UNIVERSITY OF HANNOVER
DEPARTMENT OF HORTICULTURE
INSTITUTE FOR PLANT DISEASES AND PLANT PROTECTION

POTENTIAL OF ENTOMOPATHOGENIC FUNGI ALONE AND IN COMBINATION WITH *BACILLUS THURINGIENSIS* FOR CONTROL OF *HELICOVERPA ARMIGERA* (HÜBNER) (LEPIDOPTERA: NOCTUIDAE) IN TOMATOES

Thesis

by

NGUYEN THI HANH NGUYEN

Thesis submitted in partial fulfilment for the requirements of the degree
Master of Science in Horticulture

September, 2004

DECLARATION

I, Nguyen Thi Hanh Nguyen, hereby declare that the work presented in this thesis is my own work, which has not been and will not be submitted for a degree in any other university.

I certify that this thesis has been supervised by:

Prof. Dr. Hans-Michael POEHLING

Prof. Dr. Christian BORGEMEISTER

Prof. Dr. Dieter Martin HÖRMANN

NGUYEN THI HANH NGUYEN

ACKNOLEGEMENTS

I wish to thank and acknowledge the Vietnamese Government for sponsoring my studies in these two years, Institute of Plant Diseases and Plant Protection (IPP) in Hannover University for admitting and providing me a great working environment and Vietnamese National Institute of Plant Protection for allowing me to leave for this study.

I wish to express my deepest gratitude to Prof. Dr. Christian Borgemeister for excellent advice, constructive criticism, stimulation, logical support, excellent critical editing work and patience. Without his help, this work may not be in this complete. I greatly appreciate and specially thank Prof. Dr. Hans-Michael Poehling for his very valuable guidance, criticism and sympathies in my work and providing excellent working conditions at IPP. Special thanks also go to Dr. Gisbert Zimmermann (Institute for Biological Control of the Federal Biological Research Centre for Agriculture and Forestry) for his insightful comments, helpful suggestions, training and supports and his comment to open this study. I would also thank Prof. Dr. Dieter Hörmann for his encouragement and guidance.

I thank all friends for their friendship and encouragement.

Finally, great thanks come to the encouragement and motivation of my family.

ABSTRACT

The susceptibility of third instar larvae of *Helicoverpa armigera* to 7 strains of 3 entomopathogenic fungi (EPF) species, i.e., *Beauveria bassiana, Metarhizium anisopliae* (*M.a*) and *Paecilomyces fumosoroseus* (*P.fr*) was tested under laboratory conditions. Three best-performing strains in 3 species/strains i.e. *B. bassiana* 124, *M. anisopliae* 79 and *P. fumosoroseus* 14 were selected to examine their effectiveness against different larval and pupal stages of *H. armigera*. Sublethal doses were determined for each of the above mentioned EPF strain and for a commercial *Bacillus thuringiensis* (*Bt*) product, i.e., Xentary, and were subsequently tested both in individual bioassays as well as in combinations of an EPF and the *Bt* product.

All strains/species showed substantially high efficacy against third larval instars of *H. armigera*, resulting in 68 to 100% corrected mortality. Higher efficacy levels were detected in *P. fumosoroseus* and *B. bassiana* compared to *M. anisopliae* strains. The more virulent strains resulted in shorter median survival time (MST) of *H. armigera*. With 3.3 days MST was shortest in *P.fr* 14 and with 8.1 days longest in *M.a* 97. However, only significant differences in MST were found between *P.fr* 14 and two *M.a* strains.

Paecilomyces fumosoroseus 14 showed the highest virulence at the lowest LC50 (2.3E+04 conidia/ml), followed by *B. bassiana* 124 (3.6E+04 conidia/ml) and *M. anisopliae* 79 (1.4E+05 conidia/ml). The LC50 for third instar *H. armigera* in Xentary was estimated at 0.0054 mg/ml.

High effectiveness of three above selected EPF species/strains was recorded in all tested larval instars of *H. armigera*, i.e., from second to fifth instars. No significant differences in susceptibility of different larval stages were found. However, second larval instars seemed to be more susceptible to the pathogens, as shown by the shorter MST. Fifth instar larvae responded slower than the younger instars. On the last evaluation date mortality ranged between 82 and 100% across strains and development stages of *H. armigera*. Comparatively lower mortality was recorded in *M.a* 79 compared to the two other strains though even for this strain between 82 to 98% of the *H. armigera* larvae were dead at the end of the experiment.

EPF showed high potential against pupa of *H. armigera* though the effectiveness varied with application methods. Highest mortality levels were recorded when using the immersion technique (100%) followed by soil-inoculation (74.4 to 97.8%). In contrast, almost no effect was detected after soil surface contamination.

In addition to high virulence, intensive sporulation of the EPF on cadavers was recorded in all tested *P. fumosoroceus* and *B. bassiana* strains. Sporulation of *M. anisopliae* on cadavers was lower, with the best result (75%) obtained in fifth instars. Interestingly, sporulation was observed in 100% failed emerged pupae of all tested EPF, even in pupae pretreated with *M. anisopliae,* which showed poor sporulation in larvae cadavers.

In an *in vitro* experiment on interactions between EPF and *Bt* only limited antagonism was found and depended on the agar medium used. More pronounced effects were recorded on LB compared to MEPA medium. In combination bioassays of EPF and *Bt* additive effects of the two pathogens against third instar larvae *H. armigera* could be shown. A twice as high CM (75.2%) was archived when larvae were exposed to the combined effect of EPF and *Bt* compared to individual impact of either an EPF or the *Bt* product.

Our results suggest that EPF alone and in combination with a commercial *Bt* product can efficiently control larval instars of *H. armigera*.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

EPF	Entomopathogenic fungi
Bt	*Bacillus thuringiensis*
NPV	*Nucleopolyhedrovirus*
B.ba	*Beauveria bassiana*
M.a	*Metarhizium anisopliae*
P.fr	*Paecilomyces fumosoroseus*
MST	Median survival time
CM	Corrected mortality
LC50	Lethal concentration
LT50	Lethal time
IPCS	International programme on chemical safety

1 STATEMENT OF RESEARCH PROBLEM

Helicoverpa armigera Hübner (Lepidoptera: Noctuidae) is a widespread polyphagous agricultural pest of worldwide economic importance (Zalucski, 1986; Fitt, 1989; Ranasinghe *et al.*, 1998; Cunningham *et al.*, 1999). This insect pest is considered to be one of the widest distributed of any agricultural pests (Fitt, 1989, King, 1994), and is the key pest of several cultivated and uncultivated crops, and known by various common names such as cotton bollworm, African bollworm, fruit borer and corn earworm (King & Coleman, 1989; Fitt, 1989; Cameron *et al.*, 1995; Romeis & Shanover, 1996; Kranthi *et al.*, 1997; Han *et al.*, 1999). However, the distribution of *H. armigera* is restricted to the old world (King, 1994).

Its high polyphagy, mobility and fecundity enable *H. armigera* to adapt and survive in even unsuitable habitats and in turn to colonize and successfully exploit different agricultural systems (Fitt, 1989). The larvae have a distinct feeding preference for high nitrogen plant structures, reproductive structures and growing points, enabling them to influence the crop yield directly. In addition, feeding on high value crops with low tolerance against *H. armigera* like cotton, tobacco, sweet corn, and tomato that have low damage thresholds, often results in severe crop losses and damage (Fitt, 1989; Jayaraj *et al.*, 1989).

One of the most important host plants of *H. armigera* is tomato, *Lycopersicum esculentum* (Miller) (Solanaceae), a worldwide commercially important vegetable crop both for the fresh fruit market and the processing food industry (Artheton & Harris, 1996). In addition to *H. armigera* a number of other insect pests attack tomatoes such as thrips, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae), cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), white fly, *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae), and leaf defoliator, e.g. *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae). Among these, *H. armigera* is considered to be the major pest, particularly in processing tomatoes (Mann, 2000; Arno *et al.*, 2002). With a very low threshold, once attacked by this insect pest, tomato fruits usually are no longer marketable. *Helicoverpa armigera* can damage up to 30% of fruits in unsprayed late season tomato crops in New Zealand, whereas the acceptable level of damage is 5% (Cameron *et al.*, 2001) and cause yield losses of 50% in India (Jayaraje *et al.*, 1989).

Managing *H. armigera* is a challenge to producers. Currently, control of *H. armigera* mainly relies on the applications of synthetic insecticides (Fitt, 1989; King, 1994; Ahmad *et al.*, 1997; Gunning *et al.*, 1998; Torres-Villa *et al.*, 2002). Management of *H. armigera* is becoming increasingly difficult due to frequent development of resistance to many classes of insecticides in the pest (Armes *et al.*, 1996). The intensive and frequent applications of synthetic insecticides, regardless of the economic threshold, resulted in the development of resistant pest strains, which in turn prompted farmers to apply more and more pesticides (Fitt, 1989; Ahmad *et al.*, 1999). In addition, abusive use of insecticides causes health hazard to farmers by intoxication because of inappropriate application techniques, and to consumers through, among others, high pesticide residues in food products. Control failures due to *H. armigera* resistance and reduced susceptibility to synthetic insecticides have been recorded worldwide (Cameron *et al.*, 1995; Armes *et al.*, 1996; Ahmad *et al.*, 1997; Regupathy *et al.*, 1997; Han *et al.*, 1999; Martin *et al.*, 2000).

Although chemical pesticides continue to be the main pest control agents, interest in bio-pesticides is growing as they are increasingly showing promise for the protection of agricultural crops. Some bio-pesticides are already applied commercially and some are presently undergoing laboratory and field tests. Commercially used bio-pesticides include bacteria-based products like *Bacillus thuringiensis* (*Bt*) and entomopathogenic viruses like *Nucleopolyhedroviruses* (*NPVs*), which have been successfully implemented for control of Heliothinae in general and for *H. armigera* in particular (King & Coleman, 1989; Jayaraj *et al.*, 1989; Carner & Yearian, 1989; King, 1994).

Bacillus thuringiensis, both as spray formulations and in *Bt*-transgenic cultivars, is a promising alternative to the use of synthetic insecticides for *H. armigera* control. However, frequent applications of *Bt* by farmers, among others because of the low persistence of *Bt* under ultra violet light (Roush, 1997), can lead to the development of *Bt*-resistant pest strains as already evidenced in diamond back moth (DBM) *Plutella xylostella* L. (Lepidoptera: Plutellidae) (Marone & Macintosh, 1993; Tabashnik, 1994; Schnepf *et al.*, 1998; International programme on chemical safety (IPCS), 1999; Nester *et al.*, 2002), and rises concern that this can also result in reduced susceptibility of *H. armigera* to *Bt*. *Bt* often has an antifeedant effect in larvae and is less effective against older larval stages.

Several times entomopathogenic fungi (EPF) have been proposed as potentially valuable biocontrol agents of *Heliothis* and *Helicoverpa* spp. (King & Coleman, 1989; King, 1994). However, so far no detailed studies have been carried out on the potential of these pathogens to control *H. armigera.* Entomopathogenic hyphomycetes fungi like *Metarhizium anisopliae* (Metsch.) Sorokin (*M.a*), *Beauveria bassiana* (Bals.) Vuillemin (*B.ba*) and *Nomuraea rileyi* (Fallow) Samson have also been pointed out as potentially useful biocontrol agents for control of *Heliothis* and *Helicoverpa* spp., mainly because of their environmental safety and higher pest selectivity compared to synthetic insecticides (Tang & Hou, 1998; King & Coleman, 1989; Carner & Yearian, 1989; Jayaraj *et al.*, 1989). Alternative use of EPF to *Bt* in pest control may decreases the application intensity of *Bt,* reducing frequent exposure of insect to *Bt* which can lead to resistance selection. However, their slow rate of kill compared to synthetic insecticides often enables pests to continue damaging the crops after an EPF treatment (Quinlan, 1988; Greathead, 1994).

A sub-lethal combination of both pathogens, *Bt* and EPF, may be advantageous at reduce the cost required for each agent alone while still cause high effect in insect control. Moreover, mixed actions of both pathogens can broader the mechanism of action, possibility reduces the risk of resistance development. The main objective of this study was to critically evaluate the potential of EPF for control of *H. armigera* and to optimize biocontrol with microorganism by a combination strategy. Therefore (i) the efficacy of each agent alone has to be checked, then (ii) the possibly interaction between both agents when applied together should be examined and (iii) the possibility of reducing the application dose rates of the bio-agents in order to reduce the cost required for each agent alone has to be tested.

In this study, the efficacy of several species/strains of EPF, i.e., *M.a*, *B.ba* and *Paecilomyces fumosoroseus* (Wize) (*P.fr*) have been evaluated against third instar larvae of *H. armigera.* Subsequently, the three best-performing strains of each EPF species were further evaluated against different larval stages (L2 to L5) and the pupa of *H. armigera*. Then sub-lethal doses of the one EPF species were combined with low doses of two commercial *Bt* products. With this research we do not attempt to completely replace synthetic insecticides or *Bt* for *H. armigera* control but try to develop a potentially alternative and environmentally safer control strategy that could become part of an integrated pest management (IPM) approach to combat this important pest.

This study has been conducted in laboratories of the Institute of Plant Disease and Plant Protection (IPP) in Hannover and the Institute for Biological Control of the Federal Biological Research Centre for Agriculture and Forestry (BBA) in Darmstadt between 2003 and 2004.

2 HYPOTHESES

High pathogenicity of some EPF species/strains of *B. bassiana*, *M. anisopliae*, and *P. fumosoroseus* is expected against immature stages of *H. armigera*. The combination of sub-lethal doses of the two entomopathogens (*Bt* and EPF) is hypothesized to yield significantly higher control levels in *H. armigera* than individual applications of EPF and *Bt* at the same doses. As *Bt* is a comparatively fast acting bio-pesticide, presumably it will play its role during the first days after treatment, whereas because of their slow rate of kill, EPF will impact the pests at a later stage.

3 OBJECTIVES

- To evaluate the effects of several entomopathogenic fungi such as *B. bassiana, M. anisopliae* and *P. fumosoroseus* against different larval instars of *H. armigera*.

- To test the effects of these fungi on *H. armigera* pupae.

- To estimate the effects of the combination of sub-lethal doses of the best performing EPF and *Bt* against *H. armigera* larvae.

4 LITERATURE REVIEW

4.1 Biology of *Helicoverpa armigera* (Hübner)

Helicoverpa armigera belongs to the order Lepidoptera, family Noctuidae. The genus has been recently renamed from *Heliothis* to *Helicoverpa*. The genus *Helicoverpa* was first applied to *H. zea* (Boddie) and *H. armigera* by Hardwick in 1965 since these species and other Heliothis species are not morphologically close related (Poole, 1989; Fitt, 1989; King & Coleman, 1989; King, 1994). However, not all taxonomists were convinced with this morphological distinction of *Helicoverpa* from all other species in the Heliothinae (Poole, 1989). Until 1989, Fitt considered *H. armigera* and *H. zea* being members of the noctuid genus *Heliothis*. However, in the recent past the term *Helicoverpa* has been widely used and to date seems to be accepted by most researchers.

The two most important species in the genus *Helicoverpa* are *H. zea* and *H. armigera*. Both have very similar external appearance and the identification can be only made through an examination of the male and female genitalia (Poole, 1989). In addition, their bio-geographical distribution also supports the taxonomic separation of both species, since *H. armigera* is distributed throughout the old world, whereas *H. zea* is restricted to the new world (Poole, 1989; Fitt, 1989; King, 1994).

4.1.1 Distribution

Helicoverpa armigera is considered as one of the most widely distributed agricultural pests (Fitt, 1989) as well as in the *Helicoverpa* group (King, 1994). Worldwide, *H. armigera* occurs throughout Africa, India, Central and South East Asia to Japan, Philippines, the Middle East, Southern Europe, Eastern and Northern Australia, New Zealand and many Eastern Pacific Islands (Poole, 1989; Fitt, 1989; King, 1994).

4.1.2 Life cycle

The biology and ecology of *H. armigera* have been comprehensively reviewed by Fernald and Shepard (1955), Fitt (1989) and King (1994).

The eggs are sub-spherical with a flattened base and approximately 24 longitudinal ribs. After being laid, they have a whitish or creamy colour. During the development of the embryo, the egg becomes a central reddish-brown band and gradually turns greyish brown before hatching. Egg duration is about 2-5 days depending on the temperature of incubation (King, 1994).

Larvae develop through 5 to 7 instars, with six being most common (Fernald & Shepard, 1955; Fitt, 1989; King, 1994). The duration of larval development depends on the temperature as well as the nature and quality of the host. For instance, in nature, the duration of the larval stage varies from 12.8 to 21.3 days on maize (*Zea mays* L.) at temperatures between 24-27.2°C, and from 15.2 to 23.8 days on maize and tomatoes (*Lycopersicon esculentum* Mill., [Solanaceae]), respectively. Newly hatched larvae have a translucent, yellowish white and brown to black head capsule. The second instar is similar to the first one but slightly darker in body colour. The brown colour predominates in the third instar and is generally darker in later instars. Weight and colour vary considerably with diet. The heaviest larvae feed on cotton and the lightest ones on tomatoes and sorghum. The size of a fully grown larva can vary from 35 to 42 mm in length. The nutrient factors, of which carotene is of major importance, play an important role in the colour of *H. armigera* larvae. At the end of the larval development, the last instar drop or crawl to the ground and enters the soil to pupate.

The pupa is typical for Noctuidae and of about 14-22 mm length and across the thorax 4.5-6.5 mm in width. It is brown, smooth surfaced, and surround at both ends. Pupation occurs at 2.5-17.5 cm soil depth depending on the hardness and wetness of the soil. The pre-pupal stage lasts for 1-4 days. *Helicoverpa armigera* pupal duration varies with temperature, e.g., from 10-14 days under field conditions in central India (King, 1994). Pupae exhibit a facultative diapause under adverse conditions (e.g., in short photoperiods and low temperature). However, in tropical regions *H. armigera* hardly ever diapause under field conditions (Fitt, 1989).

The adult is a stout-bodied moth of 18-19 mm in length and has a wing span of 35-40 mm. The female is grey-brown, darker than the pale creamy coloured male. Moths emerge at a circadian rhythm, starting at dusk until midnight. Adults feed, hence the adult longevity largely depends on the availability of food and food quality for adults and larvae. Adult lifespan also depends on initial weight and temperature. Female longevity is greater than

male. In southern Africa, moth longevity in captivity varied from 1-23 days for male and 5-23 days for female (King, 1994). Average fecundity is 700 eggs up to a maximum of 1,600 egg per female (Fernald & Shepard, 1955; King, 1994).

4.1.3 Behaviour

Newly hatched larvae may crawl and occasional feed on the plant surface before settling down and feeding at their preferred sites, i.e., flowers, buds, bolls and fruits. While eating, a part or their entire body hides inside the feeding site pushing faeces out, which indicates its presence. Larvae usually partially damage fruits and then move to another. This behaviour makes them a highly destructive insect pest. Moulting occurs on the upper surface of the leaf while exposing to sunlight to enhance the drying of the new cuticle. Cannibalism is often found in third instar and older larvae. The full-fed larvae drop to the ground and enter the soil for pupation (King, 1994). Pupal diapause may be induced by winter or summer conditions, which enables the population to survive during adverse environmental conditions.

The high mobility of the adult moths contributes to *H. armigera*'s pest status in particular and to members of the subfamily Heliothinae in general. Their migratory capacity enables them to exploit and take advantage of widely distributed sources of host plants and to escape from natural enemies. Moth feeding is a prerequisite for mating and oviposition. Un-fed moths also mate and lay eggs but they entirely depend on the larval diet. Diet-fed quality of the adults impacts on the peak and/or delay of their oviposition. The mating starts on the third night after emergence, and peaks on the fourth night. In captivity, infertile eggs may be laid, but these are rare under natural conditions (King, 1994).

Eggs are laid singly on different parts of host plants, depending on plant species and the physiological development stage of the plants. However, in general egg can be found on or near the flowers or fruits. The peak of oviposition is usually synchronized to the peak of flowering or nectar production of host plants (King, 1994; Cunningham *et al.*, 1998).

4.1.4 Host range

Helicoverpa armigera, like other Heliothinae spp., is highly polyphagous, feeding on a wide and diverse range of cultivated and wild host plants in a larger number of plant families.

Worldwide it is recorded on more than 60 cultivated and an equal number of wild host plants, e.g., cotton (*Gossypium* spp. [Malvaceae]), maize, chickpea (*Cicer arietinum* L.), pigeon pea (*Cajanus cajan* L. Millsp. [both Fabaceae]), sorghum (*Sorghum bicolor* L. Moench. [Poaceae]), sun flower (*Helianthus annuus* L. [Asteraceae]), soybean (*Glycine max* L. Merr.), groundnut (*Arachis hypogaea* L. [both Fabaceae]), and tomatoes (Cunningham *et al.*, 1999; Cameron *et al.*, 2001). The predominant host families include the Fabaceae, Solanaceae, Malvaceae, Asteraceae and Poaceae (King, 1994).

Because of its broad spectrum of host plants and high mobility, an increase in the diversity of cropping systems cannot reduce the problem. Even in periods of low availaibility of crop hosts uncultivated hosts like weeds or boundary plants are an important source for maintaining or initial build-up of the first spring generation of *H. armigera* before the mayor host plants become widely available (King, 1994).

Adult moths show a certain grade of discrimination among potential host species which has been termed a 'hierarchy of preference'. For instance, maize and sorghum are often preferred over other crops (Fitt, 1989; King, 1994; Cunningham *et al.*, 1999).

However, learning, defined as a change in behaviour with experience, may advantage moths to response to certain variable environments by improved foraging. Cunningham *et al.* (1999) showed that experiences with particular host plant species significantly increased the probability of selection of that plant species for subsequent oviposition and host acceptance. The preferred host species may be ignored by *H. armigera* in the prevalence and abundance of other hosts, which they have already experienced. For instance, on the same field *H. armigera* preferred abundantly present cotton over less abundantly present pigeon pea, though the latter plant is believed to be the more attractive host plant (Cunningham *et al.*, 1999). Repeated nectar foraging on a particular plant species increased its probability of being selected for feeding and ovipositing by *H. armigera* (Cunningham *et al.*, 1998, 1999).

4.1.5 Economic importance

Helicoverpa armigera is among the most destructive agricultural insect pest (Fitt, 1989), among other reasons, because of its feeding preference for plant structures that have high nitrogen contents, i.e., reproductive structures and growing points. Therefore *H. armigera*

directly affects the crop yield. Moreover, it often prefers high value crops such as cotton, tobacco (*Nicotiana tabacum* L. [Solanaceae]), sweet corn and tomato. Consequently only low damage thresholds to *H. armigera* are tolerable in these crops. Thirdly, its physiological, behavioural and ecological characteristics enable it to adapt and survive in even unsuitable habitats and then colonize and exploit agricultural system successfully (Fitt, 1989).

The polyphagy of *H. armigera* greatly affects its population dynamics. Population may develop simultaneously and continuously by exploiting a succession of different cultivated and uncultivated host plants within a region and throughout a season. Populations can persist at low densities in seemingly unsuitable area since adult moths are able to locate suitable host plants for larval survival (Zalucki *et al.*, 1986; Fitt, 1989).

Noctuids possess two strategies to adapt to the seasonality of their habitats: (i) spatial redistribution by migration and (ii) diapause through cold and dry periods. In contrast to some Noctuids that are obligatory migrants like *Spodoptera exempta* (Walker), Heliothinae species are facultative migrants, i.e., they respond to poor local conditions in terms of food or climate (Fitt, 1989). The high fecundity plus short life cycle provide *H. armigera* with a high capacity for population increase.

Severe economic losses both from direct yield reductions and from high costs for synthetic insecticides are often required to control *H. armigera*. For instance high economic yield losses caused by *H. armigera* were reported in India where 80 and 90% of the world chick pea and pigeon pea, respectively is cultivated (Jayaraj *et al.*, 1989). In 1989 the same authors reviewed crop losses in pulse, chick-pea and pigeon peas in India and estimated them to exceed $300 million per year. Up to 90% pod damage can be observed in chick pea in northern India (Fitt, 1989; King & Coleman, 1989; King, 1994). Yield losses in Indian sorghum, cotton and tomato due to *H. armigera* were 18-20%, 41-56% and 40-50%, respectively (Jayaraj *et al.*, 1989). Losses in the order of $25 million were attributed to *H. armigera* and *H. punctigera* (Wallengren) on many crops in Australia (Fitt, 1989). Up to 30% of damaged fruits by *H. armigera* in processing tomato was recorded in the East coast of New Zealand, whereas the commercially acceptable level is 5% fruit damage (Cammeron *et al.*, 2001). Cammeron *et al.* (1995, 2001) developed an economic threshold of one larva per plant for *H. armigera* in IPM processing tomatoes.

4.2 Chemical control

4.2.1 Current status of use of synthetic insecticides for *H. armigera* control

Management of *Heliothis* and *Helicoverpa* spp. still relies heavily on synthetic insecticides (King & Coleman, 1989; King, 1994; Ahmad *et al.*, 1997; Torres-Vila *et al.*, 2002) due to their availability, portability and potential for quick prevention of serious plant damage by larvae, especially in high value crops. Insecticides used to control *H. armigera* are found in several groups like organoclorine (e.g., endosulfan), carbamates, organophosphates, and pyrethroids (King, 1994; Armes *et al.,* 1996; Torres-Vila *et al.*, 2002). Among these, the last group is the most important due to its high efficacy against pests and low toxicity to mammals and some parasitoids and predators (King & Coleman, 1989).

King (1994) reviewed the history of using insecticides in control of *H. armigera*. The wide spectrum insecticides DDT and methyl parathion, widely recommended against cotton pests in 1950s, 1960s and 1970s, initially performed well. However, these insecticides then were found attacking natural enemies, thus promoting severe outbreaks by a wide range of secondary pests. In addition, the high mammalian toxicity, pollution of the environment and since the 1970s increasing resistance to these insecticides restricted their use and subsequently favoured application of the less acutely toxic insecticide endosulfan against Heliothinae bollworm. However, endosulfan use declined during the late 1970s and 1980s also because of resistance development, and it was more and more overtaken by synthetic pyrethroids.

Pyrethroids have long been considered the most cost-effective insecticides against lepidopterous pests in cotton and other crops (Ahmad *et al.*, 1997; Martin *et al.*, 2003). They are less toxic to mammals and to some parasitoids and predators (King & Colleman, 1989).

4.2.2 Resistant status

Management of *H. armigera* has become increasingly difficult because it developed resistance to most of chemical classes of insecticides (Armes *et al.,* 1996). The resistant development is influenced by genetic, ecological, behavioural and agronomic factors (Fitt, 1989). Pyrethroids resistance in *H. armigera* may be conferred through three mechanisms: detoxification by mixed function oxidise, nerve insensitivity and delayed penetration.

The excessive and widespread reliance on pyrethroids in particular and on synthetic insecticides in general, combined with calendar applications without reference to economic thresholds, lead to the rapid development of resistance in *H. armigera*, which in turn prompted farmers to apply more and more insecticides (Fitt, 1989; Ahmad *et al.*, 1999). Cameron *et al.* (2001) claimed that in tomatoes more than half of the insecticide applications were unnecessary. Moreover Fitt (1989) stated that *H. armigera* is usually most abundant in cropping areas rather than on uncultivated hosts. Consequently a high proportion of the populations are regularly exposed to insecticides, promoting the selection of insecticide resistance.

In the 1990ies many serious outbreaks of *H. armigera* have been recorded in Pakistan, mainly caused by poor management of conventional insecticides (Ahmad *et al.,* 1999). A number of cases of resistance to pyrethroids in *H. armigera* have been reported across the world, e.g., in West Africa (Martin *et al.*, 2000; Ochou & Martin, 2002), Pakistan (Ahmad *et al.*, 1997), New Zealand (Cameron *et al.*, 1995), India (Regupathy *et al.*, 1997), China (Han *et al.*, 1999; Guilin *et al.*, 2002), Nepal (Armes *et al.*, 1996), and Australia (Ramasingle *et al.*, 1988; Gunning *et al.*, 1998).

Many efforts have been made to develop management strategies that promote the use of pyrethroids since they have low mammalian toxicity and some of them are comparatively less toxic to some parasitoids and predators. For instance use of organophosphorus insecticides in alternative or combination with pyrethroids to control *H. armigera* can reduce the frequent use of the latter. So far, very few resistant cases of *H. armigera* to organophosphorus insecticides have been recorded, for example in Pakistan (Ahmad *et al.*, 1999). The synergism with organophosphorus insecticides even showed significant increased toxicity of pyrethroids hence suppressing resistance development in the pest (Regupathy *et al.*, 1997).

Although resistance of *H. armigera* to pyrethroids has been recorded, there is still some fluctuation in resistance depending on seasons, as reported in India (Regupathy *et al.*, 1997), or the 'history' of chemical control in the previous pest generations (Han *et al.*, 1999).

4.3 Cultural control

Although cultural control is rarely very successful, especially in such a highly destructive and mobile pest like *H. armigera*, many such practices can contribute, together with other management tactics, to the control of this pest. Methods include changing in planting dates, removing of crop residues, and destruction of wild hosts. In addition, trap cropping has been recommended using highly susceptible host plants, though so far with little success (Fitt, 1989; King, 1994; Cunningham *et al.*, 1999) because trap crops have only narrow attractive 'window' to target insects at their squaring stage, which is very short. Moreover increasing the plant biodiversity in large-scale intensive cropping systems is for many reasons, among them primarily agronomic ones, not easy to realise (Fitt, 1989). However, small areas of high value crops with low tolerance to damage by *H. armigera* such as tomatoes and ornamental crops have been suggested to be mixed with sorghum, a more tolerant hosts to the pest (Cunningham *et al.*, 1999).

4.4 Biological control

The development of resistance to chemical insecticides and concerns of their negative effects on environmental and human safety have provided a strong impetus for the development of biological control for *H. armigera*. The intensive use of synthetic insecticides has been the result of increasing resistance, resurgence following the destruction of natural enemies and the up-surge of secondary pests. The high costs of synthetic insecticides and the greater awareness of their environmental hazards, including pesticide residues in food, in the general public have generated increasing interest in biological pest control.

A diverse assemblage of biocontrol agents is currently under consideration for control of *H. armigera*, including parasitoids and predators, *nucleopolyhedrovirus* (NPV), *Bacillus thuringiensis* (*Bt*) and entomopathogenic fungi.

4.4.1 Predators and parasitoids

The naturally occurring predators and parasitoids are important in regulating *Heliothis* and *Helicoverpa* spp. populations. In some places they can suppress pest populations below economic thresholds in the absence of insecticides (Fitt, 1989; King & Coleman, 1989). Fitt

(1989) argued that natural enemies may suppress a pest population within a particular field but have little effect on the populations in an entire region. In addition, in cotton natural enemies not always are capable keep *H. armigera* damage below the economic threshold level (Van Den Berg & Cook, 1995).

Conservation of natural enemies has preserved and increased their effectiveness. They may also be propagated and released to increase their numbers in the nature. Introduction and establishment of additional natural enemies may also be attempted. Romeis and Shanover (1996) have comprehensively reviewed the arthropod natural enemies of *H. armigera* in India. Six egg parasitoid species from two families, more than 69 larval and larval-pupa parasitoid species, five pupal parasitoid and about 60 predator species were recorded in *H. armigera* on many host plants.

Among the egg parasitoids, *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) is found in significant numbers in eggs of *H. armigera.* Mass releases of several *Trichogramma* spp. in the field have been made on different crops including tomatoes. *Campoletis chlorideae* Uchida (Hymenoptera: Ichneumonidae) is the most important larval, and larval-pupa parasitoids. Parasitism rate is dependent on the host plants (King, 1994; Romeis & Shanover, 1996).

Predators have received less attention than parasitoids as natural control agents of Heliothinae. The most promising ones are chrysopids and ants (Van Den Berg & Cook, 1995; Romeis & Shanover, 1996).

4.4.2 *Nucleopolyhedrovirus*

Among the pathogens of *H. armigera*, including viruses, bacteria, fungi, protozoa and nematodes, the *nucleopolyhedrovirus* (NPV) has been most extensively studied for its pathogenicity, mass production, safety and field efficacy in control of the pests on chick pea, pigeon pea, field bean, cotton and tomato (Jayaraj *et al.*, 1989). According to Carner and Yearian (1989), of all microbial biocontrol agents available in the market, NPV seems to be the most promising one due to its ability to kill small larvae before they cause damage, and its great potential for causing epizootics.

Virulence of NPV isolated from different locations differs and maybe as high as causing more than 90% mortality in Heliothinae species (Jayaraj *et al.*, 1989). At low and moderate infestation levels, the virus can lead to comparable levels as synthetic insecticides but not at high infestation levels (King & Coleman, 1989; Carner & Yearian, 1989; King, 1994). The younger instar larvae are more susceptible to the virus than the older ones, and their LT50 is also shorter (Jayaraj *et al.*, 1989). However, NPV performs inconsistently since it is rapidly degraded by ultraviolet light (UV) and high temperatures (King & Coleman, 1989; Jayaraj *et al.*, 1989; Carner & Yearian, 1989; King, 1994). To overcome this problem, UV-protectants are suggested to apply along with the virus. NPV infections in *H. armigera* are also said to increase the susceptibility of *H. armigera* larvae to insecticides (Jayaraj *et al.*, 1989). However, according to King (1994) mixed applications of NPV and insecticides have generally been not better than individual treatments with the pathogen or insecticides.

4.4.3 *Bacillus thuringiensis*

4.4.3.1 General aspects

Bacillus thuringiensis (*Bt*) is a gram positive, spore forming bacterium that forms a parasporal crystal during the stationary phase of its growth cycle. It was first isolated and defined as an insect pathogen of flour moth larvae in 1915 by Berliner. To date, *Bt* formulation insecticide was accounted for 1% insecticide market (Maron & Macintosh, 1993; Tapperser, 1998; Nester *et al.*, 2002) and 90% of all biopesticide commercial sales (Glare & O'Callaghan, 2000). The insecticidal activity was attributed to the parasporal crystals. *Bt* strains have been isolated worldwide from many habitats including soil, insect, stored-products, dust and deciduous and coniferous leaves (Schnepf *et al.*, 1998; IPCS, 1999; Glare & O'Callaghan, 2000). *Bt* spores can survive for several years after spray application, although rapid decline in population and toxicity have been recorded.

To date, more than 100 sequences of crystal protein genes (encoding Cry- and Cyt-proteins) are known (Schnepf *et al.*, 1998). The classification of *Bt* subspecies based on the serological analysis of the flagella (H) antigens was introduced in the early 1960s (Schnepf *et al.*, 1998; IPCS, 1999). Crystal serology has shown that a particular crystal type may be produced by more than one H-serovar. Up to the end of 1998, over 67 subspecies have been identified.

The genes that code for toxic proteins are divided into four classes: Cry I-Lepidoptera specific, Cry II-Lepidoptera and Diptera specific, Cry III-Coleoptera specific and Cry IV-Diptera specific (Höfte & Whiteley, 1989).

The virulence of *Bt* was attributed to a number of extra-cellular compounds including phospholipases, heat-labill toxin activities, B-endotoxins, proteases, chitinnases and secreted vegetative insecticidal protein (VIPS) (Dulmage *et al.*, 1981; Schnepf *et al.*, 1998; IPCS, 1999). Among these delta-endotoxins or crystals, the protein formed during sporulation, are the most prominent of a number of virulence factors (Schnepf *et al.*, 1998) and the principle insecticidal component presently used in commercial preparations of *Bt* (Fast, 1981).

Generally, the toxin structure of crystal proteins possesses three domains in a three-dimensional structure. Domain I is responsible for the formation of lytic pores in the intestinal epithelium of target organisms. Domain II and III play a role as receptor binding and activity in insect midgut, respectively (Schnepf *et al.*, 1998).

Bt is the most widely used biologically produced pest control agent. Its Cry proteins are high specifity for different insect groups. The highest number of target species is found in Lepidoptera with around 400 *Bt*-susceptible species. *Bt* was found to be environmentally a comparably safe microbial insecticide since it has no mammalian toxicity and a rather limited host range, thus limiting its harmful effects on non-target species, while safe for human and other non-target insects. These favour its future development (Burgerjon & Martouret, 1971; Dulmage *et al.*, 1981). *Bt* can proliferate within the bodies of its weakened or dead host insects (Schnepf *et al.*, 1998; IPCS, 1999). The use of *Bt* has increased because of the increasing occurrence of pest strains resistant to many synthetic insecticides (Tapperser, 1998; Nester *et al.*, 2002).

The mode of action for the Cry proteins differ completely from the modes of action of synthetic insecticides, making them key components in integrated pest management (IPM) strategies aimed at preserving natural enemies and managing insect resistance (IPCS, 1999).

Recently the *Bt* gene that codes for the insecticidal crystal protein has been expressed in transgenic varieties of several important crop species. Transgenic plants expressing the δ-endotoxin gene from *Bt* offer the potential for more efficacious deployment of *Bt*-toxins,

resulting in high level of protection against several important pest species (Schnepf *et al.*, 1998; IPCS, 1999).

4.4.3.2 Factors influencing activity of *Bt*

Spray formulations of *Bt* are relatively unstable in the environment, with rain and temperature and particularly UV light considerably affecting the efficacy of *Bt* (Falcol, 1971). Consequently, often frequent reapplications of *Bt* are needed to assure a high control level.

In International programme on chemical safety (IPCS) (1999) the effect of environmental factors on the life time of *Bt* after application has been comprehensively reviewed. Solar radiation appears to be the environmental factor most damaging to the stability of *Bt* insecticidal crystal protein (ICP). *Bt* spores are inactivated rapidly when exposed to UV radiation. The combination effect of sunlight, leaf temperature and vapour pressure deficit appeared to contribute more to the reduction of bioactivity than any other single factor. *Bt* spores also can be affected by the surface to which they are applied. Plant chemicals can inactivate *Bt* or influence its infectivity.

4.4.3.3 Mode of action

The mode of action of *Bt* has been well documented by several authors (e.g., Bon *et al.*, 1971; Burgerjon & Martouret, 1971; Cooksey, 1971; Fast, 1981; Sebesta *et al.*, 1981; Morris *et al.*, 1996; Schnepf *et al.*, 1998; IPCS, 1999; Glare & O'Callanghan, 2000).

The mode of action depends on the crystal alone or a combined action of spores and crystals. Different isolates of *Bt* produce different delta-endotoxins that differ both in quantitative and qualitative terms. These delta-endotoxins kill insect species with varying degrees of efficacy (Dulmage *et al.*, 1981).

To be active, the *Bt*-treated leaves must be eaten by herbivorous insects. Within 1-7 h later, the pH level in the insect's blood increases while the pH in the gut falls. Then the *Bt* crystal is activated by protease in the juice of the insect's midgut at pH 8-10.5, giving rise to the actual toxin, a protein fragment. The activated protein continues to transverse the peritrophic membrane and binds to specific receptors on the midgut epithelium, forming a pore and leading to a loss of the trans-membrane potential, followed by a cell lysine leakage of the

midgut content and paralysis 18 h after digesting the crystals and eventually causing the death of the insect.

Bt spores germinate after the gut has been damaged and begin vegetative growth, producing other insecticidal toxins.

4.4.3.4 Use of *Bacillus thuringiensis* for control *H. armigera*

Bt has been used worldwide for control of *H. armigera*, both as spray formulations and in transgenic crops. *Bt* is an important and indispensable bio-insecticide (Roush, 1997) that can serve as an alternative to synthetic insecticides for control of *H. armigera* (Shen *et al.*, 1998).

Arno *et al.* (2002) showed that less than 3% of tomato fruits were damaged by *H. armigera* following a *Bt* treatment. Since the 1990s *Bt* is widely used in China for *H. armigera* control because of high levels of resistance to synthetic insecticides (Zhao *et al.*, 2002). For instance, in 1994 in Hebei, China about 1,000 tons of *Bt* formulations were used on 100,000 ha cotton. In laboratory experiments *Bt* strains with the endotoxin Cry2Aa and Cry1Ac were recorded to induce 99.3% and 89.9% mortality in *H. armigera,* respectively, at a concentration of 0.5 µg/ml (Babu *et al.*, 2002).

There is only a limited range of crystal proteins toxic to *H. armigera* at dosages that could be acceptable for commercial use, i.e., Cry 1Ab, Cry 1Ac, Cry 1Aa, Cry2Ab and Vip3A (Liao *et al.*, 2002). Padidam (1992) and Charabarti *et al.* (1998) found Cry 1Ac protein was the most potent toxin against *H. armigera* followed by Cry 1Aa, Cry 2Aa and Cry 1Ab. Different *H. armigera* populations collected from different locations and at different times of the year showed differences in susceptibility to *Bt* var. *kurstaki* (Gujar *et al.*, 2000; Jalali *et al.*, 2004).

Besides causing the death of treated larvae, *Bt* also severely inhibits larval growth (Fitt *et al.*, 1994; Chakrabarti *et al.*, 1998; Gujar *et al.*, 2000; Lenin *et al.*, 2001; Jalali *et al.*, 2004).

Transgenic plants expressing the δ-endotoxin gene of *Bt* offer the potential for more efficacious deployment of *Bt* and increase the persistence of the *Bt* toxin compared to spray *Bt* leading to significant reduction in requirement insecticidal protein for pesticides to control lepidopterous pests and application of expensive *Bt* formulation whose use has increased recently (Fitt *et al.*, 1994; Roush, 1997)). Transgenic cotton plants expressing Cry 1Ab

showed good efficacy for lepidopterous species (Fitt *et al.*, 1994; Olsen and Daly, 2000; Sun *et al.*, 2002) and 100% *H. armigera* mortality in transgenic tomatoes (Kumar & Kumar, 2004).

4.4.3.5 Resistance status

It was originally believed that insects could not develop resistance to *Bt* toxin since *Bt* and insect have coevolved. However, from the mid-1980s a number of insect populations of several pests could be selected for resistance to *Bt* in the laboratory, e.g., Indian meal moth *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), Colorado beetle *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae), and tobacco bud worm *Heliothis virescens* (Fabricius) (Lepidoptera: Noctuidae) (Marone & Macintosh, 1993; Tabashnik, 1994; Schnepf *et al.*, 1998). However, selection for resistance in the laboratory may be very different from that in the field. To date Diamond back moth *Plutella xylostella* L. (Lepidoptera: Plutellidae) is the only insect species reported to have developed resistance to *Bt* var. *kurstaki* in the field in some areas of Hawaii, Florida, Indonesia, Malaysia, Central America and the Philippines (Marone & Macintosh, 1993; Tabashnik, 1994; Schnepf *et al.*, 1998; IPCS, 1999). The resistance was defined to some but not all crystal protein.

So far no resistance of *H. armigera* to *Bt* formulations and *Bt* transgenic plants has been reported in the field. However, Fitt (1989) and Fitt *et al.* (1994) discussed that among *Helicoverpa* species, *H. armigera* is the one most likely to develop resistance to *Bt* cotton because it appears to be more consistently associated with cropping areas and hence stronger exposed to pesticide selection.

Laboratory experiments have shown the decrease in susceptibility to Cry 1Ac protein from *Bt* and *Bt* transgenic cotton in some *H. armigera* populations (Liang *et al.*, 1998; Dang & Gunning, 2002). Moreover, there is evidence of declining levels of *Bt* expression once the transgenic plant starts to senesce, consequently allowing bigger *H. armigera* larvae to survive and complete their development during the late season although their growth was severely retarded (Fitt *et al.*, 1994; Olsen & Daly, 2000; Sun *et al.*, 2002; Liao *et al.*, 2002). These survivors pose a serious risk to the sustainability of this technology because they will facilitate resistance development in pest populations.

With the increasing use of single *Bt* genes in transgenic plants, the risk of resistance is expected to be much higher (Glare & O'Callaghan, 2000). In laboratory experiments, Dang and Gunning (2002) reported reduced susceptibility in field collected *H. armigera* to single gene transgenic cotton varieties compared to two-gene varieties. There might be cross-resistance between *Bt* subspecies although the incidence is low (Marone & Macintosh, 1993; Tabashnik, 1994; Glare & O'Callaghan, 2000). On the contrary, Wu *et al.* (2002) affirmed that there is no resistance of *H. armigera* to Cry 1Ac protein encoding in transgenic cotton in China as awareness in other publication e.g., Shen *et al.* (1998) and Liang *et al.* (2000).

Different tactics have been developed for resistance management in *Bt* including use mixtures of toxins, mix or alternate with other insecticides and use of refuges for both *Bt* as spray formulations and transgenic plants (Tabashnik, 1994; Glare & O'Callaghan, 2000). Therefore, EPF with their pathogenicity against insect are among possibilities alternate or mix with *Bt* in these resistance management stragtegies.

4.4.4 Entomopathogenic fungi

4.4.4.1 General aspects

Entomopathogenic fungi (EPF) belong to four general divisions, i.e., Oomycetes, Zygomycetes, Chitrydiomycetes and Deuteromycetes, of which according to Bartlett and Jaronski (1988), the latter group is much broader in its host spectrum and can be grown on more generalized media. Furthermore most EPF belong to the Deuteromycetes (fungi imperfecti) (Samson, 1981), within the class Hyphomycetes. The most common genera of hyphomycetous fungi include *Aspergillus*, *Beauveria*, *Culicinomycetes*, *Hirsutella*, *Metarhizium*, *Nomurea*, *Paecylomyces*, *Tolypocladium* and *Verticilium* spp. (Inglis *et al.*, 2001). The most studied species of entomogenous fungi, among others, are *Metarhizium anisopliae* (Metsch.) Sorokin and *Beauveria bassiana* (Bals.) Vuill. (Gillespie, 1988; Bartlett & Jaronski, 1988; Feng *et al.*, 1994; Vey *et al.*, 2001).

Members of the Hyphomycetes are characterized by mycelial forms that bear asexual spores, termed 'conidia', borne on specialized conidiogenous cells. Conidiogenous cells are often formed on simple or branched hyphae, termed 'conidiophores', or from aggregations of

conidiophores. Fungi in this group have lost the ability to produce or rarely produce sexual spores (Inglis *et al.*, 2001).

Hyphomycetes fungi can cause epizootics in insect populations under field conditions which can be important for the natural regulation of pests. An epizootic is the result of a complex interaction among the host, the pathogen and the environment over time. Insect density and behaviour, and the ability of a pathogen to cycle and disperse can influence epizootic development and can affect the dispersal of an entomopathogen (Inglis *et al.*, 2001). EPF have been worldwide isolated from a variety of insects or soil. Fungi Hyphomycetes are able to over-winter in cadavers as conidia or mycelia as special resting spores (Gillespie, 1988).

EPF have one of the widest host ranges among the pathogens of arthropods. They attack many orders including Coleoptera, Lepidoptera, Diptera and Homoptera. Among EPF, *B. bassiana* and *M. anisopliae* have much wider host ranges compared to other species (Inglis *et al.*, 2001).

Insect death may result from a combined action, including depletion of nutrients, physical obstruction or invasion of organs and toxicosis. Different EPF produce different toxins to weaken the defence response of an insect before they can proliferate in the insect haemocoele (Inglis *et al.*, 2001). *Metarhizium* spp. are known to produce dextruxin. *Beauveria* spp. produce a number of toxins, including beauvericin, bassianolide, beauveriolide, bassianin, tenellin and oosprin. *Paecylomyces* spp. produce beauvericin (Vey *et al.*, 2001).

EPF can be easily cultured and produced on artificial solid and liquid media, forming conidia and blastospores, respectively. They can not grow in distilled water, however a prolonged period of soaking (up to 20 h) in distilled water activated an initial pre-swelling phase of germination, promoting spherical growth and germ-tube formation once a nutrient was provided (Dillon & Charnley, 1990). Being successfully used for control of insects, mass production systems for EPF are required that are rapid and provide easy growth, high productivity, which are inexpensive, allow long term storage and require low labour-input (Feng *et al.*, 1994). These authors have intensively reviewed a number of simple and successful fermented technologies for mass production of EPF in general and *B. bassiana* in particular, using inexpensive and available substrates such as wheat bran, rice, cotton seed shell, corn flour, bean flour, wheat cellophane and others. Mineral media were also developed

for submerged fermentation, which was considered to be the best technology to satisfy the requirements of commercial production and practical application (Feng *et al.*, 1994).

In principle, it is possible to use both types, i.e., conidia and blastospores for control of insect pests (Inch *et al.*, 1986; Lane *et al.*, 1991a; Kleepies & Zimmermann, 1994). The mass production of blastospores is easier to control and manipulate than that for conidia. While Lane *et al.* (1991b) found that the virulence of blastospores of *B. bassiana* was significantly lower than that of conidia against the green leaf hopper, *Nephotettix virecens* (Distant) (Hemiptera: Cicadellidae), Kleespies and Zimmermann (1994) proved that virulence of blastospore of *M. anisopliae* after storage was compatible to fresh ones and even better than that of fresh conidia against adult and third instar locust, *Locusta migratoria* L. (Orthoptera: Acrididae). Blastospores of *B. bassiana* loose viability rapidly during storage (Lane *et al.*, 1991b). However, viability of blastospores of *M. anisopliae* after storage depends on the fungal strains, the liquid media used and storage temperature (Kleepies & Zimmermann, 1994).

a. *Beauveria bassiana* (Bals.) Vuillemin

This EPF forms a dense white mycelium, covering the host exoskeleton. Conidiogenous cells are usually densely clustered (or whorled or solitary), colourless, with globose or flask-like base and denticulate (toothed) apical extension (rachis) bearing one conidium per denticle, conidia aseptate. Conidia are nearly globose, $\leq$ 3.4 μm (Humber, 1997). Hosts infected by *B. bassiana* have a dusty white appearance; hence *B. bassiana* is commonly referred to as 'white muscardine' (Bartlett & Jaronski, 1988).

b. *Metarhizium anisopliae* (Metsch.) Sorokin

The mycelium of *M. anisopliae* is often woolly, covering affected hosts. Conidiophores grow in compact patches; individual conidiophore is broadly branched, densely intertwined. Conidiogenous cells with rounded to conical apices arrange in dense hymenium. Conidia are aseptate cylindrical or ovoid, forming chains and usually aggregated into prismatic of cylindrical columns or a solid mass of parallel chains. Conidia are pale to bright-yellow-green, olivaceous, sepia or white in mass, $\geq$ 9 μm (Humber, 1997). Sporulated mycelium of

M. anisopliae produces a green colour usually called 'green muscardine' (Bartlett & Jaronski, 1988).

c. *Paecilomyces fumosoroseus* (Wize) Brown and Smith

Conidiophores of this EPF are usually well developed, synematous in many species, bearing whorls of divergent branches and conidiogenous cells (phialides), colourless to pigmented (but not black, brown or olive). Conidiogenous cells with a distinct neck and base flask-to narrowly awl-shape or nearly globose borne singly or in a group in whorls of conidiophores, on short side branches or in apical whorls. Conidia are aseptate, hyaline to colour forming in dry divergent chains, ≤ 4 µm and rosy-tan to smoky-pink in mass (Humber, 1997).

4.4.4.2 Factors influencing efficacy of EPF

Fungal pathogens require extended periods of moderate temperatures and high humidity after treatment for spore germination, colonisation, subsequent host killing and outgrowth on the cadavers. Pathogenicity of EPF is determined by a variety of factors, including the physiology of the host, and the physiology of the fungus in interaction with environmental conditions. (Inglis *et al.*, 2001). For instance, the developmental stage and the insect diet play an important role in the development of the disease. Inadequate insect nutrition can lead to increased susceptibility to EPF.

A variety of environmental factors have been shown to have dramatic effects on the germination, growth and pathogenicity of EPF and thus affect their efficacy against insect pests. These factors include solar radiation, temperature, water availability, precipitation and wind. Conida, hyphal bodies and hyphae of all taxa of hyphomycetous fungi are highly susceptible to damage by solar radiation, especially UVB. However, the susceptibility differs among taxa and strains within species. *B. bassiana*, *M. anisopliae* and *P. fumosoroseus* are among the most resistant species to sunlight (Inglis *et al.*, 2001).

The ambient temperature influences the rate of infection and time to death of insects treated with EPF. The optimum temperature for most EPF germination, radial growth and pathogenic activity is at 25°C (Hallworth & Magan, 1999; Ekesi *et al.*, 1999; Inglis *et al.*, 2001; Dimbi *et al.*, 2004), but infection and disease can occur at temperatures ranging between 15-30°C

(Inglis *et al.*, 2001). There might also be a strong relationship between temperature characteristics and place of origin of an EPF (Vidal *et al.*, 1997; Inglis *et al.*, 2001).

In the natural environment, water availability is a critical abiotic parameter determining spore germination, growth and infection. Most EPF require at least 95% relative humidity at the insect surface for germination, germ-tube extension and infection to occur (Hallworth and Magan, 1999). Not only is water essential for propagule germination, but it also regulates conidiogenesis on cadavers that have died from mycosis (Inglis *et al.*, 2001). Conversely ambient humidity in some cases does not affect infectivity of insects to EPF such as *B. bassiana* and *M. anisopliae.* In these cases, the ability of these fungi to germinate and infect the host is attributed to sufficient moisture within microhabitats. Humidity, in combination with temperature, influence evaporation of spray droplets, which can result in the loss of small spray particles containing EPF conidia and thereby affecting efficacy of EPF to the targets. Moisture can also have a significant effect on the persistence of fungal inocula and conidia's viability (Inglis *et al.*, 2001). In most of EPF, fungal conidia are most stability under cool and dry conditions.

The germination rate of EPF is also species- and strain-dependent (Hywel-Jones & Gillespie, 1990; McCammon & Rath, 1994). For instance, when investigating the effects of temperature on spore germination in three isolates of *M. anisopliae* and that of *B. bassiana,* Hywel-Jones and Gillespie (1990) found that the lag phases and germination rates within and between strains and species were temperature-dependent. Three tested *M. anisopliae* isolates generally had faster rates of germination and shorter lag phase duration compared to the tested *B. bassiana* isolates, resulting in higher germination levels in *M. anisopliae* strains. Based on the difference in germination rate and temperature range for germination it was possible to separate *M. anisopliae* species into 3 groups: the cold-active strains, those germinate at 5°C; heat-active strains, which germinate at 37°C and meso-thermo-active strains, which germinated at neither 5°C nor 37°C (McCammon and Rath, 1994). Speed of EPF penetration with hyphae into and proliferation in insect were also found to be different among isolates of species e.g., *P. fumosoroseus* when used against DBM *Plutella xylostella* (Altre & Vandenberg, 2001).

4.4.4.3 Mode of action

Bartlett and Jaronski (1988), Gillespie (1988), Feng *et al.* (1994), Goettel and Inglis (1997) and Inglis *et al.* (2001) have described the infection cycle of EPF. Initially the conidia, the infectious unit of an EPF, after landing on the host, germinate, and subsequently penetrate the insects' cuticle immediately after contact with their integuments. To penetrate the cuticle, EPF utilize a combination of enzymatic and mechanical mechanisms. Enzymes involved in this step include exoproteases, endoproteases, esterases, lipases, chitinases and chitobiases. Once the fungus reaches the heamocoele, it grows as hyphal bodies, spreads and proliferates inside the insect's body as mycelium and kills the host within 3-14 days after infection, depending on the spore dosage, humidity and temperature.

Following the death of the host insect, the fungus often grows saprotrophically within the host. Soon after the death of the host, particularly at high relative humidity levels, hyphae emerge from the cadaver; they produce conidiogenuois cells, and sporulation occurs on the host surface. Eventually, conidia are released, thus providing new inoculums for further infections. If conditions are unfavourable, the fungi can remain and survive inside the cadavers for several months, eventually producing spores once favourable conditions occur.

4.4.4.4 Use of entomopathogenic fungi for control of *H. armigera*

Entomopathogenic fungi have shown promising results against lepidopterous pests including Heliothinae species (King & Coleman, 1989). Some are known to naturally infect larvae of Heliothinae species, e.g., *B. bassiana* and *Nomureae rileyi* (Farlow) (Deuteromyces: Hyphomycetes) (Carner & Yearian, 1989). However, no or very few attempts have been made to study the control potential of EPF in *H. armigera* (King & Coleman, 1989; Jayaraj *et al.*, 1989).

In a laboratory experiment, high efficacy (90.5-100% mortality) was recorded after fourth instar larvae of *H. armigera* were fed with corn silks and leaves of soybean, tomato and chrysanthemum previously treated with 1E+07 conidia/ml of a *N. rileyi* strain, originally isolated from a naturally infected *H. armigera* (Tang and Hou, 1998). In laboratory experiments more than 90% mortality was induced when first and third instar larvae of *H. armigera* were treated with the hyphal bodies of 2E+06 fungal cells/ml of *N. rileyi*

(Holdom & Klashorst, 1986). In fifth instar larvae of *H. armigera* 25 and 96.7% mortality were recorded after the soil surface had been treated with 1E+07 conidia/ml and 1E+09 conidia/ml of *N. rileyi*, respectively (Tang & Hou, 1998).

High efficacy of *B. bassiana* against *H. armigera* has also been recorded in China and India. Gopalakrishnan and Narayanan (1990) recorded 60-100% mortality in all stages of *H. armigera* when dipped into a *B. bassiana* suspension of 1E+07 conidia/ml in laboratory experiments. More than 80% mortality were recorded in third instar *H. armigera* initially sprayed with 1E+05 conidia/ml of *B. bassiana* wild types and benomyl transformants one, which can grow well with a presence of benomyl, a fungicide (Sandhu *et al.*, 2001). There was no significant loss in the pathogenicity to *H. armigera* third instars of the transformants compared to wild types. After spraying chickpeas with 2.8E+07 conidia/ml of *B. bassiana* against *H. armigera* in a field trial in India, Hem and Ahmad (1997) observed that pod damage in the treated plots was only 6.8% and a yield of 2,377 kg/ha was recorded, whereas in the untreated control the damage level was 16.3% and a yield of only 1,844 kg/ha was obtained. Manjula and Padmavathammia (1999) observed higher larval mortality in *H. armigera* when applying higher concentrations of *B. bassiana*. Natural occurrence of *B. bassiana*, *N. rileyi*, *M. anisopliae, B. brongniartii* was also found in infected *H. armigera* in the field (Abbaiah *et al.*, 1988; Nurindah & Indrayani, 1989; Hua *et al.*, 1999)

Although *B. bassiana* can infect all larval stages of *H. armigera,* younger instars tend to be more susceptible to *B. bassiana* than older ones. In addition, temperature and relative humidity also affect the infectivity of *B. bassiana* in *H. armigera* (Sun *et al.*, 2001). These authors observed that at 25°C and 95% relative humidity (RH), the highest proportion of larvae died immediately after treatment.

5 MATERIAL AND METHODS

5.1 Insects

Helicoverpa armigera were obtained from BBA and reared individually in small plastic cubicles (1.8 x 1.8 x 1.8 cm) containing a sufficient amount of artificial diet for the entire larval development. The artificial diet contained 20 g Agar, 2 g Nipagin, 2 g Benzoic acid, 2 g Frisomycin, 8 g vitamin mixture for insect, 6 g Ascobic acid, 2 g Wesson salt, 40 g yeast, 40 g wheat germ, 4 g sunflower oil and 125 g white bean flour per 1 liter diet. This diet is routinely used in BBA to rear *H. armigera*. The pupae were kept in plastic boxes (13 x 15 x 10 cm) on a layer of tissue paper. Emerged adults were then transferred to plastic cylinders (diameter 25 cm, height 40 cm) and feed with cotton buds soaked with 10% sucrose. The insect rearing was carried out in a climate chamber at 25 ± 2°C, 65 ± 5% relative humidity (RH) and a photoperiod of L16: D8.

5.2 Plants

A hybrid Thai tomato variety, namely Phuang Chom Poo (East-west seed Co. Ltd., Bang Bue Thong Nonthaburi, Thailand) was used.

5.3 Pathogens

5.3.1 EPF and *Bt* sources

In this study seven strains/species of EPF were selected from the EPF selection of Dr. G. Zimmermann, BBA (table 1) including two *Metarhizium anisopliae* (*M.a*), three *Beauveria bassiana* (*B.ba*) and two *Paecilomyces fumosoroseus* (*P.fr*) strains. The selection was partly based on the history of the EPF, i.e., the original host from which they were isolated and the geographic origin. In addition, three strains/species were selected based on results of a previous study on EPF for *H. armigera* control (Hassani, 2000), i.e., *M.a* 79, *B.ba* 108 and *P.fr* 14. Moreover, two commercial *Bt* formulations which are officially registered in Germany, namely Raupenfrei Xentary (*Bacillus thuringiensis* var. *aizawai*) (Bayer AG, Monheim, Germany) and Turex (*B. thuringiensis* var. *aizawai*) (Ciba-Geigy GmbH, Frankfurt, Germany), were used in the experiments.

Table 1: List of EPF and *Bt* products used in the screening bioassays

No.	Pathogen		Description	
	EPF	**Strain**	**Host**	**Origin**
1	*Metarhizium anisopliae*	79	*Agrotis segetum*	Germany
2	*M. anisopliae*	97	Semi-looper (undefined)	Philippines
3	*Beauveria bassiana*	108	*Hepialus* sp.	Germany
4	*B. bassiana*	124	Commercial EPF product	China
5	*B. bassiana*	139	Commercial EPF product	China
6	*Paecilomyces fumosoroseus*	14	*Bemisia tabaci*	Pakistan
7	*P. fumosoroseus*	15	*Bemisia tabaci*	Taiwan
	Bt		**Strain**	**Provider**
1	Raupenfrei Xentary		*Bacillus thuringiensis* var. *aizawai.*	Bayer AG, Monheim, Germany
2	Turex		*Bacillus thuringiensis* var. *aizawai.*	Ciba-Geigy GmbH, Frankfurt, Germany

5.3.2 Reactivation of isolates

All EPF strains/species from the frozen storage sources (-80°C) were passed through *H. armigera* once and then re-isolated on an antibiotic medium developed by BBA containing 2% glucose, 0.5% Soya peptone, 0.5% yeast extract, 1.5% agar, 0.003% streptomycine-sulfat and 0.005% chloramphenicol. This host-passage was supposed to activate and increase the fungal pathogenicity. Thereafter, EPF were cultured on malt extract peptone agar (MEPA), containing 3% malt extract, 0.5% Soya peptone and 1.8% agar, following the protocol of Kassa *et al.* (2002). The fungal cultures were kept in an incubator at 25°C and 70% RH under dark conditions for 10-14 days.

5.3.3 Preparation of spore suspensions and viability test

Initially re-activated conidia were harvested from the MEPA culture (see previous section) by scratching the surface of the colony with an inoculating needle. By this tool, several conidia were then transferred to 1 ml Eppendorf tube containing 1 ml 0.1% Tween 80. Thereafter, the Eppendorf tube with the harvested conidia was agitated by a Vortex for about three minutes to uniformly suspend the hydrophobic propagules. In the following this solutions is referred to as the stock suspension. The spore concentration was counted in a Thoma chamber (haemocytometer).

Subsequently, a viability test for the different EPF strains/species was carried out prior to their subsequent use in the experiments. For this, a new solution was prepared by making a

dilution from the stock suspension, and adjusting its concentration to 1E+06 conidia/ml following the method described by Goettel and Inglis (1997). Three droplets of 1 µl were placed on a Petri dish (diameter 3 cm) containing MEPA. Thereafter these Petri dishes were incubated at 25°C, 70% RH under dark conditions for 24 hours. The conidia were then stained with lactophenol cotton blue and checked under the microscope. Only spores with a germ tube as long as the conidia width were considered to have germinated (Kassa *et al.*, 2002). In all tested strains/species more than 90% of the conidia had germinated.

5.4 Screening the efficacy of EPF against third instar larvae of *H. armigera*

In this study we used the immersion method as described by Goettel and Inglis (1997). *Helicoverpa armigera* third instar larvae were dipped for 10 seconds singly into an EPF suspension containing 1E+07 conidia/ml. The control larvae were dipped into an 0.1% Tween 80-only solution. Dipped larvae were allowed to freely crawl on a Petri dish to remove the excess moisture before placing them individually on to a moistened filter paper in a Petri dish (diameter 7 cm). A piece of a tomato leaf was additionally added as food source for the larvae. Leaves were replaced with fresh ones and water was added whenever needed. The Petri dishes with the *H. armigera* larvae were kept in a climate chamber at 27°C, 65 ± 5% RH and 16: 8 L: D photoperiod.

Mortality of *H. armigera* was assessed daily over a period of two weeks or until all larvae in a treatment had died during this period of time. Dead larvae were transferred to a Petri dish equipped with a moistened cotton piece to observe the sporulation of the EPF on the cadavers. Sporulating cadavers were controlled under the stereo microscope to verify whether the insect had died because of an EPF infection. Thirty individuals were tested in each treatment, and the experiments were repeated three times overtime. The three best-performing EPF strains of each species, i.e., *M.a* 79, *B.ba* 124 and *P.fr* 14, were then selected for the subsequent experiments.

5.5 Estimating the susceptibility of different larval stages of *H. armigera* to *M. anisopliae* 79, *B. bassiana* 124 and *P. fumosoroseus* 14

The same immersion method as used in the screening test was extended to evaluate the pathogenicity of the selected EPF species/strains at the same concentration, i.e., 1E+07

conidia/ml, to other larval stages of *H. armigera*, i.e., L2, L4, and L5 (for details refer to the previous section). Twenty to 30 individuals were tested per treatment and the experiments were repeated three times overtime. Daily mortality and final sporulation of cadavers were observed for 14 days.

5.6 Dose-response experiments with four concentrations of *M. anisopliae* 79, *B. bassiana* 124 and *P. fumosoroseus* 14 against third instar larvae of *H. armigera*

The same immersion method as previously described was used in these experiments. Four different doses of *M.a* 79, *B.ba* 124 and *P.fr* 14 were prepared, i.e., 1E+07, 06, 05 and 04 conidia/ml. Daily mortality and final sporulation of cadavers were assessed over a period of 14 days. The lethal concentration 50 (LC 50), i.e., the concentration at which 50% of the organisms die and the median survival time (MST), i.e., the time until 50% of the insects die, were determined. Thirty L3 *H. armigera* per treatment were used and the experiments were repeated three times overtime.

5.7 Pathogenicity of *M. anisopliae* 79, *B. bassiana* 124 and *P. fumosoroseus* 14 against pupa of *H. armigera*

Three different inoculation methods were used in these experiments, i.e., immersion of pupa, soil inoculation and soil surface contamination. The immersion method was used in a similar manner as for the larvae (for detail see previous section). In a 28-wells plastic tray, 5 g of a commercial soil substrate (Fruhstorfer Erde, Archut GmbH, Wallenrod, Germany) was added to each well (4 x 4 x 5 cm). First 2 ml of tap water was introduced into each well. Then the *H. armigera* pupae were dipped in an EPF suspension of 1E+07 conidia/ml for 10 seconds and subsequently released to the soil surface. In the control pupae were treated with a solution 0.1% Tween 80. In the soil inoculation method, the soil in each well was first moistened with 1 ml of tap water. Then 1 ml of a 1E+07 conidia/ml EPF suspension was introduced into each well and mixed gently with the soil. Thereafter, pupae were introduced individually into each cavity. In the control, the soil was treated with 1 ml of a 0.1% Tween 80 solution. For the soil surface contamination method, plastic boxes (12 x 15 x 10) were first filled with a 1 cm layer of the soil substrate. Subsequently, 15 pupae were released into one box and then covered with another 2 cm layer of the same commercial soil substrate. A total amount of 100 g soil was used per box. The soil had been previously moistened with 20 ml of tap water per 100 g

of soil. Finally, 15 ml of a 1E+07 conidia/ml EPF suspension was applied to the soil surface. In the control the same amount of a 0.1% Tween 80 solution was used. The boxes were then incubated at 25 ± 2°C, 70% RH, and 16: 8 L: D photoperiod. The emergence of adults was monitored over a period of 2-3 weeks. On pupae that failed to develop into adults the sporulation of the fungi was observed in the same manner as for the larvae (for detail see previous section).

5.8 Estimating a sub-lethal concentration of *Bt* formulations against third instar larvae of *H. armigera*

The leaf discs spreading method was used in this bioassay as described by McGuire *et al.* (1997). For this tomato leaf discs (diameter 3 mm) were introduced into the 24-wells bioassay boxes (10 x 20 cm). Each well (diameter 1 cm and 2 cm depth) was equipped with a moist filter paper to keep the leaf discs fresh. After a series of preliminary tests, the following concentrations for Xentary were prepared: 0.015, 0.0075, 0.0032 and 0.0016 mg/ml. Two µl of these *Bt* suspensions were then pipetted on the leaf disc, using a micro pipette. In the control de-ionised water was used instead. All treated leaf discs were then placed under an airflow for about 1 hour to allow the suspensions to dry. Thereafter the L3 were introduced individually into each well. The boxes were subsequently incubated at 25 ± 2°C, 70% RH and 16: 8 L: D photoperiod. Thirty L3 *H. armigera* which had hatched within 12 hours and which were then fed with tomato leaves for one day were used per treatment. The larvae were allowed to feed on the treated leaf discs for two days before transferring them to new bioassay boxes of the same type but were then fed with the artificial diet. The mortality of *H. armigera* was observed daily over a period of seven days.

5.9 *In vitro* interactions of *Bt* and *M. anisopliae* 79, *B. bassiana* 124 and *P. fumosoroseus* 14

Two different media were used for this test: the normal MEPA medium used to culture EPF in BBA as described above and LB, a nutrient enrichment medium containing 0.5% yeast extract, 1% casein peptone, 1% Natriumchlorid and 1.2% Agar. One week prior to the test, *Bt* strains were re-isolated from the commercial formulations on Petri dish filled with LB media. For the interaction test, 2 µl of a 1E+06 conidia/ml EPF solution was first placed in the centre of a Petri dish two days prior to the introduction of two lines of *Bt,* above re-isolation, of 5 cm length left and right to the fungal inoculation point. The Petri dishes were then incubated at

25°C, 70% RH in dark conditions. The growth of the EPF colonies and *Bt* was observed daily over a period of one week, and the diameter of the fungal colonies were measured. In case of suppression the shape of a fungal colony is changed from round to ellipse. Hence colonies were measured in both developmental directions, i.e., the width and length of a colony were recorded. Finally, the distance between *Bt* and the EPF colony was also quantified. Five replications were carried out for each EPF strain and *Bt* combination.

5.10 Combined effects of a sub-lethal dose of *P. fumosoroseus* 14 and *Xentary* on third instar larvae of *H. armigera*

In theses experiments the following five treatments were tested: (i) Third instars larvae of *H. armigera* were treated with a sub-lethal concentration (i.e., 2.3 x (1E+04) conidia/ml) of *P.fr* 14 and then kept separately in Petri dishes (diameter 7 cm) containing moistened tissue paper and a tomato leaf disc (diameter 2 mm) that had been previously treated with 2 µl of a sub-lethal concentration of *Bt* (i.e., 0.0054mg/ml). (ii) Same as in (i) but *H. armigera* larvae were fed with untreated tomato leaf discs. (iii) Here the larvae were not treated with EPF but fed only with *Bt*-treated tomato leaf discs. (iv) Same as in (i) but *H. armigera* larvae were treated with *P.fr* 14 at a concentration of 1E+07 conidia/ml. (v) Same as in (iii) but the larvae were fed only with untreated tomato leaf discs. Tomato leaves were added daily as food source for the larvae. Each treatment consisted of 30 third instar larvae and was repeated three times overtime. The Petri dishes were placed in a climate chamber at 25°C, 70% RH and a 16: 8 L: D photoperiod. Mortality was recorded daily over a period of 14 days. Dead larvae were removed daily and incubated in moist chambers to observe sporulation of the fungi. (*P.fr* 14 was selected for this test based on its highest virulence toward L3 compared to other EPF, defined in dose-response experiment).

5.11 Statistical analysis

Data of experiments repeated over time were checked for homogeneity of variance using the HOVTEST = LEVENE option of SAS version 8 (SAS, 2001) and pooled only when variance homogeneity could be assumed.

Cumulative corrected mortality (CM) in percent was calculated using Abbott's formula (Abbott, 1925). Prior to analysis percentages of CM were arcsine transformed. Data was then

analysed by means of two-way ANOVA, and in case of significant *F*-values means were compared using Tukey's test. The significance level was set at $P < 0.05$.

Median survival time (MST) was computed using the LIFETEST option of SAS, which can calculate the right-censored data, the surviving organism at the end of observation. The MST was derived using Kaplan-Meier estimates. The homogeneity of the survival curves among the treatments was tested using Log-rank tests.

Probit analysis was used to analyse the median lethal dose with 95% confidence limits of both EPF and *Bt* using the PROBIT option of SAS.

Percentages of sporulated cadavers were compared between isolates, and across dosages for each experiment using two-way ANOVAs followed by Tukey's test.

6 RESULTS

6.1 Screening the efficacy of EPF against third instar larvae *H. armigera*

All tested EPF species/strains proved to be highly virulent against L3 of *H. armigera* (figure 1 and table 2). On the first evaluation date, i.e., two days after application, except for *P.fr* 14 all tested species/strains did not cause significantly higher mortality than the 0.1% Tween 80 treated control. However, thereafter mortality increased rapidly over time, leading to significantly higher mortality in all tested species/strains compared to the control ($F = 47.89$; $df = 7, 14$; $P < 0.0001$). In general highest corrected mortality levels in each EPF species were recorded in *B.ba* 124, *P.fr* 14 (both 100%) and *M.a* 79 (82%) on the last day of observation. Median survival times (MST) caused by different EPF strains/species differed (figure 1 and table 2). With 3.3 days MST was shortest in *P.fr* 14 and with 8.1 days longest in *M.a* 97. However, significant differences in MST were only found between *P.fr* 14 and two *M.a* strains (table 2).

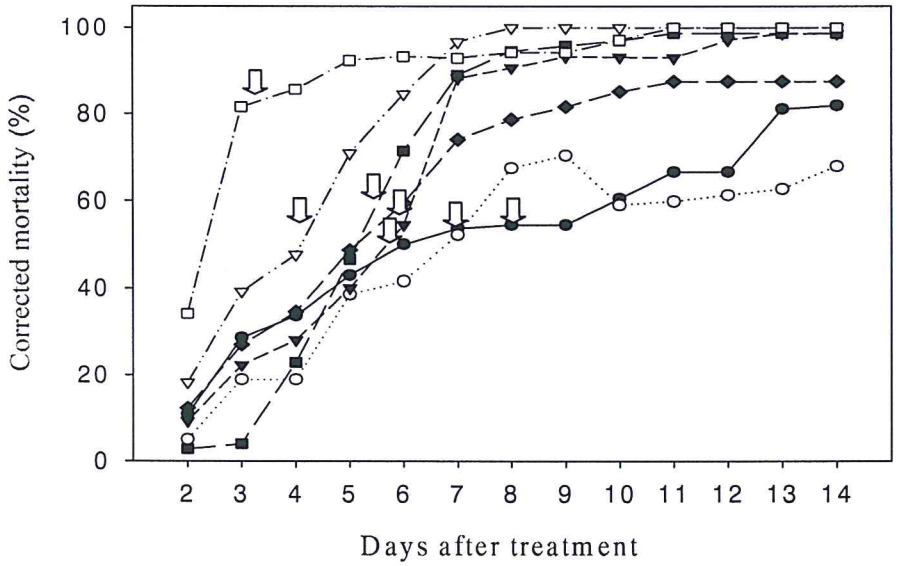

Figure 1: Cumulative corrected mortality over time and median survival time of third instar larvae of *H. armigera* exposed to different EPF species/strains (1E+07 conidia/ml) at 25°C. (-●-) *M.a* 79, (-○-) *M.a* 97, (-▼-) *B.ba* 108, (-Δ-) *B.ba* 124, (-■-) *B.ba* 139, (-□-) *P.fr* 14 and (-♦-) *P.fr* 15; (⇩) MST.

High mycosis (sporulation) was observed on cadavers previously exposed to *P.fr* and *B.ba* strains (figure 2). In addition to causing lower mortality levels, the extent of sporulation of *M.a*-treated insects was also significantly lower and did not exceed 50%.

Table 2: Cumulative corrected mortality (CM) over time and median survival time (MST) of third instar larvae of *H. armigera* exposed to different EPF species/strains (1E+07 conidia/ml) at 25°C.

Treatment	Corrected mortality (% ± SE)													MST ± SE (days)
	2 DAT[1]	3 DAT	4 DAT	5 DAT	6 DAT	7 DAT	8 DAT	9 DAT	10 DAT	11 DAT	12 DAT	13 DAT	14 DAT	
M.a 79	10.8 ± 0.8ab[2]	28.6 ± 5.3ab	33.7 ± 7.3abc	43.0 ± 6.8b*	50.0 ± 7.9bc*	53.7 ± 8.3c*	54.5 ± 5.5c*	54.5 ± 5.5c*	60.5 ± 8.8c*	66.7 ± 12.1c*	66.7 ± 12.07c*	81.2 ± 4.3bc	82.0 ± 3.6bc*	7.1 ± 0.5bc
M.a 97	5.0 ± 1.7ab	18.9 ± 12.4b	18.9 ± 12.4bc	38.5 ± 3.4b*	41.7 ± 7.4c*	52.2 ± 4.5c*	67.6 ± 14.4bc*	70.5 ± 12.6bc*	59.1 ± 1.4c*	59.9 ± 2.2c*	61.3 ± 1.4c*	62.8 ± 1.9c*	68.1 ± 3.5c*	8.1 ± 0.5c
B.ba 108	9.2 ± 5.8ab	22.3 ± 11.6b	28.1 ± 17.1bc	40.0 ± 18.1b*	54.5 ± 23.2abc*	88.2 ± 3.8ab*	90.7 ± 4.7ab*	93.2 ± 3.8ab*	93.1 ± 3.8ab*	93.1 ± 3.8ab*	97.1 ± 2.9ab*	98.6 ± 1.5a*	98.6 ± 1.5a*	6.1 ± 0.3abc
B.ba 124	18.2 ± 9.1ab	39.2 ± 19.6ab	47.6 ± 23.8ab*	71.0 ± 24.2ab*	84.6 ± 15.4ab*	96.7 ± 3.3a*	100.0 ± 0.0a*	100.0 ± 0.0a*	100.0 ± 0.0a*	100.0 ± 0.0a*	100.0 ± 0.0a*	100.0 ± 0.0a*	100.0 ± 0.0a*	4.3 ± 0.2ab
B.ba 139	2.8 ± 2.8b	4.0 ± 2.5b	22.9 ± 15.38bc	46.6 ± 7.4ab*	71.5 ± 5.6abc*	89.0 ± 1.7ab*	94.6 ± 3.2ab*	95.8 ± 2.2ab*	97.0± 1.6ab*	98.7 ± 1.3ab*	98.66 ± 1.3ab*	98.7 ± 1.3a*	98.7 ± 1.3a*	5.7± 0.2abc
P.fr 14	34.2 ± 3.2a*	81.6 ± 4.2a*	85.7 ± 4.9a*	92.4 ± 2.2a*	93.3 ± 3.5a*	92.9 ± 3.7ab*	94.2 ± 2.9ab*	94.2 ± 2.9ab*	97.1± 2.9ab*	100.0 ± 0.0a*	100.0 ± 0.0a*	100.0 ± 0.0a*	100.0 ± 0.0a*	3.3 ± 0.2a
P.fr 15	12.2 ± 10.6ab	27.0 ± 17.4b	34.6 ± 19.7abc	48.7 ± 12.8ab*	59.5 ± 12.0abc*	74.1 ± 8.6bc*	78.7± 7.1bc*	81.6 ± 7.6bc*	85.2 ± 6.5b*	87.5 ± 4.5bc*	87.5 ± 4.5bc*	87.5 ± 4.5b*	87.5 ± 4.5b*	5.9 ± 0.3abc

[1] = days after treatment; [2] = means within a column followed by the same letter are not significantly different (Tukey's test, $P < 0.05$); * denote significant differences of CM means with the untreated control. Data show the mean of three assays.

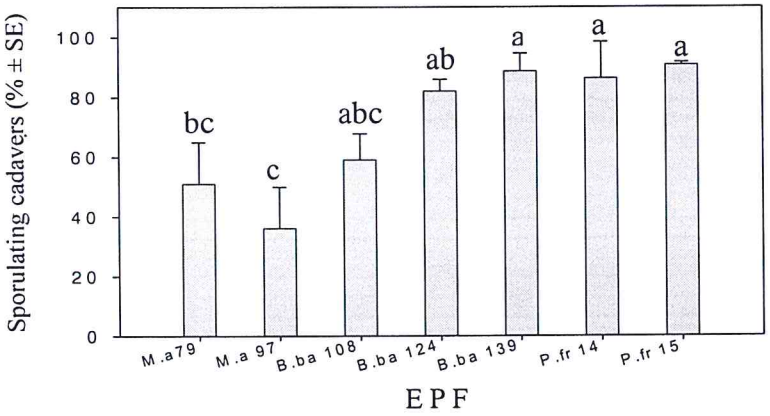

Figure 2: Percentage of sporulating cadavers of third instar larvae *H. armigera* exposed to different EPF species/strains. Means (± SE) followed by the same letter are not significantly different (Tukey's test, $P < 0.05$). Data show the mean of three assays.

Based on these results *M.a* 79, *B.ba* 124 and *P.fr* 14 were selected for further studies.

6.2 Susceptibility of different larval stages of *H. armigera* to *M. anisopliae* 79, *B. bassiana* 124 and *P. fumosoroseus* 14

From five days after treatment onwards all three strains caused high levels of mortality in the different larval instars of *H. armigera* (Figure 3 and table 3) ($F = 57.56$; df = 3, 6; $P < 0.0001$ for L2; $F = 138.49$; df = 3, 6; $P < 0.0001$ for L3; $F = 22.99$; df = 3, 6; $P = 0.0011$ for L4; and $F = 385.67$; df = 3, 6; $P < 0.0001$ for L5). On the last evaluation date mortality ranged between 82.0 and 100% across strains and development stages of *H. armigera*. Comparatively lower mortality was recorded in *M.a* 79 compared to the two other strains (figure 3 and table 3), though even for this strain between 82-98% of the *H. armigera* larvae were dead at the end of the experiment. Proportionally highest mortality was recorded between five and eight days after treatment. The different larval instars of *H. armigera* were similarly affected by the EPF, though L2 seemed to be slightly more susceptible to the pathogens and L5 responded slower than the younger instars (figure 3 and table 3).

Likewise, there was no general trend in MSTs among the different larval stages, though MSTs in L2 were shorter than in older larvae. The longest MST was recorded in L3 following a *M.a* 79 treatment. There was no significant difference between MST of *H. armigera* in the same larval instars when exposed to the three EPF except when L3 were exposed to *P.fr* 14 and *M.a* 79 (figure 3 and table 3).

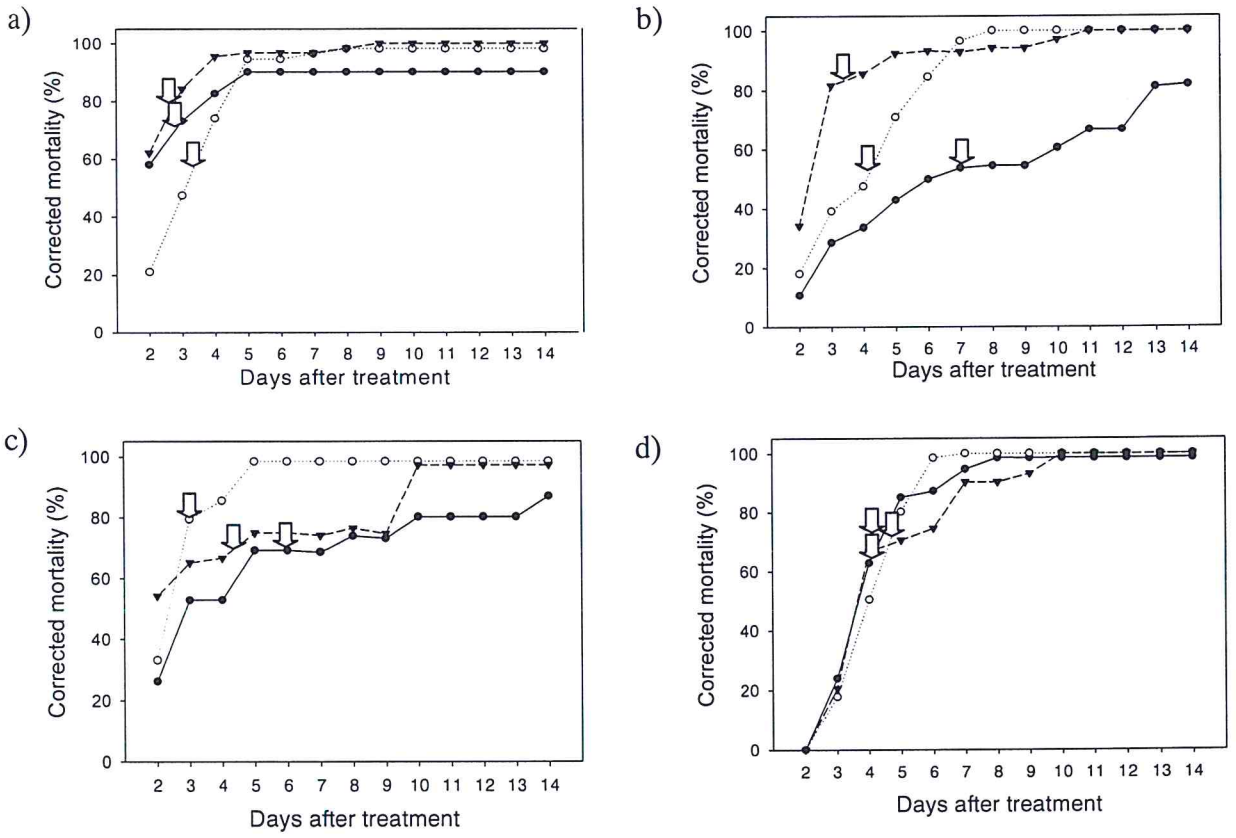

Figure 3: Cumulative corrected mortality and median survival time of L2 (a), L3 (b), L4 (c) and L5 (d) of *H. armigera* exposed to *M. anisopliae* 79, *B. bassiana* 124 and *P. fumosoroseus* 14 (1E+07 conidia/ml) at 25 °C. (-●-) *M.a* 79, (-○-) *B.ba* 124, (-▼-) *P.fr* 14; (⇩) Median survival time.

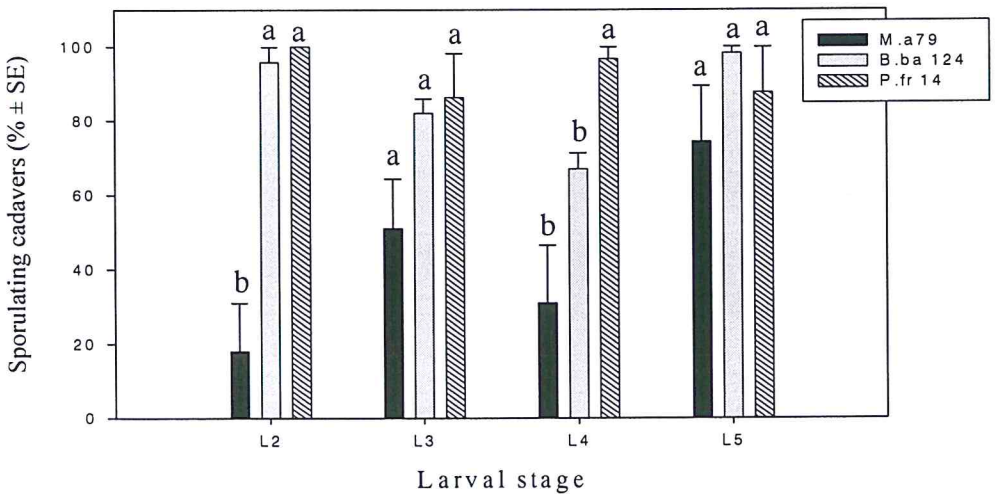

Figure 4: Percentage of sporulating cadavers of L2, L3, L4 and L5 of *H. armigera* exposed to *M. anisopliae* 79, *B. bassiana* 124 and *P. fumosoroseus* 14. Means (± SE) for the same larval instars followed by the same letter are not significantly different (Tukey's test, $P < 0.05$). Data show the mean of three assays.

Table 3: Cumulative corrected mortality (CM) and median survival time (MST) of L2 (a), L3 (b), L4 (c) and L5 (d) of *H. armigera* exposed to *M. anisopliae* 79, *B. bassiana* 124 and *P. fumosoroseus* 14 (1E+07 conidia/ml) at 25°C.

Treatment	Corrected mortality (% ± SE)													MST ± SE (days)
	2 DAT[1]	3 DAT	4 DAT	5 DAT	6 DAT	7 DAT	8 DAT	9 DAT	10 DAT	11 DAT	12 DAT	13 DAT	14 DAT	
L2														
M.a 79	58.1 ± 16.7a[2]*	73.3 ± 14.5a*	82.8 ± 12.6a*	90.3 ± 7.2a*	90.3 ± 7.2a*	90.3 ± 7.2a*	90.3 ± 7.2a*	90.3 ± 7.2b*	90.3 ± 7.2a*	90.3 ± 7.2a*	90.3 ± 7.2a*	90.3 ± 7.2a*	90.3 ± 7.2a*	2.8 ± 0.1a
B.ba 124	21.1± 12.5ab	47.5 ± 27.9ab	74.2 ± 12.9a*	94.7 ± 5.3a*	94.7 ± 5.3a*	96.5 ± 3.5a*	98.2 ± 1.8a*	98.2 ± 1.8a*	98.2 ± 1.8a*	98.2 ± 1.8a*	98.2 ± 1.8a*	98.2 ± 1.8a*	98.2 ± 1.8a*	3.3 ± 0.1a
P.fr 14	62.2 ± 6.2a*	84.4 ± 8.7a*	95.6 ± 2.4a*	96.8 ± 3.2a*	96.8 ± 3.2a*	96.8 ± 3.2a*	98.4 ± 1.6a*	100.0 ± 0.0a*	100.0 ± 0.0a*	100.0 ± 0.0a*	100.0 ± 0.0a*	100.0 ± 0.0a*	100.0 ± 0.0a*	2.6 ± 0.1a
L3														
M.a 79	10.8 ± 0.8ab	28.6 ± 5.3ab	33.7 ± 7.3ab	43.0 ± 6.8ab	50.0 ± 7.9b*	53.7 ± 8.3b*	54.5 ± 5.5b*	54.5 ± 5.5b*	60.5 ± 8.8b*	66.7 ± 12.1b*	66.7 ± 12.1b*	81.2 ± 4.3b*	82.0 ± 3.6b*	7.1 ± 0.5b
B.ba 124	18.2 ± 9.1ab	39.2 ± 19.6ab	47.6 ± 23.8ab	71.0 ± 24.2a*	84.6 ± 15.4a*	96.7 ± 3.3a*	100.0 ± 0.0a*	100.0 ± 0.0a*	100.0 ± 0.0a*	100.0 ± 0.0a*	100.0 ± 0.0a*	100.0 ± 0.0a*	100.0 ± 0.0a*	4.3 ± 0.2ab
P.fr 14	34.2 ± 3.2a*	81.6 ± 4.2a*	85.7 ± 4.9a*	92.4 ± 2.2a*	93.3 ± 3.5a*	92.9 ± 3.7a*	94.2 ± 2.9a*	94.2 ± 2.9a*	97.1 ± 2.9a*	100.0 ± 0.0a*	100.0 ± 0.0a*	100.0 ± 0.0a*	100.0 ± 0.0a*	3.3 ± 0.2a
L4														
M.a 79	26.3 ± 12.3a	52.9 ± 21.5ab	52.9 ± 21.5ab	69.3 ± 13.1a*	69.3 ± 13.1a*	68.7 ± 13.4a*	74.0 ± 10.9a*	73.2 ± 11.2a*	80.3 ± 7.8a*	80.3 ± 7.8a*	80.3 ± 7.8a*	80.3 ± 7.8a*	87.0 ± 8.7a*	5.9 ± 0.5a
B.ba 124	33.3 ± 17.6a	79.5 ± 12.6a*	85.6 ± 14.4a*	98.6 ± 1.5a*	98.6 ± 1.5a*	98.6 ± 1.5a*	98.6 ± 1.5a*	98.6 ± 1.5a*	98.6 ± 1.5a*	98.6 ± 1.5a*	98.6 ± 1.5a*	98.6 ± 1.5a*	98.6 ± 1.5a*	3.0 ± 0.1a
P.fr 14	54.2 ± 15.6a*	65.2 ± 21.6ab	66.7 ± 22.7a*	75.0 ± 23.3a*	75.0 ± 23.3a*	74.2 ± 24.1a*	76.5 ± 23.5a*	74.7 ± 25.3a*	97.3 ± 2.7a*	97.3 ± 2.7a*	97.3 ± 2.7a*	97.3 ± 2.7a*	97.3 ± 2.7a*	4.4 ± 0.4a
L5														
M.a 79	0.0 ± 0.0a	24.0 ± 7.3a*	62.9 ± 26.4a*	85.2 ± 3.7a*	87.3 ± 5.2a*	94.7 ± 3.4a*	98.6 ± 1.5a*	98.5 ± 1.5a*	98.6 ± 1.5a*	98.6 ± 1.5a*	98.6 ± 1.5a*	98.6 ± 1.5a*	98.6 ± 1.5a*	4.3 ± 0.1a
B.ba 124	0.0 ± 0.0a	17.8 ± 6.7a*	50.5 ± 22.9a*	80.3 ± 2.2a*	98.6 ± 1.5a*	100.0 ± 0.0a*	100.0 ± 0.0a*	100.0 ± 0.0a*	100.0 ± 0.0a*	100.0 ± 0.0a*	100.0 ± 0.0a*	100.0 ± 0.0a*	100.0 ± 0.0a*	4.4 ± 0.1a
P.fr 14	0.0 ± 0.0a	20.6 ± 6.1a*	66.7 ± 22.7a*	70.9 ± 14.1a*	74.6 ± 15.1a*	90.3 ± 3.7a*	90.3 ± 3.7b*	93.2 ± 5.0b*	100.0 ± 0.0a*	100.0 ± 0.0a*	100.0 ± 0.0a*	100.0 ± 0.0a*	100.0 ± 0.0a*	4.7 ± 0.2a

[1] = days after treatment; [2] = means within a column for the same fungus followed by the same letter are not significantly different (Tukey's test, $P < 0.05$); * denote significant differences of CM means with the control. Data show the mean of three assays.

Like in the previous experiment, with 67-100% high proportion of sporulation was observed on cadavers previously treated with *B.ba* 124 and *P.fr* 14 (figure 4). In contrast, the extent of sporulation on cadavers previously treated with *M.a* 79 was considerably lower, particularly on L2 and L4.

6.3 Efficacy of *M. anisopliae* 79, *B. bassiana* 124 and *P. fumosoroseus* 14 against pupae of *H. armigera*

Among the three treatment methods, the immersion technique proved to be the best for control of *H. armigera* pupae in all three tested EPF strains, always yielding 100% prevention of adult emergence (figure 5). With 74.4 to 97.8% a considerably high efficacy was also found when pupae were released into EPF-inoculated soil, with significant differences in prevention of adult emergence between *P.fr* 14 and *B.ba* 124. In contrast the soil surface contamination method had basically no effect on *H. armigera* pupae in all EPF strains tested (figure 5). Sporulation of the fungi was evident in all pupae which failed to emerge to adults.

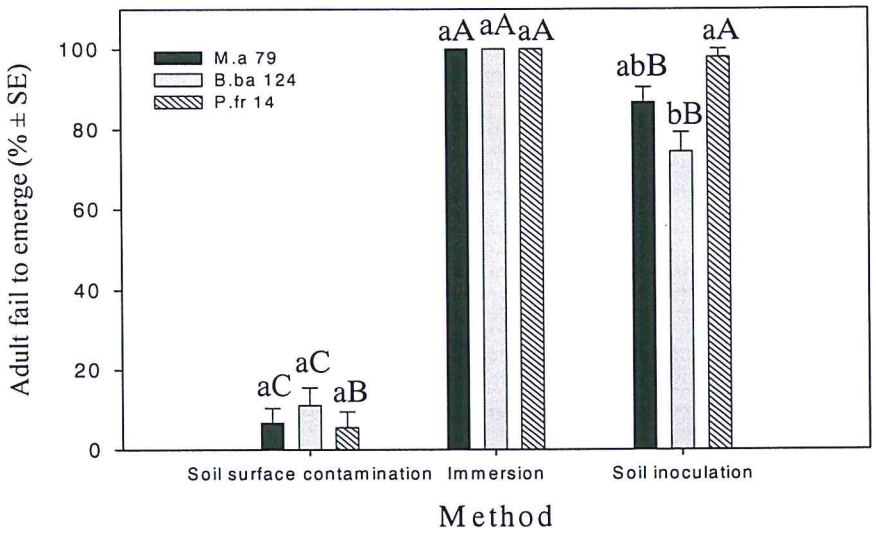

Figure 5: Efficacy of *M. anisopliae* 79, *B. bassiana* 124 and *P. fumosoroseus* 14 on pupae of *H. armigera* using three different application techniques after 14 days at 25°C. Means (± SE) within a method and fungus followed by the same lower and upper case letter, respectively, are not significantly different (Tukey's test, $P < 0.05$). Data show the mean of three assays.

6.4 Evaluating dose-response of *M. anisopliae* 79, *B. bassiana* 124 and *P. fumosoroseus* 14 to third instar larvae *H. armigera*

In all three tested EPF strains the same trend of decreasing CM levels and increasing MSTs with reduced concentrations was recorded (figure 6 and table 4). The higher dosages caused higher CM and shorter MST. Ranges of CM from 1.4 to 83.3%, 11.8 to 100% and 35.5 to 96.3% were recorded across dosages in *M.a* 79, *B.ba* 124 and *P.fr* 14, respectively. Except for *M.a* 79 at 1E+04 conidia/ml on the last day of evaluation, in all EPF species and dose rate combinations significantly higher mortality than in the untreated control was recorded (figure 6 and table 4) ($F = 61.68$; df = 4, 8; $P < 0.0001$ for *M.a* 79; $F = 228.21$; df = 4, 8; $P < 0.0001$ for *B.ba* 124 and $F = 59.58$; df = 4, 8; $P = 0.0011$ for *P.fr* 14). On this and previous evaluation dates significant differences among the different dosages of the same fungus were found. In all treatments, CMs increased considerably overtime except for the lowest dose rate in *M.a* 79 and *B.ba* 124 (figure 6 and table 4).

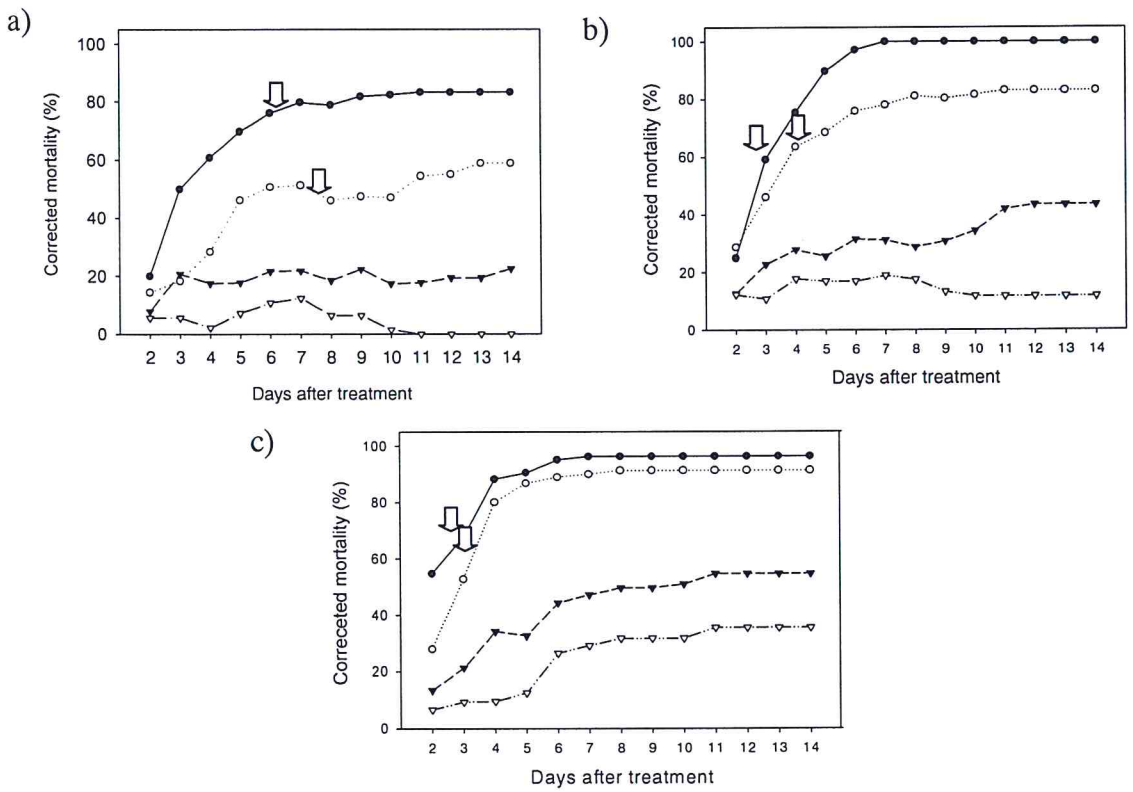

Figure 6: Corrected mortality and median survival time of L3 *H. armigera* exposed to different doses (in conidia/ml) of *M. anisopliae* 79 (a), *B. bassiana* 124 (b) and *P. fumosoroseus* 14 (c) at 25°C. (●) 1E+07, (○) 1E+06, (▼) 1E+05, (Δ) 1E+04 conidia/ml; (⇩) Median survival time.

Table 4: Corrected mortality (CM) and median survival time (MST) of L3 *H. armigera* exposed to different doses (in conidia/ml) of *M. anisopliae* 79 (a), *B. bassiana* 124 (b) and *P. fumosoroseus* 14 (c) at 25°C.

Treatment	Corrected mortality (% ± SE)													MST ± SE
	2 DAT[1]	3 DAT	4 DAT	5 DAT	6 DAT	7 DAT	8 DAT	9 DAT	10 DAT	11 DAT	12 DAT	13 DAT	14 DAT	(days)
a. *M.a* 79														
1E+07	20.0 ± 3.8a[2]*	37.3 ± 4.7a*	47.1 ± 6.9a*	55.9 ± 2.9a*	62.2 ± 4.6a*	67.9 ± 4.9a*	64.9 ± 4.7a*	69.3 ± 4.2a*	69.1± 4.1a*	74.2 ± 1.7a*	74.2 ± 1.7a*	78.9 ± 3.5a*	83.3 ± 3.0a*	6.2 ± 0.5a
1E+06	14.4 ± 7.2a*	18.4 ± 7.7ab	28.4 ± 2.8ab*	46.0 ± 10.3ab*	50.7 ± 8.0a*	51.2 ± 7.8a*	45.9 ± 10.2ab*	47.4 ± 9.1ab*	46.9 ± 9.2ab*	54.3 ± 5.7a*	55.9 ± 5.7a*	58.9 ± 3.2a*	58.9 ± 3.2b*	7.6 ± 0.5a
1E+05	7.7 ± 3.9ab	20.7 ± 10.3ab	17.5 ± 5.5b*	17.7 ± 3.7bc*	21.5 ± 3.8b*	21.7 ± 5.6b*	18.5 ± 6.5bc*	22.3 ± 4.0bc*	17.4 ± 7.4bc*	17.8 ± 7.3b*	19.3 ± 6.0b*	19.3 ± 6.0b*	22.5 ± 3.9d	-
1E+04	5.5 ± 2.9ab	5.5 ± 5.5b	3.5 ± 3.5c	7.1 ± 3.5dc	10.8 ± 3.7b*	12.4 ± 2.8b*	7.8 ± 5.8dc	7.8 ± 5.8dc	2.9 ± 1.4dc	1.4 ± 1.4c	1.4 ± 1.4c	1.4 ± 1.4c	1.4 ± 1.4d	-
b. *B.ba*124														
1E+07	25.1 ± 7.5a*	59.2± 5.7a*	75.5 ± 2.7a*	89.7 ± 1.0a*	97.1 ± 2.9a*	100.0 ± 0a*	100.0 ± 0a*	100.0 ± 0a*	100.0 ± 0a*	100.0 ± 0a*	100.0 ± 0a*	100.0 ± 0.0a*	100.0 ±0.0a*	3.2 ± 0.1a
1E+06	28.8 ± 6.7a*	46.1± 9.3ab*	63.6 ± 5.6a*	68.6 ± 6.9b*	75.9 ± 2.4b*	78.0 ± 5.0b*	81.1 ± 6.0b*	80.3 ± 5.8b*	81.5 ± 5.7b*	83.0 ± 6.0b*	83.0 ± 6.0b*	83.0 ± 6.0b*	83.0 ± 6.0b*	4.6 ± 0.3a
1E+05	12.6 ± 2.1a*	22.8± 4.3bc*	27.9 ± 2.4b*	25.6 ± 4.8c*	31.6 ± 5.1c*	31.2 ± 4.8c*	28.8 ± 7.2c*	30.8 ± 9.7c*	34.3 ± 10.2c*	41.9 ± 9.6c*	43.5 ± 8.1c*	43.5 ± 8.1c*	43.5 ± 8.1c*	-
1E+04	12.3 ± 4.7a*	10.8± 4.3c*	17.8 ± 5.1b*	17.0 ± 4.3c*	17.0 ± 4.3c*	18.9 ± 6.9c*	17.6 ± 7.9c*	13.2 ± 6.5c*	11.8 ± 7.2c*	11.8 ± 7.2d*	11.8 ± 7.2d*	11.8 ± 7.2d*	11.8 ± 7.2d*	-
c. *P.fr* 14														
1E+07	54.7 ± 13.6a*	67.8 ± 21.3a*	88.3 ± 6.1a*	90.5 ± 4.8a*	95.2 ± 3.1a*	96.3 ± 2.1a*	96.3 ± 2.1a*	96.3 ± 2.1a*	963 ± 2.1a*	96.3 ± 2.1a*	96.3 ± 2.1a*	96.3 ± 2.1a*	96.3 ± 2.1a*	3.0 ± 0.2a
1E+06	28.0 ± 5.4ab*	52.8 ± 20.8ab*	80.1 ± 6.0a*	86.8 ± 4.6a*	89.0 ± 3.5a*	90.0 ± 3.2a*	91.3 ± 3.2a*	91.3 ± 3.2a*	91.3 ± 3.2a*	91.3 ± 3.2a*	91.3 ± 3.2a*	91.3 ± 3.2a*	91.3 ± 3.2a*	3.6 ± 0.2a
1E+05	13.4 ± 6.9bc	21.3 ± 14.5abc	34.2 ± 8.4b*	32.7 ± 7.9b*	44.3 ± 7.9b*	47.2 ± 6.8b*	49.6 ± 6.7b*	49.6 ± 6.7b*	50.9 ± 5.6b*	54.6 ± 7.0b*	54.6 ± 7.0b*	54.6 ± 7.0b*	54.6 ± 7.0b*	-
1E+04	6.6 ± 7.0bc	9.3 ± 7.0bc	9.5 ± 5.2bc	12.5 ± 4.5b*	26.5 ± 10.8b*	29.2 ± 11.8b*	31.8 ± 13.0b*	31.8 ± 13.0b*	31.8 ± 13.0b*	35.5 ± 9.6b*	35.5 ± 9.6b*	35.5 ± 9.6b*	35.5 ± 9.6b*	-

[1] = days after treatment; [2] = means within a column for the same fungus followed by the same letter are not significantly different (Tukey's test, $P < 0.05$); * denote significant differences of CM means with the control. Data show the mean of three assays.

In general, the MSTs increased with decreasing dosages (figure 6 and table 4). The MSTs in all *P.fr* 14 treatments were slightly shorter than in the other two fungi, followed by those of *B.ba* 124. MSTs at the two lowest dose rate tested (i.e., 1E+04 and 1E+05 conidia/ml) could not exactly be estimated due to the high numbers of surviving larvae at the end of the evaluation. However, no significant difference was recorded between MSTs of *H. armigera* exposed to doses 1E+06 and 1E+07 conidia/ml.

Correspondingly a dose-dependent sporulation rate was found on cadavers previously treated with *B.ba* 124 and *P.fr* 14, with a tendency of higher sporulation in the latter compared to the former fungus (figure 7). In contrast, irrespective of the dose rates, no fungal growth was recorded in *M.a* 79. The same result also recorded at the lowest dose of *B.ba* 124 (i.e., 1E+04 conidia/ml).

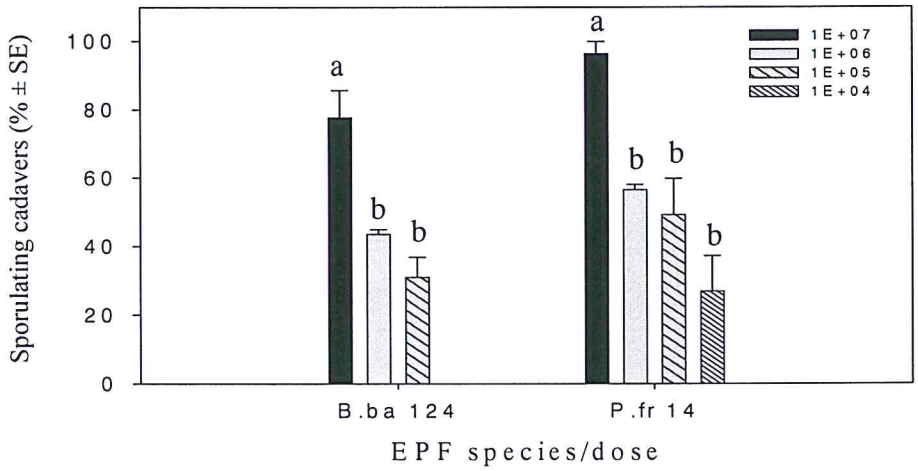

Figure 7: Percentage of sporulating cadavers of *H. armigera* L3 exposed to different doses (in conidia/ml) of *B. bassiana* 124 and *P. fumosoroseus* 14. Means (± SE) for the same fungus followed by the same letter are not significantly different (Tukey's test, $P < 0.05$). Data show the means of three assays.

The estimated LC50 values based on the mortality trends across dosages are presented in table 5. *P.fr* 14 showed the highest virulence with the lowest LC50 (2.3E+04) conidia/ml), followed by *B.ba* 124 (3.6E+04) conidia/ml) and *M.a* 79 (1.4 E+05) conidia/ml).

Table 5: Dose-response calculation (conidia/ml) for *M. anisopliae* 79, *B. bassiana* 124 and *P. fumosoroseus* 14 in third instar larvae of *H. armigera.*

Fungus	LC50 (conidia/ml)	95% confidence intervals	
		Lower	Upper
M.a 79	1.4E+05	8.0E+04	2.4E+05
P.fr 14	2.3E+04	1.0E+04	4.0E+04
B.ba 124	3.6 E+04	2.0E+04	5.6E+04

6.5 Estimating the sub-lethal concentration of *Bt* formulations against third instar larvae of *H. armigera*

The LC50 value of Xentary across dosages in third instar larvae of *H. armigera*, based on the mortality trends, was calculated to be 0.0054 mg/ml with lower and upper 95% confidence intervals of 0.0038 and 0.0078, respectively.

6.6 *In vitro* interactions of *Bt* and *M. anisopliae* 79, *B. bassiana* 124 and *P. fumosoroseus* 14

Results of the *in vitro* interaction tests among the three EPF species and the two *Bt* products are shown in figure 8 and table 6.

On the MEPA medium, although the fungal colonies in the combined treatments grew slightly less than in fungus-only colonies, in *M.a* 79 and *P.fr* 14 the differences were only significant towards the end of the experiment. In contrast, in combinations of *B.ba* 124 and the two *Bt* products the growth of the fungal colonies was at most observation dates significantly reduced to the fungus-only treatment. The two *Bt* products did not differ to a great extent in their effects on colony growth in the three EPF species.

However, on LB medium, in all EPF species a considerable, and in most cases significant reduction in the size of the fungal colonies was recorded in the combined treatments. These colonies grew smaller, both in terms of width and length of colonies. These differences became apparent three days after inoculating the fungal colonies with the two *Bt* products. On both media little differences were found between Xentary and Turex, the *Bt* products.

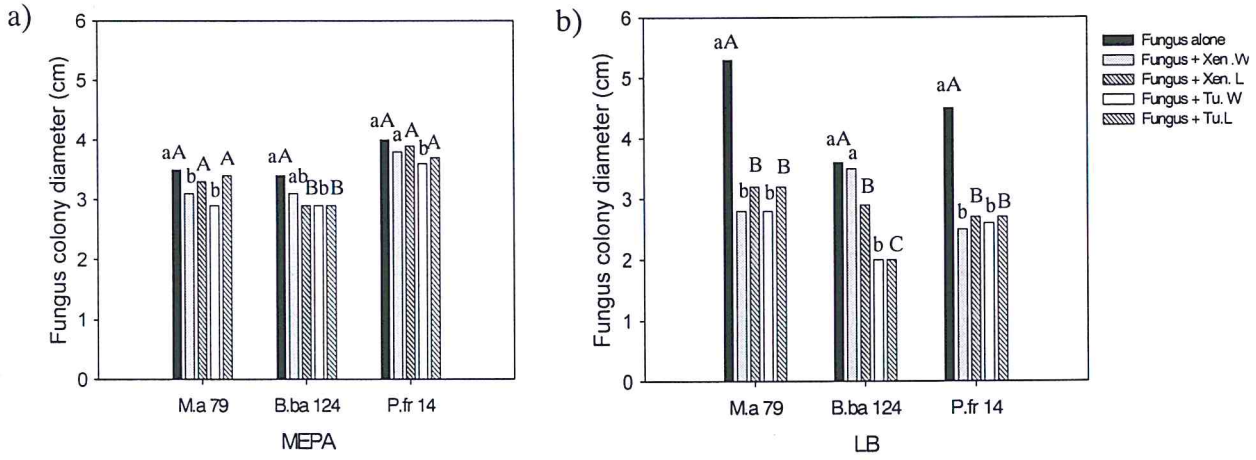

Figure 8: *In vitro* interaction between *M. anisopliae* 79 (*M.a* 79), *B. bassiana*124 (*B.ba* 124) and *P. fumosoroseus* 14 (*P.fr* 14) and Turex (Tu.) and Xentary (Xen.) on MEPA (a) and LB (b) media at seven days after inoculating the fungi. Means of the width (W) and length (L) of fungal colonies for a given fungus species followed by the same lower and upper case letter, respectively, are not significant different (Tukey's test, $P < 0.05$). Data show the means of three assays.

6.7 Combined effects of a sub-lethal dose of *P. fumosoroseus* 14 and *Xentary* on third instar larvae of *H. armigera*

Significantly higher CM in *H. armigera* was recorded following applications of both the combined treatment of sub-lethal doses of *P.fr* 14 and Xentary and the application of *P.fr* 14 at the high dose (i.e., 1E+07 conidia/ml) compared to sole applications of a sub-lethal dose of the fungus and/or *Bt* (figure 9 and table 7). *H. armigera* treated with *P.fr* 14 at a dose rate of 1E+07 conidia/ml resulted in highest CM compared to all other treatments over time. CM in the other treatments increased steadily. From five days after the treatment onwards a significant higher mortality in all treatments compared to the control was found ($F = 123.01$; $df = 4, 8$; $P < 0.0001$). Consistenly significantly higher CM in the combined compared to the two individual sub-lethal dose rate treatments were recorded from the sixth days after treatment onwards. On the last day of observation, almost double the extent of CM was recorded in the combination treatment compared to the individual ones.

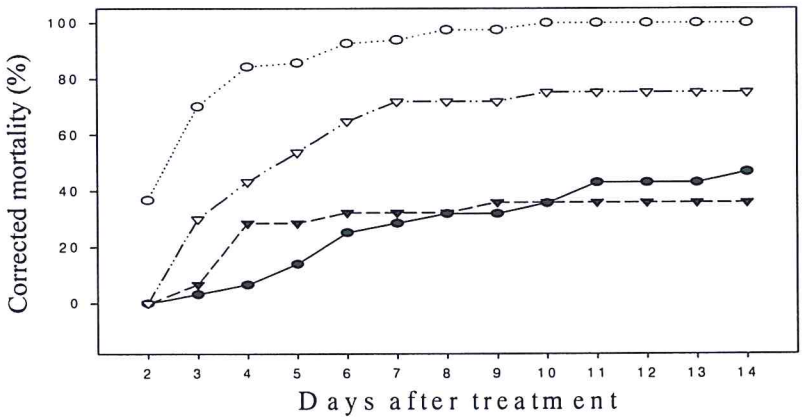

Figure 9: Cumulative corrected mortality over time of third instar larvae of *H. armigera* exposed to a sub-lethal and a high concentration of *P. fumosoroseus* 14 and a sub-lethal concentration of Xentary and the combination of the sub-lethal concentrations of *P. fumosoroseus* 14 and Xentary at 25°C. (●) *P.fr* 14 at 2.3E+04 conidia/ml, (○) *P.fr* 14 at 1E+07 conidia/ml, (▼) Xentary (0.0054 mg/ml), (Δ) Xentary and *P.fr* 14 in combination.

Considerable mycosis with high extent of sporulation was recorded on all cadavers previously treated with *P.fr* 14 (figure 10). No significant differences were found between cadavers treated with the sub-lethal concentration of *P.fr* 14, alone or in combination with *Bt.* However, significantly higher sporulation was detected on cadavers previously treated with the high dose rate of *P.fr* 14 (figure 10).

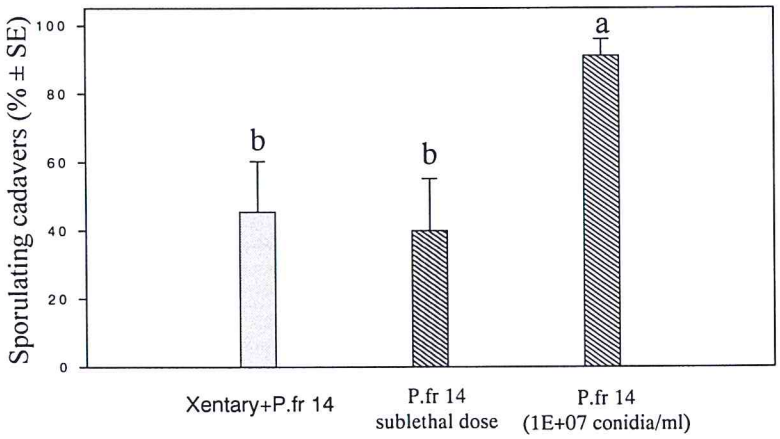

Figure 10: Percentage of sporulating cadavers of third instar *H. armigera* previously exposed to a sub-lethal (2.3E+04 conidia/ml) and a high (1E+07 conidia/ml) concentration of *P. fumosoroseus* 14 and a sub-lethal concentration (0.0054 mg/ml) of Xentary and the combination of the sub-lethal concentrations of *P. fumosoroseus* 14 and Xentary. Means (± SE) followed by the same letter are not significantly different (Tukey's test, $P < 0.05$). Data show the means of three assays.

Table 6: *In vitro* interactions between *M. anisopliae* 79, *B. bassiana* 124 and *P. fumosoroseus* 14 and Turex and Xentary on LB and MEPA media

Treatment	Medium	Diameter of fungal colonies (cm)										Distance EPF/Bt (cm)
		0 day	3 day		4 day		5 day		6 day		7 day	
		EF	EF[1]	EF(W[2])	EF(L[3])	EF(W)	EF(L)	EF(W)	EF(L)	EF(W)	EF(L)	
M.a 79	LB	1b	2.8a	3.5a	-	4.2a	-	4.7a	-	5.3a	5.3 a	
M.a 79 + Xentary		1b	2.5b	2.7b	3.0b	2.8b	3.2b	2.8b	3.2bc	2.8b	3.2b	0
M.a 79 + Turex		1.3a	2.5b	2.7b	3.0b	2.8b	3.2b	2.8b	3.2b	2.8b	3.2b	0.1
M.a 79	MEPA	1.2b	2a	2.4a	-	2.8a	-	3.2a	-	3.5a	3.5 a	
M.a 79 + Xentary		1.2b	1.9a	2.4a	2.4a	2.7a	2.7a	2.9ab	3.0a	3.1b	3.3a	0.2
M.a 79 + Turex		1.3a	2a	2.4a	2.4a	2.6a	2.8a	2.8b	3.1a	2.9b	3.4a	0
B.ba 124	LB	0.9a	1.8b	2.2a	-	2.7a	2.7a	3.2a	3.2a	3.6a	3.6a	
B.ba 124 + Turex		0.6a	1.9a	2.3a	-	2.9a	2.6a	3.2a	2.7b	3.5a	2.9b	0
B.ba 124 + Xentary		0.9a	1.7b	1.7b	-	1.8b	1.8b	1.9b	1.9c	2.0b	2.0c	0
B.ba 124	MEPA	0.9a	1.8a	2.2a	-	2.6a	2.6a	3.1a	3.1a	3.4a	3.4a	
B.ba 124 + Turex		0.8a	1.7a	2.1a	-	2.5a	2.4a	2.9b	2.9b	3.1ab	2.8b	0.3
B.ba 124 + Xentary		0.9a	1.5a	1.8b	-	2.0b	2.0b	2.5c	2.5c	2.9b	2.9b	0
P.fr 14	LB	1.2a	2.5a	3.1a	-	3.6a	-	4.1a	-	4.5a	-	
P.fr 14+ Turex		1.2a	2.3b	2.4b	2.6b	2.5b	2.6b	2.5b	2.7b	2.5b	2.7b	0.4
P.fr 14 + Xentary		1.1a	2.2b	2.4b	2.5b	2.5b	2.6b	2.5b	2.7b	2.6b	2.7b	0.4
P.fr 14	MEPA	1.2a	2.3a	2.8a	-	3.1a	-	3.5a	-	4a	-	
P.fr 14 + Turex		1.2a	2.3a	2.7a	2.7a	3.1a	3.2a	3.4a	3.5a	3.8a	3.9a	0
P.fr 14 + Xentary		1.2a	2.3a	2.7a	2.7a	3.0a	3.0a	3.3a	3.4a	3.6b	3.7a	0

[1] = fungal colony, [2, 3] = width (W) and length (L) of the fungal colonies; means within a column for the same entomopathogenic fungus (EF) species and medium followed by the same letter are not significantly different (Tukey's test, $P < 0.05$).

Table 7: Cumulative corrected mortality over time of third instar larvae of *H. armigera* exposed to a sub-lethal (2.3E+04 conidia/ml) (*P. fr* 14-s) and a high (1E+07 conidia/ml) (*P. fr* 14-h) dose of *P. fumosoroseus* 14 and a sub-lethal dose (0.0054 mg/ml) of Xentary and the combination of the sub-lethal doses of *P. fumosoroseus* 14 and Xentary (Xen.) at 25°C.

Treatment	Corrected mortality (% ± SE)												
	2 DAT[1]	3 DAT	4 DAT	5 DAT	6 DAT	7 DAT	8 DAT	9 DAT	10 DAT	11 DAT	12 DAT	13 DAT	14 DAT
P.fr 14-s	0.0 ± 0.0b[2]	3.3 ± 3.3b	6.7 ± 3.3c	14.1 ± 3c*	25.2 ± 4.1c*	28.5 ± 3.3b*	31.9 ± 5.2c*	31.9 ± 5.2c*	35.6 ± 2.2c*	43.0 ± 1.5c*	43.0 ± 1.5c*	43.0 ± 1.5c*	46.7 ± 4.6bc*
P.fr 14-h	36.7 ± 3.8a*	70.1 ± 10.2a*	84.4 ± 2.2a*	85.7 ± 0.5a*	92.7 ± 2.2a*	93.9 ± 1.4a*	97.5 ± 2.5a*	97.5 ± 2.5a*	100.0 ± 0.0a*	100.0 ± 0.0a*	100.0 ± 0.0a*	100.0 ± 0.0a*	100.0 ± 0.0a*
Xentary	0.0 ± 0.0b	6.7 ± 6.7b	28.5 ± 9.7bc*	28.5 ± 9.7cb*	32.2 ± 6.5c*	32.2 ± 6.5b*	32.2 ± 6.5c*	35.9 ± 4.4c*	35.9 ± 4.4c*	35.9 ± 4.4c*	35.9 ± 4.4c*	35.9 ± 4.4c*	35.9 ± 4.4c*
Xen. + *P.fr*	0.0 ± 0.0b	30.0 ± 5.8ab*	43.3 ± 6.7b*	53.7 ± 6.7b*	64.8 ± 8.1b*	71.9 ± 8.7a*	71.9 ± 8.7b*	71.9 ± 8.7b*	75.2 ± 6.9b*	75.2 ± 6.9b*	75.2 ± 6.9b*	75.2 ± 6.9b*	75.2 ± 6.9b*

[1] = days after treatment; [2] = means within a column followed by the same letter are not significantly different (Tukey's test, $P < 0.05$); * denote significant differences of CM means with the untreated control. Data show the mean of three assays.

7 DISCUSSIONS

All seven tested EPF strains/species showed substantially high efficacy against third larval instars of *H. armigera* in the screening tests, corroborating previous findings of Hassani (2000), the only so far researcher worked slightly detail on efficacy of EPF against *H. armigera*. However in our study we recorded higher mortality in *P.fr* and *B.ba* strains (87.5 to 100%) compared to the two tested *M.a* strains (68-82%). The difference can be attributed to different application methods. In Hassani's (2000) study *H. armigera* larvae were sprayed with fungal suspensions in a potter spray tower whereas in this study they were dipped individually into fungal suspensions. According to Goettel and Inglis (1997) when using the dipping method there is a higher probability for the insects to come into contact with the conidia, often leading to higher percentage of mortality compared to other application methods. This may also explain the shorter MSTs of *H. armigera* L3 in this study. In general the more virulent strains resulted in shorter MSTs in *H. armigera* L3, though significantly different only between *P. fumosoroseus* and *M. anisopliae* strains.

Pathogenicity of EPF is determined by a variety of factors, including the physiology of the host, and the physiology of the fungus in interactions with various environmental factors. The germination rate and speed of penetrating hyphae and their proliferation in the insect hosts are species/strain-dependent (Hywel-Jones & Gillespie, 1990; McCammon & Rath, 1994; Altre & Vandenberg, 2001). So far only *B. bassiana* and *N. rileyi* were recorded as naturally infecting pathogens of *Heliothis* and *Helicoverpa* spp. (Carner & Yearian, 1989). Only few EPF species/strains have been tested so far against *H. armigera* under field and laboratory conditions, and emphasis was mainly placed on *B. bassiana* and *N. rileyi* (Jayanthi & Padmavathamma, 1996, Sandhu *et al.*, 2001). The good performance of *M. anisopliae* and especially *P. fumosoroseus* strains in this study suggests a good potential of EPF against *H. armigera*. Based on their performance in the initial screening tests we selected *M. anisopliae* 79, *P. fumosoroseus* 14 and *B. bassiana* 124 for detailed studies on their dose-response to L3 and their efficacy against other larval stages and the pupa of *H. armigera*.

In addition to high virulence, intense sporulation of EPF on cadavers is a key factor for proliferation and spreading of the fungi, and thus a prerequisite for successful use of EPF in biological control (Inglis *et al.*, 2001). The percentage of fungal growth on cadavers varied

among larval instars of *H. armigera* and EPF species tested. Although conidiogenesis on cadavers usually require periods of high humidity (≥ 70%), conidia are formed on cadavers independently of fluctuations in relative humidity as long as the cadavers do not rapidly desiccate (Inglis *et al.*, 2001). Moreover according to Hallworth and Magan (1999), *B. bassiana* and *M. anisopliae*, among some other EPF, can independently infect insects without the effect of ambient humidity as long as moisture is sufficiently available on the host cuticle. The reason for the poor extent of sporulation on cadavers previously treated with *M. anisopliae* 79 may be the fact that almost all of the collected cadavers were dry. Additionally, *M. anisopliae* is also known to be a relatively weak saprophyte (Thomas *et al.*, 2003).

In general, different developmental stages of insects differ in their susceptibility to infections by entomopathogenic Hyphomycetes, and usually younger stages are more vulnerable to fungal diseases (Manjula & Padmavathamma, 1999; Inglis *et al.*, 2001). However, in our study no consistent trend in terms of effectiveness of the three EPF species on different development stages of *H. armigera* was observed. High effects were recorded in all tested larval instars, i.e., L2 to L5, although the L2 seemed to be more susceptible than older instars, as also shown by their shorter MST values.

With 100% and 74.4-97.7% both the immersion and soil inoculation methods, respectively, largely prevented adult emergence following an EPF treatment of *H. armigera* pupae. In contrast, very poor results were obtained using the soil surface contamination method. Initially we did not expect *H. armigera* pupae to be very susceptible to EPF since their thick cuticle is believed to prevent penetration by entomopathogenic fungi (St. Lejer, 1993; Hajeck & St. Leger, 1994). Our result supported previous reports on efficacy of EPF against pupa stages though they were on other insects i.e., fruit fly. High efficacies (32-94%) of three African tephritid fruit fly *Ceratitis* spp. were found when released the last larval stage in the soil previously treated with 1E+08 conidia/ml of *M. anisopliae* and *B. bassiana* (Ekesi *et al.*, 2002). Different *M. anisopliae* isolates were also found to cause 37.9-98.7% CM in last larval stage of Mexican fruit fly *Anastrepha ludens* (Diptera: Tephritidae) previuosly dipped in *M. anisopliae* suspension (1E+08UFC/ml) (Lezama-Gutierrez *et al.*, 2000). However, no effect was found when treated with *B. bassiana* (1-1.6E+08 conidia/ml) by the same method (De La Rosa *et al.*, 2002). The high mycosis occured in the dead pupae will ensure the transmission of EPF in the soil.

Vänninem *et al.* (2000) discussed the impact of different soil types on the persistency and penetration of *M. anisopliae* and *B. bassiana* in the soil. Although their penetration and persistency remarkably increased two years after the treatment, during the first year always a rapid decline was observed. The effect of different soil types to the efficacy of *M. anisopliae* prohibiting the emergence of adults Mexican fruit fly *A. ludens* was also reported with the more effect in loam soil compared to sandy loam one (Lezama-Gutierrez *et al.*, 2000). Moreover, when *B. bassiana* was applied to the soil surface as aqueous conidial spray, Storey *et al.* (1989) recorded a decrease in abundance of up to 85-95% within only 12 days after the application. However, mixing *B. bassiana* with the soil resulted in high persistency of the EPF (Gaugler *et al.*, 1989). This may explain the strong effects of the tested EPF on adult emergence in the soil inoculation compared to the soil surface contamination method. Our results suggest that EPF can be effectively used also against pupae of *H. armigera* and further research should target at the development of suitable control techniques and pricise application time in the field soil. Prophylactic application of EPF before target at pupae stage has been found to be more effective than curative application (Zimmermann, 1994; Ekesi *et al.*, 2002).

In the dose-mortality study, mortality and extent of sporulation in *H. armigera* increased with increasing EPF concentrations, and likewise lead to a reduction in MST values. Our results corroborate the results of other dose-mortality studies of EPF against insects e.g., *A. ludens* (De La Rosa *et al.*, 2002) and *Sitophilus zeamais* (Motsch) (Coleoptera: Curculiolidae) (Kassa *et al.*, 2002). Moreover the greater the tendency toward cadaver sporulation previously treated with higher conidia concentrations of EPF in the inoculum which was found in this study also coincides with the report of De La Rosa *et al.* (2002). The determined LC50 values of *M. anisopliae* 79 and *P. fumosoroseus* 14 against third instar larvae were lower than the ones reported by Hassani (2000). Again differences in application methods may explain these discrepancies.

Sub-lethal concentrations of *Bt* to *H. armigera* were also reported by other authors. However, since either different *Bt* products and toxins or methods or larval stages were tested, rendering comparation of the LC50 values measured in this study difficult. For instance, LC50 of Cry 1Ac to newly hatched larvae was defined at 0.11-06.1 µg/ml diet for *H. armigera* populations collected from different places in India (Jalali et al., 2004). Four µg AI/g diet was the LC50 of Dipel to the third instar *H. armigera* after 7 day (Liao et al., 2002).

In combined infections of two pathogens, complex interactions between the pathogens and the host occur. The interactions of the two agents can be negative, i.e., antagonism, or positive, i.e., synergistic or aditive (Brousseau *et al.*, 1998; Pingel & Lewis, 1999; Thomas *et al.*, 2003). Our results revealed only limited *in vitro* antagonism between the tested EPF species and the two *Bt* products. More pronounced effects were observed on the LB compared to the MEPA media. Possibly, LB medium is more suitable for growth of bacteria resulting in faster growth of *Bt* compared to that on MEPA medium as observed and hence competed nutrient with fungal colony in the same Petri dish for growth. Fungal colonies in the combined treatments continued to grow, though with a lower speed, indicating that both microbial agents may be applied together. However, the *in vitro* interaction maybe differs from that in bioassay. For instance, *M. anisopliae* was recorded decreasing its pathogenicity and sporulation when combined with an *B. bassiana* isolate against locust *Shistocerca gregaria* (Forskal) though no obvious inhibition zone was found in mixed *in vitro* culture of 2 fungal colonies (Thomas *et al.*, 2003)

In the bioassay on combined effects of sub-lethal doses of *P. fumosoroseus* 14 and Xentary, significantly higher CM of *H. armigera* L3 were recorded in the combined treatment compared to the sole applications of the fungus and the *Bt* product. One of the most important paradigms in microbial control is that 'stressed' animals are more susceptible to entomopathogens than non stressed ones (Inglis *et al.*, 2001). Stress can influence the insects' susceptibility to diseases (Costa *et al.*, 2001). The chronic sub-lethal exposure to one pathogen may not kill the insect but either reduces the host cellular defences or promotes an active immune response of an insect, causing an effective depletion of host haemocytes and hence indirectly enhances the effect of the second pathogen (Koppenhöfer *et al.*, 2000; Thomas *et al.*, 2003). According to Thomas *et al.* (2003), a competition for nutrients within the host heamocoel is likely to occur in mixed infections by *M. anisopliae* and *B. bassiana* resulting in a reduction of pathogenicity in and sporulation of *M. anisopliae* toward locust *S. gregaria* (Forskal). However, in EPF and *Bt* the route of infection within an insect host varies (for detail refer to 4.4.3.3 and 4.4.4.3, the mode of action of *Bt* and EPF, respectively). EPF penetrate through the cuticle and enter into the hemolymph, where they grow and subsequently release their toxins (for detail refer to 4.4.4.3), whereas *Bt* binds to the midgut epithelial tissues (for detail refer to 4.4.3.3). While *Bt* acts faster after digestion by the insect, EPF need time to germinate and penetrate into the cuticle. Exposure to sub-lethal doses of *Bt* may result in delayed development of insect (Glare & O'Callaghan, 2000) and thus facilitates

the infection process of the fungus. Sub-lethal dose of *Bt* also cause reduction in larval and pupal weight and size, reduction in pupation or adult emergence, occational loss or reduction of adult fecundity and reduce egg viability (Glare & O'Callaghan, 2000).

Enhancement or no inhibition between bio-toxins or chemical pesticides is prerequisites for combined applications of two agents, a strategy which is often successfully used in resistance management programs (Roush 1997). Synergism of some biocontrol agents or toxins has been demonstrated, e.g., in a laboratory experiment with a sub-lethal dose of *Bt* and that of destruxins (the *M. anisopliae* mycotoxins) against Spuce budworm (Brousseau *et al.*, 1998), or in the field between both low and high doses of *Bt* and an intermediate dose of *B. bassiana* against Colorado potato beetle (Wraight & Ramos, 2001). Hassani (2000) demonstrated the potential synergism between *Bt* and EPF against *S. litura* and *A. gossypii* on cotton. Acute and chronic sub-lethal exposure of Colorado potato beetle to *Bt* did not alter their subsequent susceptibility to *B. bassiana* (Costa *et al.*, 2001). Contrary, antagonism or inhibition can also occur in mixtures of other toxins with either *Bt* or EPF. Combined applications of *Bt* and *Anagrapha falcifera multiple NPV* caused no additive effects on the mortality of lepidopteran maize pests when compared with both pathogens alone or mortality caused by the virus was reduced with the addition of *Bt* (Pingel & Lewis, 1999). Azadirachtin also was found to be moderately antagonistic to *P. fumosoroseus* (James, 2003). The antagonisms were also reported in combination of three EPF strains/species i.e., *B. bassiana*, *M. anisopliae* and *P. fumosoroseus* with two entomopathogenic nematode species i.e., *Heterorhabditis indica* and *Steinernema carpocapsae* for control of peacan weevil *Curculio carye* (Coleoptera: Curculionidae), except the additive effect in combined *M. anisopliae* with *H. indica* (Shapiro-Ilan *et al.*, 2004)

8 RECOMMENDATIONS

The results presented in this study indicate a high potential of using EPF Hyphomycetes for control of all developmental stages of *H. armigera*. EPF, in particular *P. fumosoroseus* and *B. bassiana* strains, showed high pathogenicity against *H. armigera* and high sporulation on cadavers which is an important characteristic for the development of epizootics. High efficacy of *P. fumosoroseus* and *M. anisopliae* strains, which have so far not been recorded as naturally occurring pathogens of *H. armigera*, also indicate their potential as biocontrol agents for this pest. Despite their thick cuticle, pupae of *H. armigera* turned out to be highly susceptible to EPF infections depending on the application method. These findings merit further investigations. Results of sub-lethal combined applications of *P. fumosoroseus* and the commercial *Bt* product Xentary indicate that such combinations might become an interesting component in IPM of *H. armigera*. However, the here presented results of laboratory experiments need to be further verified under greenhouse and field conditions.

REFERENCES

Abbaiah, K.; Satyanarayana, A.; Rao, K. T. and Rao, N. V., 1988. Incidence of a fungal disease on *Heliothis armigera* larvae in Andhra Pradesh, India. *International Pigeonpea Newsletters* **8**, 11

Abbott, W. S., 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* **18**, 265-267

Ahmad, M.; Arif, M. I. and Attique, M. R., 1997. Pyrethroid resistance of *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Pakistan. *Bulletin of Entomological Research* **87**, 343-347

Ahmad, M.; Arif, M. I. and Attique, M. R., 1999. Patterns of resistance to Organophosphate insecticides in field population of *Helicoverpa armigera* in Pakistan. *Pesticide Science* **55**, 626-632

Altre, J. A. and Vandenberg, J. D., 2001. Penetration of cuticle and proliferation on hemolymph by *Paecilomyces fumosoroseus* isolates that differ in virulence against Lepidopteran larvae. *Journal of Invertebrate Pathology* **78**, 81-86

Armes, N. J. and Pandey, R., 1996. Pyrethroid resistance in *Helicoverpa armigera* in Nepal. *Resistant Pest Management Newsletter* **7**:1
Available: *http://whalonlab.msu.edu/rpm news/* 12.2002

Arno, J.; Gabarra, R.; Roig, J. and Fosch, T., 2002. Integrated pest management for processing tomatoes in the Ebro Delta (Spain). ISHS Acta Horticulture 487: VI International symposium on processing tomato and workshop on irrigation and fertilizations of processing tomato
Available: *http://www.actahort.org/books/487/487_28.htm* 12.02

Artheton, J. G. and Harris, G. P., 1996. Flowering. In *The Tomatoes crop.* Ed. Artheron, J. G. and Rudich, J., Chapman and Hall, 176-194

Babu, A. B.; Udagasuriyan, V.; Mariam, M. A.; Sivakumar, N. C.; Bharathi, M. and Balasubrabramanian, G., 2002. Comparative toxicity of Cry 1Aa delta-endotoxins of *Bacillus thuringiensis* against *Helicoverpa armigera*. *Crop Protection* **21**: 9, 817-822

Bartlett, M. C. and Jaronski, S.,T., 1988. Mass production of entomopathogeneous fungi for biological control of insects. In *Fungi in biological control system.* Ed. Burge, M. N., Manchester University Press, 61-85

Bon, R. P. M. and Boyce, C. B. C., 1971. The thermotable exotocin of *Bacillus thuringiensis*. In *Microbial control of insects and mites*. Ed. Burges, H. D. and Hussey, N. M., Academic Press London-New York, 275-302

Brousseau, C.; Charpentier, G. and Belloncik, S., 1998. Effects of *Bacillus thuringiensis* and *Destruxins* (*Metarhizium anisopliae* mycotoxins) combinations on Spuce budworm (Lepidoptera: Tortricidae). *Journal of Inverterbrate Pathology* **79**, 262-268

Burgerjon, A. and Martouret, D., 1971. Determination and significance of host spectrum of *Bacillus thuringiensis*. In *Microbial control of Insects and Mites*. Ed. Burges, H. D. and Hussey, N. M., Academic Press London –New York, 305-322

Cameron, P. J.; Walker, G. P.; Herman, T. J. B. and Wallace, A. R., 2001. Development of economic thresholds and monitoring systems for *Helicoverpa armigera* (Lepidoptera: Noctuidae) in tomatoes. *Journal of Economic Entomology* **94**: 5, 1104-1112

Cameron, P. J.; Walker, G. P. and Herman, T. J. B., 1995. Status of resistant to Fenvaralate in tomato fruitworm, *Helicoverpa armigera*
Available: *http://www.hortnet.co.nz/publications/nzpps/proceedings/95/95_3.htm* 12.02

Carner, G. R. and Yearian, W. C., 1989. Development and use of microbial agents for control of *Heliothis* spp. In USA. IOBC of *Heliothis*: Increasing the effectiveness of natural enemies. New Delhi, India 11-15 November 1985, 467-481

Charabartin, S. K.; Mandaokar, A.; Kumar, P. A. and Sharma, R. P., 1998. Efficacy of Lepidopteran specific δ–endotoxins of *Bacillus thuringiensis* against *Helicoverpa armigera*. *Journal of Invertebrate Pathology* **72**, 336-337

Cooksey, K. E. 1971. The protein crystal toxin of *Bacillus thuringiensis*: Biochemistry and mode of action. In *Microbial control of insects and mites*. Ed. Burges, H. D. and Hussey, N. M., Academic Press London-New York, 247-273

Costa, S. D.; Barbercheck, M. E. and Kenedy, G. G, 2001. Mortality of Colorado beetle (*Leptinotarsa decemlineata*) after sub-lethal stress with the CryIIIA δ-endotoxin of *Bacillus thuringiensis* and subsequent exposure to *Beauveria bassiana*. *Journal of Invertebrate Pathology* **77**, 173-179

Cunningham, J. P.; West, S. A. and Wright, D. J., 1998. Learning in nectar foraging behaviour of *Helicoverpa armigera*. *Ecological Entomology* **23**, 363-369

Cunningham, J. P.; Zalucki, M. P. and West, S. A., 1999. Learning in *Helicoverpa armigera* (Lepidoptera: Noctuidae): A new look at the behaviour and control of polyphagous pest. *Bulletin of Entomological Research* **89**, 201-207

Dang, H. T. and Gunning, R., 2002. Evidence of the shift in susceptibility to *Bacillus thuringiensis* delta-endotoxin Cry IAc in Australian *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Resistant Pest Management Newsletter* **11**: 2 Available: *http://whalonlab.msu.edu/rpmnews/vol.11-no2/rpm_research.htm.* 12.2002

De La Rosa, W.; Lopez, F. L. and Liedo, P., 2002. *Beauveria bassiana* as a pathogen of the Mexicanfruit fly (Diptera: Tephritidae) under laboratory conditions. *J. Econ. Entomol.* **95**: 1, 36-43

Dillon, R. J. and Charnley, A. K., 1990. Initiation of germination in conidia of the entomopathogenic fungus, *Metarhizium anisopliae. Mycological Research* **94**: 3, 299-304

Dimbi, S.; Maniania, N. K.; Lux, S. A and Mueke, J. M., 2004. Effect of constant temperature on germination, radial growth and virulence of *Metarhizium anisopliae* to three species of African tephritid fruit flies. *Biocontrol* **49**, 83-94

Dulmage, H. T. and co-operators, 1981. Insecticidal activity of isolates of *Bacillus thuringiensis* and their potential for pest control. In *Microbial control of pests and diseases 1970-1980*. Ed. Burges, H. D., Academic Press, 193-223

Ekesi, S.; Maniania, K. and Ampong-Nyarko, K., 1999. Effect of temperature on germination, radial growth and virulence of *Metarhizium anisopliae* and *Beauveria bassiana* on *Megalurothips sjostedti. Biocontrol Science and Technology* **9**, 177-185

Falcol, L. A., 1971. Use of bacteria for microbial control. In *Microbial control of insects and mites*. Ed. Burges, H. D. and Hussey, N. M. Academic Press London-New York, 67-90

Fast, P. G., 1981. The crystal toxin of *Bacillus thuringiensis*. In *Microbial control of pests and diseases 1970-1980*. Ed. Burges, H. D., Academic Press, 223-248

Feng, M. G.; Poprawski, T. J. and Khachatourians, G. G., 1994. Production, formulation and application of the entomopathogenic fungus *Beauveria bassiana* for insect control: current status. *Biocontrol Science and Technology* **4**, 3-34

Fernald, H. T. and Shepard, H. H., 1955. Applied Entomology. Fifth edition. Mac Grow-Hill Book Company, INC, 255-257

Fitt, G. P., 1989. The ecology of *Heliothis* species in relation to agro-ecosystem. *Ann. Rev. Entomol.* **34**, 53-57

Fitt, G. P.; Mares, C. L. and Llewellyn, D. J., 1994. Field evaluation and potential impact of transgenic cottons (*Gossypium hirsutum*) in Australia. *Biocontrol Science and Technology* **4**, 535-548

Gaugler, R. S. D.; Costa, S. D. and Lashom, J., 1989. Stability and efficacy of *Beauveria bassiana* soil inoculations. *Environ. Entomol.* **18**, 412-417

Gillespie, A. T., 1988. Use of fungi to control pests of agricultural importance. In *Fungi in biological control system*. Ed. Burge, M. N., Manchester University Press, 37-60

Glare, T. R. and O'Callaghan, M., 2000. *Bacillus thuringiensis*: Biology, ecology and safety. John Wiley and Sons, Ltd.

Goettel, M. S. and Inglis, G. D., 1997. Fungi: Hyphomycetes. In *Biological Techniques. Manual of Techniques in Insect Pathology*. Ed. Lacey, L. A., Academic Press, 213-247

Gopalakrishnan, C. and Narayanan, K., 1990. Studies on the dose-mortality relatioship between the entomopathogenic pathogen *Beauveria bassiana* (Bals.) Vuillemin and *Heliothis armigera* Hübner (Lepidoptera: Noctuidae). *Journal of Biological Control* **4**: 2, 112-115

Greathead, D.J., 1994. Biological Control. In *Insect Pest of Cotton*. Ed. Mathews, G.A. and Tunstall, J.P., CAB International, 463- 476

Guilin, C.; Runxi, L.; Xuelin, D.; Mingjiang, H. and Mingxun, D., 2002. Study on the gossypol sterilant to control resistant cotton bollworm (*Helicoverpa armigera*). *Resistant Pest Management Newsletter* **12**: 1, 22-24

Gujar, G. T.; Kumari, A.; Kalia, V. and Chandrashekar, K., 2000. Spatial and temporal variation in susceptibility of the American bollworm, *Helicoverpa armigera* (Hübner) to *Bacillus thuringiensis* var. *kurstaki* in India. *Current Science* **78**:8, 995-1000

Gunning, R. V.; Moores, G. D. and Denoshire, A. L., 1998. Insensitive Acetycholinesterase causes resistance to Organophosphates in Australia *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Pest. Sci.* **54**, 319-320

Hajek, A. E. and Leger, R. J. St., 1994. Interactions between fungal pathogens and insect hosts. *Annu. Rev.Entomol.* **39**, 293-322

Hallsworth, J. E. and Magan, N., 1999. Water and temperature relations of growth of the entomogenous fungi *Beauveria bassiana, Metarhizium anisopliae* and *Paecilomyces farinosus. Journal of Invertebrate Pathology* **74**, 261-266

Han, Z.; Wang, Y.; Zhang, Q.; Li, X. and Li, G., 1999. Dynamics of Pyrethroid resistance in the field population of *Helicoverpa armigera* (Hübner) in China. *Pesticide Science* **55**, 462-466

Hassani, M., 2000. Development and proving of biocontrol methods based on *Bt* and entomopathogenic fungi against the cotton pests *Spodoptera lituralis*, *Helicoverpa armigera* (Lepidoptera: Noctuidae) and *Aphids gossypii* (Homoptera: Aphididae). PhD thesis

Hem, S. and Ahmad, R., 1997. Field evaluation of *Beauveria bassiana* (Balsamo) Vuillemin against *Helicoverpa armigera* (Hübner) infecting chickpea. *Journal of Biological Control* **11**: 1-2, 93-96

Holdom, D. G. and Klashorst, G. V. D, 1986. Sporulation by hyphal bodies of *Nomurae rileyi* and subsequent infection of *Heliothis* spp. *Journal of Invertebrate Pathology* **48**, 242-245

Höfte, H. and Whiteley, H. R., 1989. Insecticidal crystal proteins of *Bacillus thuringiensis. Microbiol. Rev.* **53**, 242-255

Hua, F.; Liang, F.; Qin, F.; Yan, W.; Jun, H.; Fan, X.; Feng, S.; Fu, Y.; Wang, R. and Hu, M., 1999. Field studies on the occurrence and epidemiology of entomopathogenic diseases infecting *Helicoverpa armigera* in Hebei. *Chinese Journal of Biological Control* **15**:1, 27-30 (abstract)

Humber, R. A., 1997. Fungi: identification. In *Manual of techniques in insect pathology*. Ed. Lacey, L. A., Academic Press, 153-185

Hywel-Jones, N. L. and Gillespie, A. T., 1990. Effect of temperature on spore germination in *Metarhizium anisopliae* and *Beauveria bassiana. Mycological Research* **94**: 3, 389-392

Inch, J. M. M.; Humphreys, A. M.; Trinci, A. P. J. and Gillespie, A. T., 1986. Growth and blastospores formation by *Paecilomyces fumosoroseus,* a pathogen of brown plant hopper (*Nilaparvata lugens*). *Transactions of the British Mycological Society* **87**: 2, 215-222

Inglis, G. D.; Goettel, M. S.; Butt, T. M. and Strasser, H., 2001. Use of *Hyphomycetous* fungi for managing insect pests. In *Fungi as biocontrol agent progress, problems and potential.* Ed. Butt, T. M.; Jackson, C. and Magan, N., CABI publishing, 23-60

International programme on chemical safety (IPCS), 1999. Environmental health criteria 217. *Bacillus thuringiensis.* World health organization

Jalali, S. K.; Mohan, K. S.; Singh, S. P.; Manjunath, T. M. and Lalitha, Y., 2004. Baseline-susceptibility of the old-world bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) populations from India to *Bacillus thuringiensis* Cry1Ac insecticidal protein. *Crop Protection* **23**, 53-57

James, R. R., 2003. Combining Azadirachtin and *Paecylomyces fumosoroseus* (Deuteromycotina: Hyphomycetes) to control *Bemisia argentifolii* (Homoptera: Aleyrodidae). *J. Econ. Entomol.* **96**:1, 25-30

Jayanthi, P. D. K. and Padmavathamma, K., 1996. Cross infectivity and safety of nuclear polyhedresis virus, *Bacillus thurnigiensis* subsp. *kurstaki* Berliner and *Beauveria bassiana* (Balsamo) Vuill. to pests of groundnut (*Arachis hypogaea* Linn.) and their natural enemies. *Journal of Entomological Research* **20**: 3, 211-215

Jayaraj, S.; Rabindra, R. J. and Narayanan, K., 1989. Development and use of microbial agents for control of *Heliothis* spp. (Lepidoptera: Noctuidae) in India. In *Proceedings of the workshop on biological control of Heliothis: Increasing the effectiveness of natural enemies.* New Delhi, India. 483-503

Kassa, A.; Zimmerman, G.; Stephan D. and Vidal S., 2002. Susceptibility of *Sitophilus Zea Mais* (Motsh.) (Coleoptera: Curculionidae) and *Prosterphanus truncatus* (Horn.) (Coleoptera: Bostrichidae) to entomopathogenic fungi from Ethiopia. *Biocontrol Science and Technology* **12**, 727-736

King, A. B. S. and Coleman, R. J., 1989. Potential for biological control of *Heliothis* species. *Ann. Rev. Entomol.* **34**, 53-75

King, A.B.S., 1994. Heliothis/Helicoverpa (Lepidoptera: Noctuidae). In *Insect pest of cotton.* Ed. Matthews, G. A. and Tunstall, J. P. CAB International, 21-75

Kleespies, R. G. and Zimmermann, G., 1994. Viability and virulence of blastospores of *Metarhizium anisopliae* (Metch.) Sorokin after storage in various liquids at different temperatures. *Biocontrol Science and Technology* **4**, 309-319

Koppenhöfer, A. M., Grewal, P. S. and Kaya, H. K. 2000. Synergism of imidacloprid and entomopathogenic nematodes against white grubs: the mechanism. *Entomologia Experimentalis et Applicata* **94**, 283-293

Kranthi, K. R.; Armes, N. J.; Rao, N. G. V.; Raj, S. and Sundaramurthy, V. T., 1997. Seasonal dynamics of metabolic mechanisms mediating Pyrethroid resistance in *Helicoverpa armigera* in central India. *Pest. Sci.* **50**, 91-98

Kumar, H. and Kumar, V., 2004. Tomato expressing Cry 1Ab insecticidal protein from *Bacillus thuringiensis* protected against tomato fruit borer, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) damage in the laboratory, green house and field. *Crop Protection* **23**, 135-139

Lane, B. S.; Trinci, A. P. J. and Gillespie, A. T., 1991a. Endogenous reverses and survival of blastospores of *Beauveria bassiana* harvested from carbon- and nitrogen-limited batch culture. *Mycological Research* **95**, 821-828

Lane, B. S.; Trinci, A. P. J. and Gillespie, A. T., 1991b. Influence of cultural conditions on the virulence of conidia and blastospores of *Beauveria bassiana* to green leafhopper, *Nephotettix virescens*. *Mycological Research* **95**, 829-833

Lenin, K.; Mariam, M. A. and Udayasuriyan, V., 2001. Expression of a cry2Aa gene in an acrystalliferous *Bacillus thuringiensis* strain and toxicity of Cry2Aa against *Helicoverpa armigera*. *World journal of Microbiology and Biotechnology* **17**, 273-278

Lezama-Gutierrez, R.; Trujillo-De La Luz, A.; Monila-Ochoa, J.; Rebolledo-Dominguez, O.; Pescador, A. R.; Lopez-Edwards, M. and Aluja, M., 2000. Virulence of Metarhizium anisopliae (Deuteromycotina: Hyphomycetes) on *Anastrepha ludens* (Diptera: Tephritidae): Laboratory and field trials. *J. Econ. Entomol.* **93**: 4, 1080-1084

Liang, S. J.; Jun, Z.; Dong, W.; Wen, L. and Fei, Z., 1998. Early resistance of *Helicoverpa armigera* (Hübner) to *Bt* and its relation to the effect of transgenics cotton lines expressing *Bt* toxin on the insect. *Acta-entomologica-Sinica* **41**: 1, 8-14

Liang, G., Tan, W. and Guo, Y., 2000. Study on screening and inheritance mode of resistance to *Bt* transgenic cotton in *H. armigera*. *Acta Entomol. Sin.* **43**, 57-62

Liao, C.; Heckel, D. G. and Akhurst, R., 2002. Toxicity of *Bacillus thuringiensis* insecticidal proteins for *Helicoverpa armigera* and *Helicoverpa punctigera* (Lepidoptera: Noctuidae), major pests of cotton. *Journal of Invertebrate Pathology* **80**, 55-63

Mann, L., 2000. Tomatoes topics-news and information for the processing tomato industry. ISSN 1038-3522 **9**: 1
Available: *http://www.aptrc.asn.au/tomato topics March 2000.pdf*.12.02

Manjula, K. and Padmavathammia, K., 1999. Effect of insect pathogens on larvae of *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae). *Entomol.* **24**: 1, 71-74

Marrone, P. G. and Macintosh, S. C., 1993. Resistance to *Bacillus thuringiensis* and resistance management. In *Bacillus thuringiensis, an environmental biopesticide: Theory and practice*. Ed. Entwistle, P. F.; Cory, J. S. Bailey, M. J. and Higgs, S., John Wiley and Sons, 221-233

Martin, T.; Ochou, G. O.; Hala–N'Klo, F.; Wassal, J. and Waissayre, M., 2000. Pyrethroid resistance in cotton bollworm *Helicoverpa armigera* in West Africa. *Pest Management Science* ***56***, 549-554

McCammon, S. A. and Rath, A. C., 1994. Separation of *Metarhizium anisopliae* strains by temperature dependent germination rate. *Mycological Research* **98**: 11, 1253-1257

McGuire, M. R.; Galan-Wong, L. J. and Tamez-Guerra, P., 1997. Bacteria: Bioassay of *Bacillus thuringiensis* against Lepidoptera larvae. In *Manual of techniques in insect pathology*. Ed. Lacey, L.,A., Academic Press, 91-99

Morris, O. N.; Converse, V.; Kanagaratnam, P. and Davies, J. S., 1996. Effect of cultural conditions on spore-crystal yield and toxicity of *Bacillus thuringiensis* subsp. *aizawai* (HD 133). *Journal of Invertebrate Pathology* **67**, 129-163

Nester, E. W.; Thomashorw, L. S.; Metz, M. and Gorden, M., 2002. 100 Years of *Bacillus thuringiensis*: A critical scientific assessment
Available: *http://www.asmusa.org*. 12.02

Nurindah, A. A. A. G. and Indrayani, I. G. A. A., 1989. New records of natural enemies of *Heliothis armigera* on cotton in Indonesia. *Industrial Crops Research Journal* **2**: 1, 1-5

Ochou, G. O. and Martin, T., 2002. Pyrethroid resistance in *Helicoverpa armigera* (Hübner): Recent developments and prospects for its management in Cote d'Ivoire, West Africa. *Resistant Pest Management Newsletter* **12**: 1, 10-16

Olsen, K. M. and Daly, J. C., 2000. Plant-toxin interaction in transgenic *Bt* cotton and their effect on mortality of *Helicoverpa armigera* (Lepidoptera: Noctuidae). *J. Econ. Entomol.* **93**: 4, 1293-1299

Padidam, M., 1992. The insecticidal crystal protein CryIA(c) from *Bacillus thuringiensis* is highly toxic for *Heliothis armigera*. *Journal of Invertebrate Pathology* **59**, 109-111

Pingel, R. L. and Lewis, L. C., 1999. Effect of *Bacillus thuringiensis*, *Anagrapha falcifera* multiple nucleopolyhedrovirus and their mixture on three lepidopteran corn ear pests. *J. Econ. Entomol.* **92**: 1, 91-96

Poole, R. W., 1989. A general synopsis of the systematics of *Heliothis* and *Helicoverpa*. In *Proceedings of the Workshop on biological control of Heliothis: Increasing the effectiveness of natural enemies*. New Delhi, India, 161-171

Poston, F. L.; Pedigo, L. P. and Welch, S. M., 1983. Economic injury levels: Reality and practicality. *Bull. Entomol. Soc. Am.* **29**, 49-53

Quinlan, R. J., 1988. Use of fungi to control insect in glasshouse. In *Fungi in biological control System*. Ed. Burge, M. N., Manchester University Press, 19-37

Ranashinghe, C.; Campbel, B. and Hobbs, A., 1988. Over-expression of Cytochrome P450 CYP 6B7 mRNA and Pyrethroid resistance in Australian population of *Helicoverpa armigera* (Hübner). *Pestici. Sci.* **54,** 195-202

Regupathy, A.; Manoharan, T.; Asokan, G.; Soundaranrajan, R. P. and Armes, N. J., 1997. Synergistc suppression of Fenvarelate resistance in field population of *Helicoverpa armigera* in Tamil Nadu. *Resistant Pest Management* **9**:1, 37-39

Romeis, J. and Shanower, T. G., 1996. Arthropod natural enemy of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) in India. *Biocontrol Science and Technology* **6**, 481-508

Roush, R. T., 1997. *Bt*-transgenics crops: Just another pretty insecticide a chance for a new start in resistance management? *Pestic. Sci.* **51,** 328-334

Samson, R. A., 1981. Identification of entomopathogenic Deuteromycetes. In *Microbial control of pests and diseases 1970-1980*. Ed. Burges, H. D., Academic Press, 93-107

Sandhu, S. S.; Unkles, S. E.; Rajak, R. C. and Kinghorn, J. R., 2001. Generation of Benomyl resistant *Beauveria bassiana* strains and their infectivity against *Helicoverpa armigera*. *Biocontrol Science and Technology* **11**, 245-250

SAS Institute, 1989. SAS/STAT user's guide. Version 6, fourth edition, Vols.1&2. SAA Institute INC. Cary, NC, USA

Schnepf, E.; Crickmore, N.; Van Rie, J.; Lereclus, D.; Baum, J.; Feitelson, J.; Zeigler, D. R. and Dean, D. H., 1998. *Bacillus thuringiensis* and its pesticidal crystal proteins. *Micobiology and Molecular Biology Review* **62**: 3, 775-806

Sebesta, K.; Farkas, J.; Horska, K. and Vankova, J., 1981. Thurigiensin, the Beta-exotoxin of *Bacillus thuringiensis*. In *Microbial control of pests and diseases 1970-1980*. Ed. Burges, H. D., Academic Press, 249-283

Shapiro-Ilan, D. I.; Jackson, M.; Reilly, C. C. and Hotchkiss, M. V., 2004. Effect of combining an entomopathogenic fungi or bacterium with entomopathogenic nematodes on mortality of *Curculio carye* (Coleoptera: Curculionidae). *Biological Control* **30**, 119-126

Shen, J.; Zhou, W.; Wu, Y. and Lin, X., 1998. Early resistance of Helicoverpa armigera to *Bacillus thuringiensis* and its relation to the effect of transgenic cotton lines expressing *Bt* toxin on the insect. *Acta Entomol. Sin.* **41**, 8-14

Storey, G. K. and Gardner, W. A., 1989. Movement of aqueous spray of *Beauveria bassiana* into the profile on four Georgia soils. *Environ. Emtomol.* **18**, 835-839

St Leger, R. T., 1993. Biology and mechanism of insect-cuticle invasion by deuteromycete fungal pathogens. In *Parasites and Pathogens of insects, Vol. 2: Pathogens*. Ed. Beckage, N. E., Thompson, S. N.; Fedrici, B. A., Academic press, San Diego, CA, 211-229

Sun, J.; Tang, C.; Zhu, X.; Guo, W.; Zhang, T.; Zhou, W; Meng, F. and Sheng, J., 2002. Characterization of resistance to *Helicoverpa armigera* in three lines of transgenic *Bt* upland cotton. *Euphytica* **123**, 343-351

Sun, L. J.; Wu, K. M. and Guo, Y. Y., 2001. The pathogenicity of *Beauveria bassiana* to *Helicoverpa armigera* under different temperatures and humidities. *Acta-entomologica-Sinica* **44**: 4, 501-506

Tabashnik, B. E., 1994. Evolution of resistance to *Bacillus thuringiensis*. *Annu. Rev. Entomol.* **39**, 47-74

Tang, L. and Hou, R. F., 1998. Potential application of the entomopathogenic fungus, *Nomuraea rileyi*, for control of the corn earworm, *Helicoverpa armigera*. *Entomologia Experimentalis et Applicata* **88**, 25-30

Tapperser B., 1998. The difference between conventional *Bacillus thuringiensis* and transgenic insect resistant plants. Possible reasons for rapid resistance development and susceptibility of non-target organisms. Record in the third meeting of the open-ended working group in biosafety. 13-17/10/1998. Montreal, Canada

Thomas, M. B.; Watson, E. L. and Valverde-Garcia, P., 2003. Mixed infections and insect-pathogen interactions. *Ecology Letters* **6**, 183–188

Torres-villa, L. M.; Rodrguez-Molina, M. C.; Lacasa-Plasencia, A. and Bielza-Lino, P., 2002. Insecticide resistance of *Helicoverpa armigera* to endosulfan, carbamate and organophosphates: The Spanish case. *Crop Protection* **21**:10, 1003-1013

Van Den Berg, H. and Cock, M. J. W., 1995. Natural control of *Helicoverpa armigera* in cotton. Assessment of the role of predation. *Biocontrol Science and Technology* **5**, 453-463

Vänninem, I.; Tyni-Juslin, J. and Kokkanen, H., 2000. Persistence of augmented *Metarhizium anisopliae* and *Beauveria bassiana* in Finnish agricultural soils. *BioControl* **45**, 201-222

Vey, A.; Hoagland, R. E. and Butt, T. M., 2001. Toxic metabolites of fungal biocontrol agents. In *Fungi as biocontrol agent progress, problems and potential.* Ed. Butt, T. M.; Jackson, C. and Magan, N., CABI publishing, 311-346

Vidal, C.; Fargues, J. and Lacey L. A., 1997. Intraspecific variability of *Paecilomyces fumosoroseus*: Effect of temperature on vegetative growth. *Journal of Invertebrate Pathology* **70**,18-26

Wraight, S. and Ramos, M., 2001. Control of Colorado potato beetle with foliar application of *Beauveria bassiana* alone and in combination with *Bacillus thuringiensis*. Available: *http://www.nps.ars.isda.gov/publications/publications.htm.* 4.2003

Wu, K.; Gou, Y. and Gao, S., 2002. Evaluation of the natural refuge function for *Helicoverpa armigera* (Lepidoptera: Noctuidae) within *Bacillus thuringiensis* transgenic cotton growing areas in North China. *J. Econ. Entomol.* **96**: 4, 832-837

Zalucski, M. P., 1986. The biology and ecology of *Heliothis armigera* (Hübner) and *H. punctigera* (Wallengren) (Lepidoptera: Noctuidae) in Australia: What do we know? *Australian Journal of Zoology* **42**, 329-34

Zhao, J. Z.; Lu, M.; Fan, X. and Wei, G., 2002. Resistant monitoring of *Helicoverpa armigera* to *Bacillus thuringiensis* in North China.
Available: *http://www. Msstate.edu/entomology/v8n2/art 05.html.* 12.2002

Zimmermann, G., 1994. Strategies for utilization of entomopathogenic fungi. In *Proceedings, VIth International colloquium on invertebrate pathology and microbial control.* Society for invertebrate pathology, Montpellier, France, 67-73