (described in 1.2). Whiteflies were collected from tomato field and introduced to the eggplants in netted greenhouse. The adult whitefly laid eggs on the underside of the leaves. After emergence, the immature whiteflies fed on eggplant leaves and complete their developmental cycle. The wilted plants were replaced with the healthy ones weekly.

By these described methods, the stock cultures of host insects were maintained and sufficient numbers of insects were obtained for all experiments.

2. Stock culture of entomophagous insects of tomato insect pests

The entomophagous insects used in this study were illustrated in figure 3 which included green lacewing, *Mallada basalis* (Neuroptera: Chrysopidae), the predator of mealybug; anthocorid bug, *Wollastoniella rotunda* (Hemiptera: Anthocoridae) and mirid bug, *Macrolophus caliginosus* (Hemiptera: Miridae), the two predators of thrips and whitefly parasitoid, *Encarsia formosa* (Hymenoptera: Aphelinidae). All of them were mass reared on their insect hosts following the methods described below:

2.1 Green lacewing, *Mallada basalis*: the predator of mealybug, *Pseudococcus cryptus*

The original colony of green lacewing, *M. basalis* was laboratory-reared colony kindly provided by National Biological Control Research Center (NBCRC), National Research Council of Thailand (NRCT). Stock culture of *M. basalis* was established successfully in Insect Pathology Laboratory, Department of Entomology, Kasetsart University, Kamphaengsaen Campus. Larvae of *M. basalis* were reared in the plastic boxes, 23 cm in diameter and 11 cm in height, with tightly fitting lids having a 10 cm diameter circular wire-mesh screen for ventilation. A number of all stages of aphid were daily provided as food for green lacewing larvae until they became pupae. When the adults emerged, they were transferred to the cylindrical plastic box of 10 cm in diameter and 40 cm in height for mating and oviposition. The plastic box was covered

with fine cheesecloth and held by round rubber band. *M. basalis* adults were fed daily with honey solution and water supplied on thin sponge hung on the top of the plastic cylinder.

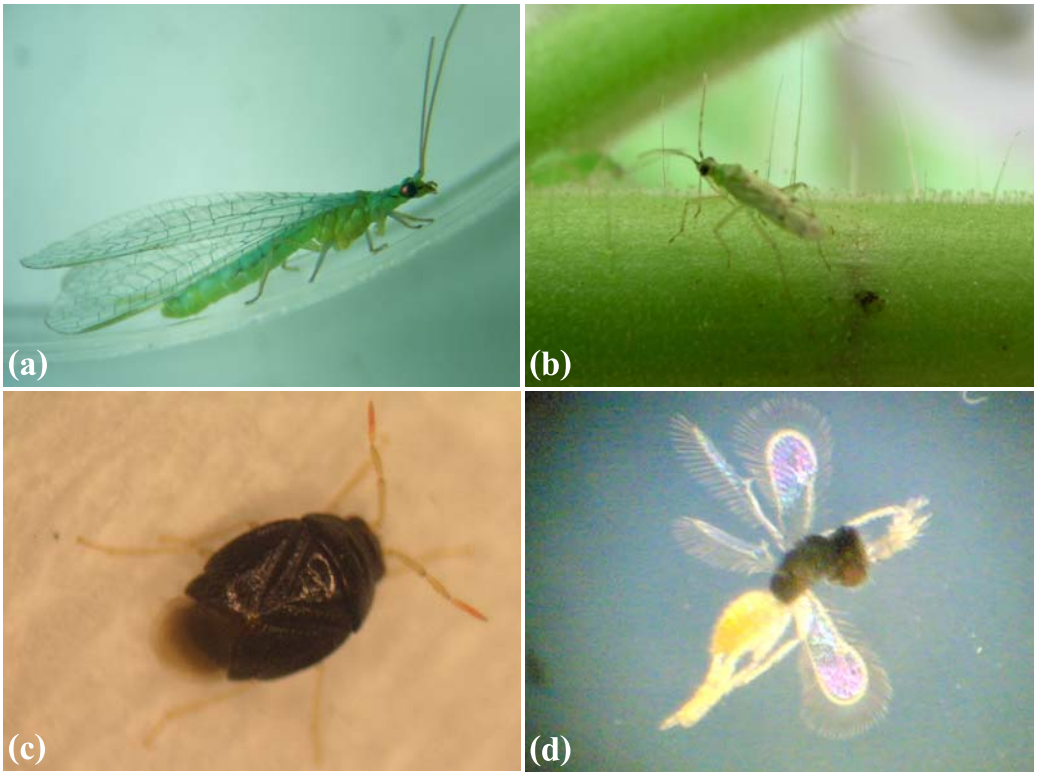

Figure 3 Important natural enemies of tomato insect pests found in Thailand
(a) green lacewing, *Mallada basalis*: the predator of mealybug (4x)
(b) anthocorid bug, *Wollastoniella rotunda*: the predator of thrips (33x)
(c) mirid bug, *Macrolophus caliginosus*: the predator of thrips (6x)
(d) whitefly parasitoid, *Encarsia formosa* (40x)

2.2 Anthocorid bug, *Wollastoniella rotunda*: the predator of thrips

Larvae of *W*. rotunda used in this study originally came from laboratory-reared colony and kindly provided by National Biological Control Research Center (NBCRC), National Research Council of Thailand (NRCT). The larvae were introduced to the netted greenhouse containing tomato plants infested with thrips, *C. claratris* (described in 1.1). Colony of *W. rotunda* was established using thrips colonized on tomato plants as food. Fresh tomato plants

were supplied in two weeks interval or when the old plants were no longer served as food for tomato thrips. The predator-prey colonies were carefully kept in balance. Adults of *W. rotunda* laid eggs on upper side of eggplant leaves. The mass rearing was conducted under environmental condition measuring at 33 ± 2^{o}C.

2.3 Mirid bug, *Macrolophus caliginosus*: the predator of thrips

Adults of mirid bug, *M. caliginosus* were released into the netted greenhouse containing tomato plants infested with thrips, *C. claratris* (described in 1.1). The stock colony of mirid bug was successfully established using thrips that colonized on tomato plants as food. Adults of mirid bug can be collected as needed for the experiment. Fresh tomato plants were replaced the old plants every 2 weeks.

2.4 Whitefly parasitoid, *Encarsia formosa*

The potted eggplants were placed in whitefly mass rearing netted greenhouse (described in 1.3) as sites of oviposition. Seven days after egg laying, immatures of whitefly emerged and within 14 days whitefly parasitoids, *E. formosa* were observed in the netted greenhouse. Parasitization occurred when *E. formosa* laid eggs in immatures of whitefly. The parasitoids fed and developed inside their host until they reached adult stage. The adult parasitoids emerged while whitefly host succumbed as the result of the parasitoid invading. A daubed of aqueous 10% honey on wax paper was provided as food for the parasitoids. Larvae of whitefly, *B. tabaci* were regularly provided as insect host for the parasitoids to produce their offsprings. In this netted greenhouse when whitefly populations were successfully established, they were naturally parasitized by *E. formosa*.

3. Preparation of fungal suspension

Panyasiri (2005) revealed that entomopathogenic fungi, *Beauveria bassiana* and *Paecilomyces fumosoroseus* were effective against thrips; *Metarhizium anisopliae* and *Hypocrella hypocreoidea* were effective against mealybug. These four fungal isolates were recovered from

insect pests found in Thailand. Therefore, they were selected for the experiment. These fungal isolates were obtained from the collection of Insect Pathology Laboratory, Department of Entomology, Kasetsart University, Kamphaengsaen Campus. They were maintained in 25% glycerol and kept in deep freezed chamber set at -80^{o}C. The fungi were recultured by transferring pieces of fungi onto PDA (Potato Dextrose Agar) plates and incubated at 25 $\pm$ 1^{o}C. When diameter of the colonies reached approximately 5-6 cm, they were harvested by scraping surface of the agar plates and suspending in aqueous sterile 0.1% tween 20 (autoclaved for 15 minutes at 121^{o}C,15 psi). The suspension was then vortexed for a second. Conidia were separated from hyphae and substrate material by filtration through a sterile sieve. Conidia count was made by using an Improved Neubauer Bright Line haemacytometer and different concentrations were prepared by ten-fold serially dilution of the original suspension with sterile distilled water. These fungal suspensions were used for evaluation of their pathogenicity and effect towards the natural enemies of tomato insect pests.

4. Pathogenicity of entomopathogenic fungi on predators of tomato insect pests

Pathogenicity of the four selected entomopathogenic fungi on the predators of tomato insect pests were determined. The fungi, *M. anisopliae* and *H. hypocreoidea* were bioassayed against green lacewing, *M. basalis*, the major predator of mealybug and the fungi *B. bassiana* and *P. fumosoroseus* against anthocorid bug, *W. rotunda* and mirid bug, *M. caliginosus*, the important predators of thrips.

4.1 Bioassay against green lacewing, *Mallada basalis* (Walker)

a. Conidial suspensions of *M. anisopliae* and *H. hypocreoidea* were prepared as described in 3. After harvesting, the spore counts for *M. anisopliae* and *H. hypocreoidea* were 4.5 x 10^{9} and 3.8 x 10^{9} conidia/ml, respectively. From these original concentrations, ten-fold serially dilutions were made. *M. anisopliae* suspensions of 4.5 x 10^{6}, 4.5 x 10^{7}, 4.5 x 10^{8} and 4.5 x 10^{9} conidia/ml and *H. hypocreoidea* suspensions of 3.8 x 10^{6}, 3.8 x 10^{7}, 3.8 x 10^{8} and 3.8 x 10^{9} conidia/ml were used as inoculums in the experiment.

b. A complete randomized design was employed for the experiment which consisted of five treatments (four concentrations of the fungus and the control), four replicates for each treatment. Each replicate consisted of 20 second-instar larvae of green lacewing.

c. Each experimental unit was prepared according to the following procedure: four hundreds of the second-instar larvae of green lacewing, *M. basalis* were picked up and carried out in plastic boxes from the above-described rearing insectary. All appliances were surface sterilized before use by dipping in 70% ethanol. Twenty larvae were removed from the plastic box with a fine paintbrush and placed on a filter paper in a Petri-dish (9 cm diameter). Each plate was then sprayed with 4 ml of each concentration of the two fungal suspensions prepared earlier and with aqueous 0.1% tween 20 for the control. The sprays were made from a 30 cm distance using a clean perfume bottle. After spraying, the immatures of green lacewing were placed on a sterile filter paper to absorb any excess droplet and were moved into plastic cups. A number of immature aphids, *A. gossypii* were introduced into the cups as food for the predators. The cup was then lidded with fine cheesecloth and held by round rubber band (Figure 4). All plastic cups were grouped together in plastic container (30 x 40 x 10 cm). The basket containers were then placed in environmental chamber regulated at 26 $\pm$ 2^{o}C. All stages of aphid were daily supplied as food. Mortality of green lacewing larvae was recorded for 7 days.

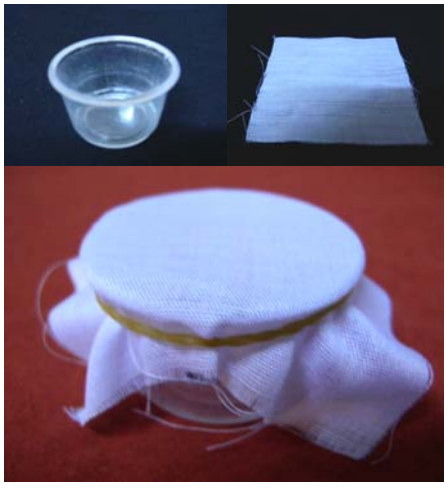

Figure 4 Plastic cup tightly covered with fine cheesecloth used for rearing predators after spray application with the selected fungi

4.2 Bioassay against anthocorid bug, *Wollastoniella rotunda*

a. Conidial suspensions of *P. fumosoroseus* and *B. bassiana* were prepared as described in 3. After harvesting, the spore counts for *P. fumosoroseus* and *B. bassiana* were 4.2 x 10^9 and 4.3 x 10^9 conidia/ml, respectively. From these original concentrations, ten-fold serially dilutions were made. *P. fumosoroseus* suspensions of 4.2 x 10^6, 4.2 x 10^7, 4.2 x 10^8 and 4.2 x 10^9 conidia/ml and *B. bassiana* suspensions of 4.3 x 10^6, 4.3 x 10^7, 4.3 x 10^8 and 4.3 x 10^9 conidia/ml were used as inoculums in the experiment.

b. A complete randomized design was employed for the experiment which consisted of five treatments (four concentrations of the fungus and the control), four replicates for each treatment. Each replicate consisted of 20 third-instar larvae of anthocorid bug.

c. Each experimental unit was prepared according to the following procedure: four hundreds of the third-instar nymphs of anthocorid bug, *W. rotunda* were picked up from the above-described rearing insectary and placed in the plastic boxes for the tests. All appliances were surface sterilized before use by dipping in 70% ethanol. Twenty of the third-instar immatures of anthocorid bug were placed into a Petri-dish (9 cm diameter). The plates were covered with lids and placed in a refrigerator set at 6 $\pm$ 2^{o}C for approximately 10 minutes to decrease bugs activity and ease fungal application. The Petri-dishes were then removed from refrigerator and 4 ml of each concentration of the two fungi prepared earlier were sprayed onto the immatures of anthocorid bug in each plate. The solution of 0.1% tween 20 was used for the control. The bugs were then gently tapped out from the plate onto a sterile filter paper to absorb any excess droplet of the fungal suspension on the body. After that the treated anthocorid bugs were transferred singly into plastic cups, covered with lids. A number of thrips, *C. claratris* from the rearing insectary were provided as insect preys for the predators. All cups were grouped together in a plastic basket container (30 x 40 x 10 cm). The containers were incubated in environmental chamber regulated at 26$\pm$2^{o}C. All stages of thrips were daily supplied as food for the tested anthocorid bugs. Mortality of *W. rotunda* was recorded for 7 days.

4.3 Bioassay against mirid bug, *Macrolophus caliginosus*

a. Conidia suspensions of *P. fumosoroseus* and *B. bassiana* were prepared as described in 3. For this experiment, the original concentrations by spore counts of *P. fumosoroseus* and *B. bassiana* were 5.4 x 10^9 and 5.0 x 10^9 conidia/ml, respectively. From these original concentrations, ten-fold serially dilutions were made and *P. fumosoroseus* spore suspensions of 5.4 x 10^6, 5.4 x 10^7, 5.4 x 10^8 and 5.4 x 10^9 conidia/ml and *B. bassiana* spore suspensions of 5.0 x 10^6, 5.0 x 10^7, 5.0 x 10^8 and 5.0 x 10^9 conidia/ml were used as inoculums in the experiment.

b. A complete randomized design was employed for the experiment which consisted of five treatments (four concentrations of the fungus and the control), four replicates for each treatment. Each replicate consisted of 20 adults of mirid bug.

c. Each experimental unit was prepared according to the following procedure: four hundreds of the adults of mirid bug, *M. caliginosus* were picked up from the above-described rearing insectary and placed in plastic boxes for the tests. All appliances were surface sterilized before use by dipping in 70% ethanol. Twenty adults of mirid bug were placed into a Petri-dish (9 cm diameter). The plates were then covered with lids and placed in a refrigerator set at 6 ± 2^{o}C for approximately 10 minutes to decrease bugs activity and ease fungal application. Mirid bugs were removed from refrigerator and 4 ml of each concentration of the two fungal suspensions prepared earlier were sprayed onto the adults of mirid bug in each plate and 0.1% tween 20 was used for the control. The bugs were then gently removed from the plate and placed on a sterile filter paper to absorb any excess droplet of the fungal suspension on the body. After that the adult mirid bugs were transferred singly into plastic cups covered with lids. Picked-up thrips from the rearing insectary were placed in the plastic cups to serve as food. All cups were grouped together in plastic basket container (30 x 40 x 10 cm) which were placed on cabinet in environmental chamber regulated at 26 ± 2^{o}C. All stages of thrips were daily supplied as insect prey for the tested mirid bug. Mortality of *M. caliginosus* was recorded for 7 days.

4.4 Statistical analysis

To ensure that death of the predators were due to the infection of the tested entomopathogenic fungi, individual insect cadaver was placed on filter paper, wetted with distilled water, in clean Petri-dishes. The plates were sealed with parafilm and incubated at 26 $\pm$ 2^{o}C for 4 days to observe outgrowth of the fungus on the insect body. The recovered fungus was identified and compared with the respective original inoculum. Mortality data of the tested insects at day 7th were corrected using Abbott's formula (Abbott, 1925) as the following equation:

$$\text{Percent corrected mortality} = \left[(C-T) / C \right] \times 100$$

C = Percentage of the control insects that are living

T = Percentage of the treated insects that are living after the experimental period.

Data were subjected to analysis of variance and the least significant difference test at the *P*= 0.05. Percentage values were normalized using arcsine transformation before each analysis. The ANOVA analysis was made using SPSS.

5. Effect of entomopathogenic fungus on entomophagous insects of tomato insect pests

Green lacewing, *Mallada basalis* is an aggressive and efficient predator of several species of insect. In addition, it can be mass-reared quite easily in laboratory and adequate numbers of each developmental stage can be obtained when needed for the experiment. With these reasons, it was selected to demonstrate possible adverse effects of entomopathogenic fungi to its progeny and to its role as effective insect predator. The harmful effects of the fungi on whitefly parasitoid, *Encarsia formosa* was also evaluated in this study.

5.1 Effect of entomopathogenic fungus on the predator

5.1.1 Effect of the fungus, *P. fumosoroseus* on green lacewing, *M. basalis*

Twenty *M. basalis* adults were collected from the above-described rearing netted greenhouse and placed in the plastic box for the treatment. Conidial suspensions of *P. fumosoroseus* were prepared as described in 3. After harvesting, suspension of *P. fumosoroseus* at 3.3 x 10^9 conidia/ml was obtained and used as inoculum for the following experiment. Adults *M. basalis* were smeared with the conidial suspension by paintbrush afterwhich they were kept in clean plastic box for mating and egg laying. To allow air circulation and prevent escaping of the predators, a 4 x 5 cm hole was cut on the lid of the plastic box and a fine mesh screen was affixed. In the control, the adults of *M. basalis* were smeared with 0.1% tween 20. *M. basalis* eggs were collected randomly from both the untreated control and fungal-treated units. The experiment consisted of four replications, 10 eggs per replication. Eggs were placed individually in plastic cups lined with a piece of filter paper and newly hatched larvae were fed with aphid, *A. gossypii*. Sterilized water was provided through a piece of moistened cotton placed on the fine mesh screen. Numbers and each developmental stage of *M. basalis* progeny in the control and fungal-treated units were observed and recorded daily.

5.1.2 Statistical analysis

Time periods for each developmental stage of the tested *M. basalis* progeny were evaluated which included egg stage, 1^{st} to 3^{th} larval instars, prepupal and pupal stages. In addition, mortality of *M. basalis* due to mycosis was also evaluated. Mean differences among the control and fungal-treated units were analyzed using ANOVA analysis with a significant level of $\alpha = 0.05$. Means were compared using Duncan's multiple range test.

5.2 Effect of entomopathogenic fungus on the parasitoid

5.2.1 Preparation of whitefly samples parasitized by the parasitoid, *E. formosa*

Two-week old potted eggplants were prepared in screen cage. Four-hundred of adults of whitefly were released in the cage for oviposition. After 24 hours, whiteflies laid eggs on the lower surface of the eggplant leaves. The egg-attached plants were then moved into plexiglass insect cage having 10 cm diameter circular entrance covered with fine cheesecloth (Figure 5).

Figure 5 Two-week old potted eggplants in plexiglass cage infested with whitefly, *Bemisia tabaci* prepared for parasitization

The whitefly eggs hatched and the larvae grew into the second instar within 11-12 days after egg laying. Fifty of the parasitoid whitefly, *E. formosa* collected from the netted greenhouse as described in 2.4 were released into the cage containing colony of second instar whitefly for parasitization. After 24 h of exposure, the parasitoids were removed from the cages and the parasitized whitefly larvae were transferrred into new cages (Figure 6) for further fungal sprays.

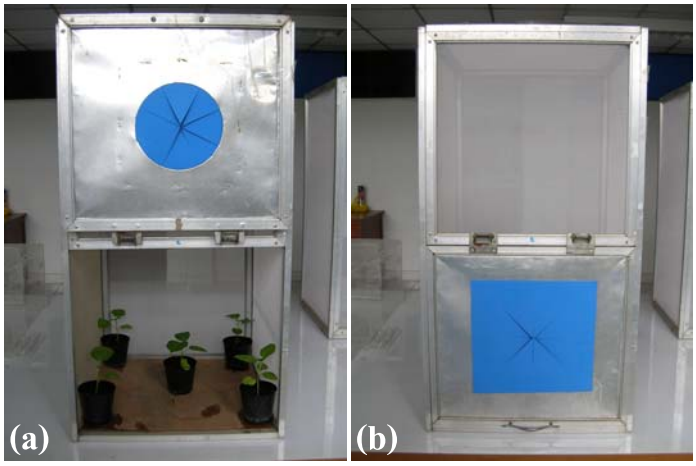

Figure 6 Insect screen cages used to determine the effect of entomopathogenic fungi on the survival of the parasitoid

(a) Screen cage with aluminium door having square rubber plate cut crosswise to ease working and prevent the entry of other small insects

(b) Screen cage with door opened to view the eggplants infested with parasitized whitefly larvae prepared for fungal sprays

5.2.2 Effect of *P. fumosoroseus* on the whitefly parasitoid, *E. formosa*

Conidial suspensions of *P. fumosoroseus* were prepared as described in 3. After harvesting, the spore count for *P. fumosoroseus* was estimated at 3.3 x 10^9 conidia/ml. From this original concentration, ten-fold serially dilutions were made. *P. fumosoroseus* suspensions of 3.3 x 10^6, 3.3 x 10^7, 3.3 x 10^8 and 3.3 x 10^9 conidia/ml were used as inoculums in the experiment. The immatures of whitefly were sprayed with each fungal concentration using 70% ethanol cleaned foggy bottle. The spray applications were made at 0 (parasitoid at egg stage), 5 (parasitoid at larval stage) and 9 (parasitoid at pupal stage) days after exposure to parasitoid. Five replications, twenty of immatures of whitefly per replicate were made for each fungal concentration. Numbers of mycosis of whiteflies, mumification of whitefly due to parasitization and adult parasitoid emergence were recorded on day 15th after parasitoid exposure. Infection of *P. fumosoroseus* was confirmed by stereo microscopic observation.

5.2.3 Statistical analysis

Data on percent mycosis of the immature whitefly, percent mummification of the parasitized whitefly and percent emergence of the adult parasitoid were analyzed using the compare means procedure in SAS. In case the ANOVA yielded significant *P*-values, means were compared using Duncan's multiple range test. A significance level of $\alpha = 0.05$ was used in all analyses.

RESULTS AND DISCUSSION

1. Pathogenicity of entomopathogenic fungi on the predators of tomato insect pests

1.1 Pathogenicity tests of the entomopathogenic fungi, *Hypocrella hypocreoidea* and *Metarhizium anisopliae* on green lacewing, *Mallada basalis*

The initial conidial suspensions of the fungi, *H. hypocreoidea* and *M. anisopliae* harvested from PDA medium, were measured 3.8×10^9 and 4.5×10^9 conidia/ml, respectively. Five concentrations, 10-fold serially diluted from the initial suspensions of the two fungi were used as inoculum for the bioassays against green lacewing, *M. basalis*. Percent corrected mortalities of green lacewing were summarized in Table 1 and 2. *H. hypocreoidea* at the concentrations of 3.8×10^6, 3.8×10^7, 3.8×10^8 and 3.8×10^9 conidia/ml caused 1.47, 2.94, 0 and 0% mortality, respectively and *M. anisopliae* at the concentrations of 4.5×10^6, 4.5×10^7, 4.5×10^8 and 4.5×10^9 conidia/ml caused 1.64, 0, 1.64 and 0% mortality, respectively

Percent mortalities of green lacewing sprayed with *H. hypocreoidea* as illustrated in Table 1, were very low and not significantly different from the controls ($P > 0.05$). Similar results were also obtained when the green lacewings were sprayed with *M. anisopliae* as shown in Table 2 ($P > 0.05$). These data indicated that the fungi, *H. hypocreoidea* and *M. anisopliae* were non-pathogenic to the green lacewing, *M. basalis*.

1.2 Pathogenicity tests of the entomopathogenic fungi, *Paecilomyces fumosoroseus* and *Beauveria bassiana* on anthocorid bug, *Wollastoniella rotunda*

The harvested conidial suspensions of the fungi, *P. fumosoroseus* and *B. bassiana* were measured 4.2×10^9 and 4.3×10^9 conidia/ml, respectively. Five different concentrations, 10-fold serially diluted from the initial suspensions were used as inoculum for the bioassays against anthocorid bug, *W. rotunda*. Percent corrected mortalities of anthocorid bug were shown in Table 3 and 4. *P. fumosoroseus* at the concentrations of 4.2×10^6, 4.2×10^7, 4.2×10^8 and 4.2×10^9 conidia/ml caused 3.92, 1.96, 0 and 1.96% mortality, respectively and *B. bassiana* at the

concentrations of 4.3 x 10^6, 4.3 x 10^7, 4.3 x 10^8 and 4.3 x 10^9 conidia/ml caused 1.61, 0, 3.23 and 3.23% mortality, respectively.

When the anthocorid bugs were sprayed with *P. fumosoroseus*, percent mortalities as shown in Table 3, were very low and not significantly different from the controls ($P > 0.05$). Spray treatment on the anthocorid bug with *B. bassiana*, gave similar results as shown in Table 4 ($P > 0.05$). These results revealed that the anthocorid bug, *W. rotunda* was not susceptible to the fungi, *P. fumosoroseus* and *B. bassiana*. The tested fungi were considered non-pathogenic to *W. rotunda*.

1.3 Pathogenicity tests of the entomopathogenic fungi, *Paecilomyces fumosoroseus* and *Beauveria bassiana* on mirid bug, *Macrolophus caliginosus*

Spore counts of the initial suspensions of the fungi, *P. fumosoroseus* and *B. bassiana* harvested from PDA medium, were 5.4 x 10^9 and 5.0 x 10^9 conidia/ml, respectively. Five different concentrations, 10-fold serially diluted from the initial suspensions were used as inoculum for the bioassays against mirid bug, *M. caliginosus*. Percent corrected mortalities of mirid bug were given in Table 5 and 6. *P. fumosoroseus* at the concentrations of 5.4 x 10^6, 5.4 x 10^7, 5.4 x 10^8 and 5.4 x 10^9 conidia/ml caused 0, 7.25, 1.45 and 2.90% mortality, respectively and *B. bassiana* at the concentrations of 5.0 x 10^6, 5.0 x 10^7, 5.0 x 10^8 and 5.0 x 10^9 conidia/ml caused 1.47, 2.94, 2.94 and 2.94% mortality, respectively.

Percent mortalities of the predator, mirid bug sprayed with *P. fumosoroseus* as shown in Table 5, were very low and not significantly different from the controls ($P > 0.05$). Similar results were obtained when the mirid bugs were sprayed with *B. bassiana* as shown in Table 6 ($P > 0.05$). These results confirmed that the fungi, *P. fumosoroseus* and *B. bassiana* were non-pathogenic to the mirid bug, *M. caliginosus*

Results from the pathogenicity tests indicated that the entomopathogenic fungi of insect tomato pests (mealybug and thrips) were non-pathogenic to predatory insects namely green lacewing, anthocorid bug and mirid bug. Similarly, Ignoffo (1981) exposed three species of

predatory insects; the convergent lady beetle, *Hippodamia convergens*, *Chrysopa carnea* and *Podisus maculiventris* and four species of parasitoids; *Voria ruralis*, *Apanteles marginiventris*, *Campoletris sonorensis* and *Telenomus proditor* to extremely high doses of *N. rileyi* in the laboratory and found that the fungus killed none of the natural enemies.

In contrast, Magalhaes *et al*. (1988) demonstrated that two species, *Coleomegilla maculate* and *Eriopis connexa*, of predators were susceptible to infection by topical application of *B. bassiana* in the laboratory. However, infection rates were considerably reduced if the predators received the inoculum only when they walked over contaminated leaf surfaces (the most likely mode of inoculum acquisition in the field). In this study, mode of fungal acquisition was the direct contact of the fungus on insect body. By this route, the predators should receive sufficient amount of the conidia to initiate infection but as observed, no incident of predator death by mycosis. Thus confirmed that *P. fumosoroseus* was not pathogenic to the predators. Magalhaes *et al*. (1988) suggested that, as species that are not physiologically susceptible to infection under optimal laboratory conditions for the fungus, where environmental and behavioral constraints to infection have been removed, are unlikely to be ecologically susceptible to infection in the field. However, physiological susceptibility in the laboratory does not necessarily translate into field ecological susceptibility, where the impact of a given fungus on a physiologically susceptible predator or parasitoid may be minimal (Jaronski *et al*., 1998). In addition, Pell and Vandenberg (2002) showed that under optimal conditions for the fungus, individuals of the aphid natural enemies, *H. convergens* (Coleopera: Coccinellidae) were killed by high dose of *P. fumosoroseus* after 72 h at 100% relative humidity in the laboratory, but they speculated that as these conditions are unlikely to be replicated in the field, this important aphid natural enemy would be unlikely to be affected by field application rates of the fungus. In similar studies, James and Lighthart (1994) showed that *H. convergens* was physiologically susceptible to *B. bassiana*, *M. anisopliae* and *P. fumosoroseus* in laboratory tests. However, in field tests only *B. bassiana* caused significant mortality in *H. convergens* populations and only early in the season when environmental conditions were favorable. Applications later in the season, when temperatures were higher than the optimum for infection, had little impact on the insect (James *et al*., 1995). Eventhough, this present study was performed under laboratory conditions which were optimized in favor of fungal growth, the tested fungi could not cause any infection in the three species of predatory insects. It

can be assumed that in the field, these major predatory insects of tomato insect pests are unlikely to be affected by the tested fungal isolates since field conditions, mostly retain insufficient humidity for spore germination and mycelial growth. Nevertheless, as suggested by many reseachers as mentioned above, it is not necessary that results from laboratory can be implied to the field directly. Further experiment in field conditions may be necessary to evaluate the impact of entomopathogenic fungi on the predatory insects. Such information will support the appropriate use of fungi as safe biological control agents for important insect species.

Table 1 Percent corrected mortality of green lacewing, *Mallada basalis* sprayed with the fungus, *Hypocrella hypocreoidea* under laboratory condition

Fungal concentration (spores/ml)	Mortality Mean ± SD	% Corrected mortality
Control	3.00 ± 0.816	0.00a
3.8 X 10^6	3.25 ± 0.957	1.47a
3.8 X 10^7	3.50 ± 2.380	2.94a
3.8 X 10^8	3.00 ± 0.816	0.00a
3.8 X 10^9	3.00 ± 1.826	0.00a

Means within the column followed by the same letter are not significantly different (P> 0.05, n=20)

Table 2 Percent corrected mortality of green lacewing, *Mallada basalis* sprayed with the fungus, *Metarhizium anisopliae* under laboratory condition

Fungal concentration (spores/ml)	Mortality Mean ± SD	% Corrected mortality
Control	4.75 ± 0.500	0.00a
4.5 X 10^6	4.75 ± 1.258	1.64a
4.5 X 10^7	4.75 ± 1.258	0.00a
4.5 X 10^8	5.00 ± 0.816	1.64a
4.5 X 10^9	4.75 ± 1.258	0.00a

Means within the column followed by the same letter are not significantly different (P> 0.05, n=20)

Table 3 Percent corrected mortality of anthocorid bug, *Wollastoniella rotunda* sprayed with the fungus, *Paecilomyces fumosoroseus* under laboratory condition

Fumgal concentration (spores/ml)	Mortality Mean ± SD	% Corrected mortality
Control	2.25 ± 1.500	0.00a
4.2×10^6	2.75 ± 0.957	3.92a
4.2×10^7	2.50 ± 1.291	1.96a
4.2×10^8	3.00 ± 0.816	0.00a
4.2×10^9	3.50 ± 0.577	1.96a

Means within the column followed by the same letter are not significantly different ($P > 0.05$, n=20)

Table 4 Percent corrected mortality of anthocorid bug, *Wollastoniella rotunda* sprayed with the fungus, *Beauveria bassiana* under laboratory condition

Fungal concentration (spores/ml)	Mortality Mean ± SD	% Corrected mortality
Control	4.50 ± 1.291	0.00a
4.3×10^6	4.75 ± 0.500	1.61a
4.3×10^7	4.50 ± 1.291	0.00a
4.3×10^8	5.00 ± 0.816	3.23a
4.3×10^9	5.00 ± 1.414	3.23a

Means within the column followed by the same letter are not significantly different ($P > 0.05$, n=20)

Table 5 Percent corrected mortality of mirid bug, *Macrolophus caliginosus* sprayed with the fungus, *Paecilomyces fumosoroseus* under laboratory condition

Fungal concentration (spores/ml)	Mortality Mean ± SD	% Corrected mortality
Control	2.75 ± 0.957	0.00a
5.4×10^6	2.75 ± 1.708	0.00a
5.4×10^7	3.25 ± 0.957	7.25a
5.4×10^8	3.00 ± 1.414	1.45a
5.4×10^9	3.25 ± 1.708	2.90a

Means within the column followed by the same letter are not significantly different ($P > 0.05$, n=20)

Table 6 Percent corrected mortality of mirid bug, *Macrolophus caliginosus* sprayed with the fungus, *Beauveria bassiana* under laboratory condition

Fungal concentration (spores/ml)	Mortality Mean ± SD	% Corrected mortality
Control	3.00 ± 1.414	0.00a
5.0×10^6	3.25 ± 0.957	1.47a
5.0×10^7	3.50 ± 1.291	2.94a
5.0×10^8	3.50 ± 1.000	2.94a
5.0×10^9	3.50 ± 1.291	2.94a

Means within the column followed by the same letter are not significantly different ($P > 0.05$, n=20)

2. Effect of entomopathogenic fungus on entomophagous insects of tomato insect pests

2.1 Effect of entomopathogenic fungus, *Paecilomyces fumosoroseus* on green lacewing, *Mallada basalis*

Effect of *P. fumosoroseus* on succeeding generation of green lacewing, *M. basalis* was investigated. Developmental cycle of progeny of the green lacewing treated with the fungus during its adult stage was carefully observed in comparison to progeny of the untreated group. The results were summarized in Table 7. The developmental cycle of untreated *M. basalis*, maintained in laboratory condition (26 $\pm$ 2^{o}C) was recorded as the followings. The duration of egg stage was 2.40 $\pm$ 0.516 days ranging from 2 to 3 days. The larva of *M. basalis* has three larval instars. The duration of the 1st, 2nd and 3rd larval instars were 2.50 $\pm$ 0.527 days, ranging from 2 to 3 days; 2.40 $\pm$ 0.516 days, ranging from 2 to 3 days and 3.90 $\pm$ 0.738 days, ranging from 3 to 5 days, respectively. The duration of prepupa stage was 1.20 $\pm$ 0.422 days, ranging from 1 to 2 days and the duration of pupal stage was 9.40 $\pm$ 0.516 days, ranging from 9 to 10 days. In fungal-treated *M. basalis*, the recorded developmental cycle was as the followings: the duration of egg stage was 2.53 $\pm$ 0.507 days, ranging from 2 to 3 days. The duration of the 1st, 2nd and 3rd larval instars were 2.67 $\pm$ 0.479 days, ranging from 2 to 3 days; 2.77 $\pm$ 0.430 days, ranging from 2 to 3 days and 3.93 $\pm$ 0.944 days, ranging from 3 to 4 days, respectively. The duration of prepupa stage was 1.27 $\pm$ 0.450 days, ranging from 1 to 2 days and the duration of pupal stage was 9.50 $\pm$ 1.042 days, ranging from 9 to 11 days.

No significant differences were detected on the duration of egg, larval, prepupal and pupal stages of the progeny of the fungal-treated and untreated *M. basalis* (P> 0.05). This indicated that longivity of the progeny of the fungal-treated green lacewing was not different from that of the untreated individuals. Green lacewing progeny from both groups grew normally to adult stage. In addition, larva, prepupa and pupa mortalities observed in the progeny of the fungal treated and untreated individuals did not differ significantly (P> 0.05). Entomopathogenic fungus, *P. fumosoroseus* had no effect on all developmental stages of the succeeding generation of the fungal-treated *M. basalis*. Percent mortality (2.5%) found in 1st instar larvae of *M. basalis* (progeny of the fungal-treated individuals) was probably due to the effect of insect handling during the experiment since at this stage, the larvae were very small and so fragile.

Joudrey and Bjornson (2007) studied on the effects of an unidentified microsporidium on the convergent lady beetle, *Hippodamia convergens* (Coleoptera: Coccinellidae) which has been used for biological control of *H. convergens*. They found that developmental times for larval and pupal stages were significantly longer for microsporidia-infected *H. convergens* than for uninfected individuals. However, no significant difference was detected for developmental times between microsporidia-infected and uninfected eggs. There was also no significant difference on the larval mortality. In contrast, our study revealed that the developmental times of *P. fumosoroseus*-treated larvae and pupae of *M. basalis* were not significantly longer than that of the untreated individuals. Nevertheless, result on larval mortality was similar in which mortalities in pathogen-treated and untreated groups were not significantly different.

Results from this study could suggest the use of the fungus, *P. fumosoroseus* in combination with the predator, *M. basalis* for effective control of important insect pests of tomato cultivated in greenhouse for example tomato thrips and mealybug. *P. fumosoroseus* spray application at any concentration did not affect habitation and development of *M. basalis* and its progeny. Therefore, there is no special precaution for the release of predator and the application of entomopathogenic fungus for insect control. They can be used together at the same time or at different time. Combined use of these two biological agents could enhance insect control efficiency. This strategy can be implied for biological control of tomato insect pests in field condition as well. Fungal applications have no deleterious effects on the predators existing naturally in the environment.

Table 7 Growth development and mortality observed in progeny of the untreated and fungal-treated green lacewing, *Mallada basalis*

	Untreated			Fungal-treated			% mortality	
	Mean development ± SD (days)	range	*n*	Mean development ± SD (days)	range	*n*	Untreated	Fungal-treated
Egg	2.40 ± 0.516c	2-3	10	2.53 ± 0.507c	2-3	10		
1st instar larva	2.50 ± 0.527c	2-3	10	2.67 ± 0.479c	2-3	9.75	0a	2.5a
2rd instar larva	2.40 ± 0.516c	2-3	10	2.77 ± 0.430c	2-3	9.75	0a	0a
3nd instar larva	3.90 ± 0.738b	3-5	10	3.93 ± 0.944b	3-4	9.75	0a	0a
Prepupa	1.20 ± 0.422d	1-2	10	1.27 ± 0.450d	1-2	9.75	0a	0a
Pupa	9.40 ± 0.516a	9-10	10	9.50 ± 1.042a	9-11	9.75	0a	0a

Means followed by the same letter are not significantly different at the 0.05 level using Duncan's multiple range test

2.2 Effect of entomopathogenic fungus, *Paecilomyces fumosoroseus* on the whitefly parasitoid, *Encarsia formosa*

Fungal-infected whitefly can be easily recognized by the mycelia outgrowth from the cadaver as observed by stereo-microscope. Percent mycosis of immatures of whitefly after spray applications of *P. fumosoroseus* was shown in Table 8. Spray treatments of *P. fumosoroseus* at the concentrations of 3.3×10^6, 3.3×10^7, 3.3×10^8 and 3.3×10^9 conidia/ml, made immediately after parasitoid exposure caused 43.33, 50.00, 73.33 and 90.00% mycosis in whitefly, respectively and mean percent mycosis was 64.17%. Percent mycosis increased in correspond to the increasing of the fungal concentration. No incident of mycosis was observed when fungal applications were made 5 and 9 days after parasitoid exposure. Based on this result, *P. fumosoroseus* was a virulent strain towards whitefly as it could cause approximately 40-90% mortality depend on the concentration of the fungus. However, fungal treatment either at high or low concentration could not induce mycosis in whitefly if the whitefly was parasitized by parasitoid especially when the parasitoid in whitefly host had reached larval or pupal stage. Kim (2005) had studied impact of the entomopathogenic fungus, *Verticillium lecanii* on development of an aphid parasitoid, *Aphidius colemani* and found that mycosis in treated aphids was significantly affected, depending on time of exposure to spores. Mycosis in aphid were high with the first three treatment times (no fungus, 1 day before and immediately exposure), but were not detected within the 5 and 7 day after exposure dates. Likewise, % mycosis observed in this study indicated that whitefly suffered with mycosis only when fungal spray application was made immediately after parasitoid exposure and once the parasitoid had established in whitefly host (approximately 5 days after parasitoid exposure), no mycosis was detected.

As the intra-host developmental time for fungal pathogens is generally shorter than that of parasitoids (Brooks, 1993). It is suggested that if a fungal pathogen infected host insect at the same time of parasitoid invading the insect, fungus can outgrowth the parasitoid and grow rapidly until it covers the whole body of the insect. However, if a fungal pathogen infected insect host after the insect has already been parasitized by parasitoid, the fungus can not grow as normal due to the competition for host resources necessary for growth development of the

fungus and parasitoid. This often resulted in high percentage of parasitism and rarely, if any that both fungal mycosis and parasitism occurred in the same insect host.

Table 8 Percent mycosis of whitefly, *Bemisia tabaci* sprayed with conidial suspensions of *Paecilomyces fumosoroseus* at different times after parasitoid exposure[a/]

Fungal suspension (conidia/ml)	n	Immediately after parasitization		5 day after parasitization		9 day after parasitization	
		Number mycosis	% mycosis	Number mycosis	% mycosis	Number mycosis	% mycosis
Control	30	0	0.00e	0	0.00e	0	0.00e
3.3×10^6	30	13	43.33d	0	0.00e	0	0.00e
3.3×10^7	30	15	50.00c	0	0.00e	0	0.00e
3.3×10^8	30	22	73.33b	0	0.00e	0	0.00e
3.3×10^9	30	27	90.00a	0	0.00e	0	0.00e
Mean[b/]	30	19.25	64.17	0	0.00	0	0.00

Means followed by the same letter are not significantly different at the 0.05 level using Duncan's multiple range test

[a/] recorded at day 15th after parasitoid exposure

[b/] Mean for all fungal concentrations, the control is excluded

n number of tested insects in each replication, 5 replicates per treatment

After parasitization, the body of the immature whitefly became darken and turned black eventually. The black cadaver due to parasitization was generally called "mummy". Successful parasitism was, therefore indicated by mummification of whitefly (Figure 7a) and emergence of the adult parasitoid from the whitefly mummy (Figure 7b). Mummification was counted based on the number of black cadavers and parasitoid exit-hole on the cadavers.

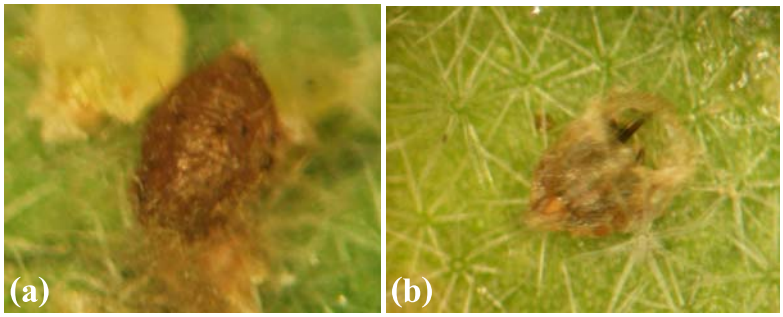

Figure 7 (a) Mummified 2nd instar larvae of whitefly, *Bemisia tabaci* parasitized by the parasitoid, *Encarsia formosa* (27x)
(b) Exuviae of whitefly after emergence of the adult parasitoid (25x)

Table 9 summarized percent mummification of immature whitefly observed after inoculation with *P. fumorososeus* at immediately, 5 day and 9 day after the exposure to *E. formosa*. The percent mummification was significantly different within fungal applications at immediately, 5 day and 9 day after exposure of whitefly to parasitoid oviposition. It was found that means of percent mummification increased in correspond to the time interval between fungal application and parasitoid oviposition. When fungal sprays were made immediately, 5 and 9 days after exposure to parasitoid, means of percent mummification were 35.84, 67.50 and 94.17%, respectively. Number of mummy whitefly observed at 9 days after exposure to parasitoid was higher than those at immediately and 5 days after parasitoid exposure. This study demonstrated that parasitoid development was slower and took longer time than the fungus. If fungal spray was made immediately after parasitoid oviposition, parasitoid can not compete with the fungus in intake the insect host. Mesquita and Lacey (2001) studied the interaction among the Russian wheat aphid *Diuraphis noxia*, its parasitoid *Aphelinus asychis* and the pathogen *P. fumosoroseus*, and revealed that treatment with the pathogen 24-96 h after parasitoid oviposition did not affect cocoon production but adult parasitoid emergence from cocoons were depressed following pathogen application 24 h after parasitism. They pointed out that when aphid hosts were treated with the fungus before exposure to parasitoids, the number of cocoons produced was significantly affected by the degree of temporal separation between fungal attack and parasitoid oviposition. When the fungus and the parasitoid attacked host aphids at the same time, the number of cocoons produced was not reduced but if the delay in

parasitoid attacked increased by 24 h, or more the number of cocoons produced declined, approaching zero when the delay was 72 h after fungal application. Their reports agreed with the results presented here which demonstrated that timing of fungal application to the insect host determined percent parasitism and adult parasitoid emergence. The parasitoid cocoon production or percent mummification increased with the delay in fungal application to the insect host after parasitoid exposure.

Percent mummification of whitefly due to *E. formosa* attack decreased following the progessive increase in the concentration of the fungus spray. When *P. fumosoroseus* at the concentrations of 3.3×10^6, 3.3×10^7, 3.3×10^8 and 3.3×10^9 conidia/ml were applied immediately after parasitoid exposure, percent mummification by *E. formosa* were 56.67, 50.00, 26.67 and 10.00%, respectively. When fungal spore treatments at the concentrations of 3.3×10^6, 3.3×10^7, 3.3×10^8 and 3.3×10^9 conidia/ml were made at 5 days after parasitoid exposure, percent mummification of 83.33, 70.00, 63.33 and 53.33% were obtained, respectively. Kim *et al*. (2005) studied impact of the fungus *V. lecanii* on development of an aphid parasitoid, *A. colemani* and reported that mummification of aphids was severely reduced following spore treatments 1 day before or 0 to 3 days after exposure to the parasitoid but there was little impact when the spore treatment was at 5 or 7 days after parasitoid application. Both direct fungal infection and malformed parasitoid larvae or pupae were observed on dissection of mummies from aphids treated with spores shortly after parasitoid exposure. The cuticle of mummified aphids sprayed with fungal spores 5 and 7 days after parasitoid exposure were not penetrated by *V. lecanii*.

Table 9 Percent mummification of whitefly, *Bemisia tabaci* due to the parasitization by *Encarsia formosa* after spraying with conidial suspensions of *Paecilomyces fumosoroseus* at different period of parasitoid exposure[a/]

Fungal suspension (conidia/ml)	n	Immediately after parasitization		5 day after parasitization		9 day after parasitization	
		Number mummy	% mummy	Number mummy	% mummy	Number mummy	% mummy
Control	30	29	96.67a	28	93.33ab	29	96.67a
3.3×10^6	30	17	56.67de	25	83.33b	28	93.33ab
3.3×10^7	30	15	50.00e	21	70.00c	29	96.67a
3.3×10^8	30	8	26.67f	19	63.33cd	27	90.00ab
3.3×10^9	30	3	10.00g	16	53.33de	29	96.67ab
Mean[b/]	30	10.75	35.84	20.25	67.50	28.25	94.17

Means followed by the same letter are not significantly different at the 0.05 level using Duncan's multiple range test

a/ recorded at day 15th after parasitoid exposure

b/ Mean for all fungal concentrations, the control is excluded

n number of tested insects in each replication, 5 replicates per treatment

Percent emergence of the adult parasitoids from the whitefly hosts that received *P. fumorososeus* spray application at 0, 5 and 9 day after parasitoid exposure, was shown in Table 10. The percent adult emergence was significantly different at timing of host infection after exposure to parasitoids. It was found that percent emergence increased in correspond to the time interval between fungal application and parasitoid oviposition. Fungal traeatment made immediately, 5 days and 9 days after exposure to parasitoid, means of percent emergence of adult parasitoid were 43.31, 90.74 and 95.63% respectively. Spraying the entomopathogenic fungus on immature of whitefly at 5 and 9 days after exposure to parasitoids were considered to have no effect on the development of *E. formosa* adults. Little impact may be observed when

the fungus was applied at 5 days after exposure to parasitoids. Timing of fungal application to the host of parasitoid is important to the successful development of the parasitoid. Mostly the outcome is affected by whether the insect hosts were first infected by the pathogen or if they were first parasitized by the parasitoid. This suggestion is documented by the results from the study of Askary and Brodeur (1999) with an isolate of *V. lecanii* infecting *Aphidius nigripes*, a parasitoid of potato aphid, *Macrosiphum euphorbiae*. Their study explained that suscessful parasitoid development was dependent upon the timing of host infection, as 30.7 and 89.2% of parasitoids survived to mummification when aphids were exposed to the fungus 2 and 4 days following parasitization. Similar results were also obtained by Fransen and van Lenteren (1994) who found that infection rates of *E. formosa*, by *Aschersonia aleyrodis* varied with timing of spore application after parasitization. The number of parasitized whitefly pupae was significantly reduced by spore treatment 1-3 days after oviposition in whitefly nymphs, whereas spore treatment at 4-10 days did not reduce parasitization (as compared with untreated controls). Although a parasitoid may not die of direct infection of its host by a pathogen, the altered nutritional status and premature death of a fungus-infected aphid host may negatively affect parasitoid survival (Brooks 1993). Interestingly, Washburn *et al*. (2000) reported that parasitized larvae of tobacco hornworm were more susceptible to viral infection than nonparasitized larvae because of immuno suppression by the parasitoid's polydnavirus.

Powell *et al*. (1986) found that the fungal pathogen, *Pandora neoaphidis* took 3-4 days to complete its development and kill its aphid host, whereas the parasitoid *Aphidius rhopalosiphi* took 8 or more days after oviposition to pupate. This suggests that entomopathogenic fungus could complete its development much faster than parasitoid in their co-host. When parasitized aphids were infected by *P. neoaphidis* fewer than 4 days after parasitism, the fungus out-competed the parasitoid for the host resources and caused host death before the parasitoid could complete its development. However, fungal spray on the host aphids at 4 or more days after parasitoid oviposition allowed parasitoids to complete their development and in some cases both *P. neoaphidis* and *A. rhopalosiphi* developed successfully within the same host. All of their findings supported our experiments which indicated that when fungal spray was made immediately after parasitoid exposure, the fungus could complete its development and caused up to 90% mycosis whereas the parasitoid could not reach their final

developmental stage in whitefly host. This study suggested that if parasitoid had already developed inside the insect host, fungus had less chance to complete its development in the same host. As the results had shown that when parasitized whitefly was exposed to fungal spray at 5 and 9 days after parasitoid exposure, the fungus could not complete its development and mummification due to parasitism occurred. Fungal spray made fewer than 5 days after parasitism, some parasitoid adult emergence were observed, but if the spray was made 9 days after parasitism, almost 100% of the parasitoid emergence was obtained.

In a similar study on the interactions among the fungus *Zoophthora radicans*, the diamondback moth *Plutella xylostella* and two of its larval parasitoids, *Diadegma semiclausum* and *Cotesia plutellae*, Furlong and Pell (2000) found that the fungal pathogen was the superior competitor for host resources. The interference competition between the fungus and each of the parasitoids were skewed in favor of the fungus and both parasitoids required a significant temporal adventage over the pathogen to develop successfully. The premature death of parasitized insects that were infected by *Z. radicans* 3 days after parasitoid oviposition resulted in the mortality of immature parasitoids. Infection of parasitized hosts 5 or more days after parasitoid oviposition reduced but did not preclude, successful parasitoid development and the negative competitive effects of *Z. radicans* on parasitoid survival decreased as the interval between parasitoid oviposition and initiation of infection increased. In some host individuals both competitors developed successfully; parasitoid formed viable pupae and the fungus produced conidia. However, the number of conidia and fitness parameters of the emerging parasitoid, both of which could have been negatively affected by interference competition was not quantified value.

Percent emergence of adult parasitoid decreased in correspond to the increasing of the fungal concentrations. Using the fungal spray at the concentrations of 3.3×10^6, 3.3×10^7, 3.3×10^8 and 3.3×10^9 conidia/ml, percents adult parasitoid emergence were 88.24, 60.00, 25.00 and 0.00%, respectively when the spray treatment were made immediately after parasitoid exposure and were 96.00, 95.24, 84.21 and 87.50%, respectively when the spray treatment were made 5 days after parasitoid exposure. This result was similar to the study of Kim *et al*. (2005) who found impact of the fungus, *V. lecanii* on development of an aphid parasitoid, *A. colemani*. The emergence of aphid parasitoids increased following fungal spore spraying at 5 or 7 days

after exposure to the parasitoid; but lower percentage of adult parasitoid emergence was obtained when the spore treatment was made at 1 day before or 0 to 3 days after parasitoid oviposition. This study also revealed that percent emergence of the adult parasitoid varied with respect to the concentration of the fungus used. Increasing the concentration of the fungus, the emergence of adult parasitoid was reduced significantly. The possible explanation was that fungus caused higher percent mortality of whitefly, as a consequence, less adult whitefly parasitoids were produced. Moreover, Fransen and van Lenteren (1994) studied the intra-host interactions among the netted greenhouse whitefly. *Trialeurodes vaporariorum*, its fungal pathogen, *Aschersonia aleyrodis* and the parasitoid, *E. formosa* and found that increasing the time between parasitoid oviposition and fungal infection decreased the competitive adventage over the pathogen and parasitoid. If parasitized hosts were treated with fungus 4 or more days after parasitoid oviposition, the parasitoid had absolute competitive adventage over the pathogen and parasitoid developments were completed with no detrimental effect on the reproductive potential of emerging adults.

The entomopathogenic fungus, *P. fumosoroseus* had proved to be highly pathogenic to whitefly and had minimal negative impact on whitefly parasitoid, *E. formosa.* The results suggest that entomopathogenic fungi can be used together with insect parasitoids for the control of insect pests. These two biological agents could act synergistically if temporal separation between fungal attack and parasitoid oviposition was properly manipulated. Fungal application should be employed with respect to safeness of parasitoid, that is when parasitoid developed into non-susceptible stage. Due to the fact that entomopathogenic fungi had no direct effect on parasitoids, the use of fungi in natural environment or field condition should have no significant impact on naturally occurring parasitoids.

Table 10 Percent of emergence of the whitefly parasitoid, *Encarsia formosa* after spraying with conidial suspensions of *Paecilomyces fumosoroseus* at different times of parasitoid exposure[a/]

Fungal suspension (conidia/ml)	n	Immediately after parasitization		5 day after parasitization		9 day after parasitization	
		Number emerge	% Adult emerge	Number emerge	% Adult emerge	Number emerge	% Adult emerge
Control	30	28	96.55a	28	100.00a	27	93.10a
3.3×10^6	30	15	88.24d	24	96.00c	28	100.00a
3.3×10^7	30	9	60.00e	20	95.24c	26	89.65a
3.3×10^8	30	2	25.00f	16	84.21d	26	96.30a
3.3×10^9	30	0	0.00f	14	87.50d	28	96.55a
Mean[b/]	30	6.5	43.31	18.5	90.74	27	95.63

Means followed by the same letter are not significantly different at the 0.05 level using Duncan's multiple range test

a/ recorded at day 15^{th} after parasitoid exposure

b/ Mean for all fungal concentrations, the control is excluded

n number of tested insects in each replication, 5 replicates per treatment

CONCLUSION

The effective fungal strains against tomato thrips, *Ceratothripoides claratris* and mealybug, *Pseudococcus cryptus*, the important tomato insect pests cultivated in greenhouse, were selected to evaluate their pathogenicity on natural enemies of their respective insect hosts. The considerable effective predators, green lacewing (*Mallada basalis*), anthocorid bug (*Wollastoniella rotunda*) and mirid bug (*Macrolophus caliginosus*) were employed in this study. Pathogenicity tests on green lacewing showed that the fungus, *Hypocrella hypocreoidea* at the concentrations of 3.8×10^6, 3.8×10^7, 3.8×10^8 and 3.8×10^9 conidia/ml caused 1.47, 2.94, 0 and 0% mortality, respectively and *Metarhizium anisopliae* at the concentrations of 4.5×10^6, 4.5×10^7, 4.5×10^8 and 4.5×10^9 conidia/ml caused 1.64, 0, 1.64 and 0% mortality, respectively. In anthocorid bug, the fungus *Paecilomyces fumosoroseus* at the concentrations of 4.2×10^6, 4.2×10^7, 4.2×10^8 and 4.2×10^9 conidia/ml caused 3.92, 1.96, 0 and 1.96% mortality, respectively, and *Beauveria bassiana* at the concentrations of 4.3×10^6, 4.3×10^7, 4.3×10^8 and 4.3×10^9 conidia/ml caused 1.61, 0, 3.23 and 3.23% mortality, respectively. In mirid bug, pathogenicity tests revealed that *P. fumosoroseus* at the concentrations of 5.4×10^6, 5.4×10^7, 5.4×10^8 and 5.4×10^9 conidia/ml caused 0, 7.25, 1.45 and 2.90% mortality, respectively and *B. bassiana* at the concentrations of 5.0×10^6, 5.0×10^7, 5.0×10^8 and 5.0×10^9 conidia/ml caused 1.47, 2.94, 2.94 and 2.94% mortality, respectively. Percentage mortality of the predators treated with the selected fungi were very low and did not differ significantly from the controls. Results suggested that the tested entomopathogenic fungi of tomato insect pests were non-pathogenic to the predators. Moreover, progeny of the fungal-treated green lacewing were not affected by *P. fumosoroseus* spray treatments. Developmental time of the egg, 1^{st}, 2^{nd} and 3^{rd} instar larva, prepupa and pupa, progeny of the fungal-treated green lacewing were not significant different from those of the untreated controls. No larval, prepupal and pupal mortalities were detected in both the fungal-treated and untreated groups.

Effects of the fungus, *P. fumosoroseus* on the whitefly parasitoid, *Encarsia formosa* were evaluated based on percentage of mycosis, mummification and adult parasitoid emergence. *P. fumosoroseus* had proved to be highly effective against whitefly since it caused approximately 40-90% mycosis (mean 64.17%) depend on the concentration of the fungus.

However, no incident of mycosis was observed when fungal applications were made 5 and 9 days after parasitoid exposure. When fungal applications were made immediataly, 5 and 9 days after exposure to parasitoid, means of percent mummification of whitefly were 35.84, 67.50 and 94.17%, respectively and means of percent emergence of adult whitefly parasitoid, *E. formosa* were 43.31, 90.74 and 95.63%, respectively. Mycosis in treated whitefly was affected, depending on time of fungal application and concentration. Means of percent mummification of whitefly host and percent emergence of parasitoid increased in correspond to the time interval between fungal application and parasitoid oviposition. Spray treatments of the entomopathogenic fungus on immatures of whitefly at 5 and 9 days after exposure to parasitoids were considered to have no effect on the development of *E. formosa* adults. Little impact may be observed when the fungus was applied at 5 days after exposure to parasitoids.

In this study, *P. fumosoroseus* had a direct impact on populations of the whitefly host. and had minimal negative effect on the whtiefly parasitoid, *E. formosa*. Timing of fungal applications to the whitefly hosts is important to the successful development of parasitoids. Effects of *P. fumosoroseus* varied with timing of spore application after parasitization. The combined use of *P. fumosoroseus* and *E. formosa* for integrated pest management of whitefly can be an effective control option but fungal spore applications should as much as possible be timed to coincide with the later developmental stage of parasitoid to conserve the parasitoids within the system.

This study provides a promising future basis for integrating the entomophagous insect with entomopathogenic fungi. Generally, there are many factors influencing interactions between biological control organisms which include arthropod behaviour, dose of the applied entomopathogen, specificity, spatial and temporal separation, as well as genetic factors. All these factors should be addressed and carefully studied in order to increase our present understanding of such a combined strategy. Moreover, for effective use of entomopathogenic fungi in combination with the release of parasitoids and predators, one should not neglect the possible direct and indirect effects of entomopathogenic fungi on natural enemies and their progeny and vice versa, under practical growing conditions. All these reliable data should be assessed when considering the safety of a microbial control agent, in order to achieve the

successful and sustainable management of insect pests, the greenhouse pests in particular, since greenhouse environment provides climatic conditions favorable for entomopathogenic fungi.

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APPENDIX

Appendix table 1 Spray application of *Hypocrella hypocreoidea* on Green lacewing, *Mallada basalis*, under laboratory condition.

Concentration (spores/ml)	Replication 1		Replication 2		Replication 3		Replication 4		% mortality
	Total	Killed	Total	Killed	Total	Killed	Total	Killed	
Control	20	3	20	4	20	2	20	3	15
3.8×10^6	20	3	20	4	20	4	20	2	16.25
3.8×10^7	20	2	20	3	20	2	20	7	17.5
3.8×10^8	20	3	20	4	20	2	20	3	15
3.8×10^9	20	5	20	4	20	2	20	1	15

Appendix table 2 Spray application of *Metarhizium anisopliae* on Green lacewing, *Mallada basalis,* under laboratory condition.

Concentration (spores/ml)	Replication 1		Replication 2		Replication 3		Replication 4		% mortality
	Total	Killed	Total	Killed	Total	Killed	Total	Killed	
Control	20	4	20	1	20	3	20	1	15
4.5×10^6	20	3	20	2	20	4	20	2	18.33
4.5×10^7	20	3	20	4	20	2	20	1	16.67
4.5×10^8	20	2	20	3	20	4	20	3	20
4.5×10^9	20	4	20	3	20	3	20	4	16.67

Appendix table 3 Spray application of *Paecilomyces fumosoroseus* on Anthocorid bug, *Wollastoniella rotunda,* under laboratory condition.

Concentration	Replication 1		Replication 2		Replication 3		Replication 4		%
(spores/ml)	Total	Killed	Total	Killed	Total	Killed	Total	Killed	mortality
Control	20	5	20	4	20	5	20	5	23.75
4.2 X 10^6	20	5	20	3	20	6	20	5	25
4.2 X 10^7	20	5	20	6	20	5	20	3	23.75
4.2 X 10^8	20	5	20	4	20	5	20	6	25
4.2 X 10^9	20	5	20	6	20	3	20	5	23.75

Appendix table 4 Spray application of *Beauveria bassiana* on Anthocorid bug, *Wollastoniella rotunda,* under laboratory condition.

Concentration	Replication 1		Replication 2		Replication 3		Replication 4		%
(spores/ml)	Total	Killed	Total	Killed	Total	Killed	Total	Killed	mortality
Control	20	5	20	4	20	3	20	6	22.5
4.3 X 10^6	20	5	20	5	20	5	20	4	23.75
4.3 X 10^7	20	6	20	3	20	5	20	4	22.5
4.3 X 10^8	20	5	20	4	20	5	20	6	25
4.3 X 10^9	20	5	20	3	20	6	20	6	25

Appendix table 5 Spray application of *Paecilomyces fumosoroseus* on Mirid bug, *Macrolophus caliginosus,* under laboratory condition.

Concentration	Replication 1		Replication 2		Replication 3		Replication 4		%
(spores/ml)	Total	Killed	Total	Killed	Total	Killed	Total	Killed	mortality
Control	20	3	20	2	20	4	20	2	13.75
5.4×10^6	20	5	20	2	20	1	20	3	13.75
5.4×10^7	20	3	20	4	20	4	20	2	20
5.4×10^8	20	3	20	5	20	2	20	2	15
5.4×10^9	20	4	20	3	20	5	20	1	16.25

Appendix table 6 Spray application of *Beauveria bassiana* on Mirid bug, *Macrolophus caliginosus,* under laboratory condition.

Concentration	Replication 1		Replication 2		Replication 3		Replication 4		%
(spores/ml)	Total	Killed	Total	Killed	Total	Killed	Total	Killed	mortality
Control	20	2	20	3	20	2	20	5	15
5.0×10^6	20	3	20	4	20	4	20	2	16.25
5.0×10^7	20	5	20	3	20	4	20	2	17.5
5.0×10^8	20	2	20	4	20	4	20	4	17.5
5.0×10^9	20	3	20	2	20	4	20	5	17.5

Appendix table 7 Analysis of Variance (ANOVA) between mortality of green lacewing, *Mallada basalis* in *Hypocrella hypocreoidea* 5 concentration.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.800	4	.200	.089	.985$^{ns/}$
Within Groups	33.750	15	2.250		
Total	34.550	19			

ns/ non significant difference (P>0.05)

Appendix table 8 Analysis of Variance (ANOVA) between mortality of green lacewing, *Mallada basalis* in *Metarhizium anisopliae* 5 concentration.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.800	4	.700	.667	.625$^{ns/}$
Within Groups	15.750	15	1.050		
Total	18.550	19			

ns/ non significant difference (P>0.05)

Appendix table 9 Analysis of Variance (ANOVA) between mortality of anthocorid bug, *Wollastoniella rotunda* in *Paecilomyces fumosoroseus* 5 concentration.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.200	4	.050	.044	.996$^{ns/}$
Within Groups	17.000	15	1.133		
Total	17.200	19			

ns/ non significant difference (P>0.05)

Appendix table 10 Analysis of Variance (ANOVA) between mortality of anthocorid bug, *Wollastoniella rotunda* in *Beauveria bassiana* 5 concentration.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.000	4	.250	.200	.934[ns/]
Within Groups	18.750	15	1.250		
Total	19.750	19			

ns/ non significant difference (P>0.05)

Appendix table 11 Analysis of Variance (ANOVA) between mortality of mirid bug, *Macrolophus caliginosus* in *Paecilomyces fumosoroseus* 5 concentration.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.000	4	.250	.129	.969[ns/]
Within Groups	29.000	15	1.933		
Total	30.000	19			

ns/ non significant difference (P>0.05)

Appendix table 12 Analysis of Variance (ANOVA) between mortality of mirid bug, *Macrolophus caliginosus* in *Beauveria bassiana* 5 concentration.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.000	4	.250	.165	.953[ns/]
Within Groups	22.750	15	1.517		
Total	23.750	19			

ns/ non significant difference (P>0.05)