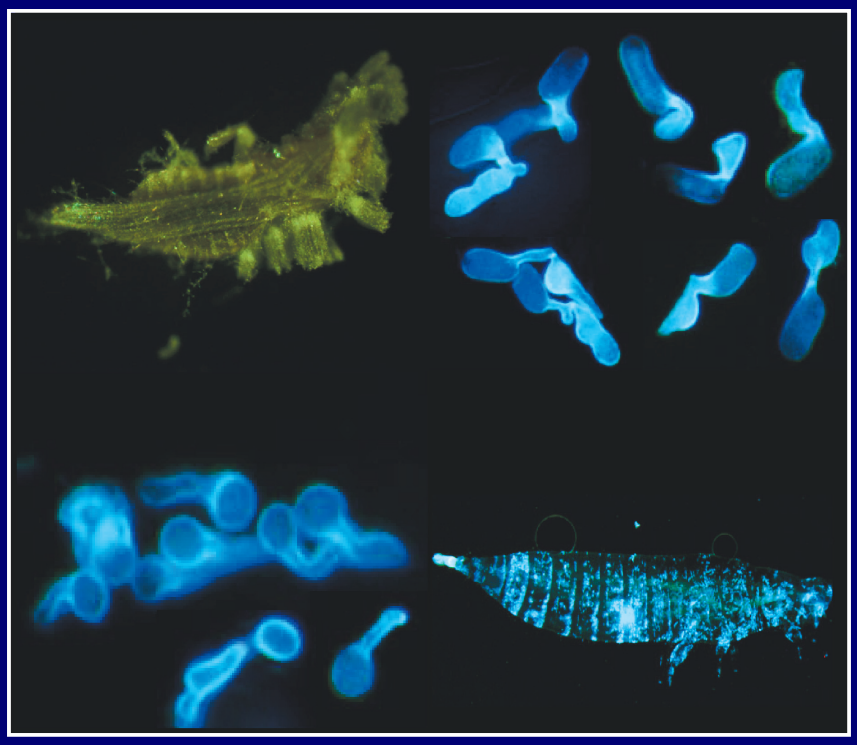
Efficiency of different entomopathogenic fungi isolates from Thailand as biological control agents against *Frankliniella occidentalis* (PERGANDE) and *Thrips tabaci* LINDEMAN (Thysanoptera: Thripidae)



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Efficiency of different entomopathogenic fungi isolates from Thailand as biological control agents against *Frankliniella occidentalis* (PERGANDE) and *Thrips tabaci* LINDEMAN (Thysanoptera: Thripidae)

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Dedication

To

My beloved parents and
all people who supported me throughout my lifetime

Efficiency of different entomopathogenic fungi isolates from Thailand as biological control agents against *Frankliniella occidentalis* (PERGANDE) and *Thrips tabaci* LINDEMAN (Thysanoptera: Thripidae)

Abstract

The present research aimed to study efficiency of 41 isolates of entomopathogenic fungi, belonging to 25 species from 11 genera isolated from different hosts in Thailand, as biological control agents against *Frankliniella occidentalis* (PERGANDE) and *Thrips tabaci* LINDEMAN (Thys., Thripidae). Firstly, the efficiency isolates was screened in the laboratory for the following characteristics: pathogenicity, virulence degree and effectiveness. In further experiments, the isolates that displayed highly efficient were subjected to studies biological and ecological characteristics, mode of infection, their efficiency under abiotic and biotic factors as well as side effects on non-target arthropods. Finally, persistence on different host plants and efficiency for controlling thrips under greenhouse conditions were investigated.

In the laboratory, the results showed that 37 isolates for *F. occidentalis* and 36 isolates for *T. tabaci* were found to be pathogenic. The 16 isolates were highly pathogenic. Fungi isolates varied significantly in term of virulence. Five isolates were superior to all other isolates according to the virulence degree. The efficiency against all stages of both thrips species showed that the susceptibility decreased from larvae over pupae to adults. *Beauveria bassiana* Bb.5335 and *Metarhizium anisopliae* Ma.7965 displayed the highest efficacy throughout the following series of screenings and were used for further experimentation. Biological characteristics of *B. bassiana* and *M. anisopliae* were dependent on environmental factors but they did not greatly affect. The process involving pathogenicity on thrips began from 12 h and was completed within 72 h after inoculation. However, temperature and life stage of thrips were found to have effects on the development of pathogenicity process. The efficacy of the two fungi was higher at 25 and 30°C than at 18 and 35°C. Those increased with increasing relative humidity and varied according to the host plant whether on crop or ornamental plants. Both thrips species reared on cucumber were highly susceptible to fungal infection but significantly less susceptible when reared on saintpaulia. *B. bassiana* and *M. anisopliae* had low side effects on non-target beneficial arthropods.

Under greenhouse conditions, it was found that *B. bassiana* and *M. anisopliae* conidia were able to persist at least 30 days on leaf/flower surface and still had efficiency to infect thrips, but dependent on host plant. The two fungi isolates have been used successfully for controlling *F. occidentalis* on cucumber in greenhouse.

Wirksamkeit von unterschiedlichen entomopathogenen Pilzisolaten aus Thailand als biologische Kontrollagenten gegen *Frankliniella occidentalis* (PERGANDE) und *Thrips tabaci* LINDEMAN (Thysanoptera: Thripidae)

Kurzfassung

Das Ziel der vorliegenden Arbeit war, die Eignung von 41 aus unterschiedlichen Wirten isolierten, entomopathogenen Pilzen aus 25 Arten von 11 Genera, als biologische Kontrollagenten gegen *Frankliniella occidentalis* (PERGANDE) und *Thrips tabaci* LINDEMAN (Thys., Thripidae) zu untersuchen. Zunächst wurden hierzu die Pathogenität, Virulenzgrad und Wirksamkeit der erfolgreichsten Isolate im Labor ermittelt. In anschließenden Experimenten wurden die wirksamsten Isolate Untersuchungen zu ihren biologischen und ökologischen Eigenschaften, der Infektionsverläufe, ihrer Wirksamkeit unter unterschiedlichen abiotischen und biotischen Faktoren sowie ihrer Nebenwirkungen auf Nutzarthropoden unterzogen. Abschließend erfolgten einige Versuche zur Persistenz an unterschiedlichen Wirtspflanzen und zur Eignung für die Bekämpfung von Thripsen unter Gewächshausbedingungen.

Die Laboruntersuchungen zeigten, dass von den 41 Isolaten 37 Isolate eine Pathogenität gegenüber *F. occidentalis* und 36 Isolate gegenüber *T. tabaci* aufwiesen. Von diesen Isolaten waren 16 hochpathogen, wobei die Pilzisolate signifikante Unterschiede in ihrer Virulenz zeigten. Fünf Isolate besaßen gegenüber allen anderen Isolaten den höchsten Virulenzgrad. Die Wirksamkeit gegenüber den unterschiedlichen Entwicklungsstadien beider Thripsarten erhöhte sich von den Larven über die Puppen zu den Adulten. Die Isolate *Beauveria bassiana* Bb.5335 und *Metarhizium anisopliae* Ma.7965 zeigten die höchste Wirksamkeit in den Untersuchungen und wurden für die weiteren Experimente verwendet. Die biologischen Charakteristika von *B. bassiana* und *M. anisopliae* waren von den klimatischen Faktoren abhängig, wurden jedoch nicht stark beeinflusst. Der Infektionsverlauf an den Thripsen begann nach 12 h und war 72 h nach der Inokulation vollendet. Jedoch übten die Temperaturen und die Entwicklungsstadien der Thripse, einen Einfluss auf die Pathogenität aus. Die Wirksamkeit der beiden Pilzisolate war bei 25 und 30°C höher als bei 18 und 35°C. Diese erhöhte sich mit zunehmender relativer Luftfeuchtigkeit und variierte zwischen den unterschiedlichen Kultur- und Zierpflanzen. Beide Thripsarten reagierten wesentlich empfindlicher auf Pilzinfektionen, wenn sie auf Gurkenpflanzen gezüchtet waren als solche, die auf Saintpaulia-Pflanzen gezüchtet wurden. *B. bassiana* und *M. anisopliae* hatten nur einen geringen Nebeneffekt auf Nutzarthropoden.

Unter Gewächshausbedingungen konnten die Konidien von *B. bassiana* und *M. anisopliae* mindesten 30 Tage auf der Blatt-/Blütenoberfläche persistent bleiben und in Abhängigkeit von der Wirtspflanze die Thripse infizieren. Die beiden Pilzisolate konnten erfolgreich zur Bekämpfung von *F. occidentalis* an Gurken im Gewächshaus verwendet werden.

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1 INTRODUCTION

The western flower thrips, *Frankliniella occidentalis* (PERGANDE) and the onion thrips, *Thrips tabaci* LINDEMAN (Thys., Thripidae) are a major serious pests of a wide range of field and greenhouse crops around the world (KARNKOWSKI and TRDAN 2002). They cause damage directly through feeding and indirectly through the transmission of lethal plant viruses (MARCHOUX et al. 1991, DEANGELIS et al. 1994). It is difficult to control these pests with insecticides because of their small sizes and cryptic habits (LEWIS 1997). Moreover, these two thrips species have long histories of developing resistances to chemical insecticides and the resistance problem is one of the main reasons for these insects (ZHAO et al. 1994, KONTSEDALOV et al. 1998). In addition, insecticides have harmful effects on natural enemies (NEMOTO 1995). This has led to increment in the efforts to search for and develop biological control methods that are species-specific and efficient.

Entomopathogens may be a good source. Many entomopathogens are important in causing natural insect mortality. They are currently being investigated for control of many important insect pests on various crops around the world, and some are commercially available. In theory, the use of entomopathogenic fungi could be much more effective than other entomopathogens, because they are able to infect thrips that are plant sucking insects through penetration of the cuticle (TANDA and KAYA 1993). The available data have revealed that at least 7 species of entomopathogenic fungi were isolated from infected *F. occidentalis* and *T. tabaci* or known to be able infect these two pests, such as *Verticillium lecanii* (ZIMMERMANN) VIÉGAS (RAVENSBERG et al. 1990), *Conidiobolus coronatus* (COSTANTIN) BATKO (HUMBER 1992), *Neozygites parvaisporo* (MACLEOD, TYRRELL & CARL) REMAUDIERE & KELLER (MAGANO DI SAN et al. 1992), *Beauveria bassiana* (BALSAMO) VUILLEMIN, *Metarhizium anisopliae* (METSCH) SOROKIN (BROWNBRIDGE et al. 1994), *Paecilomyces farinosus* (HOLM ex S.F. GRAY) BROWN & SMITH, *Paecilomyces fumosoroseus* (WIZE) BROWN & SMITH (BROWNBRIDGE 1995). Meanwhile, the fungi belonging to same species may show difference in efficiency against the same insect host.

The improvement of entomopathogenic fungi as biological control agents needs several selection criteria. Firstly, the selection of high efficiency isolates, many researchers noted that the successful in the use of entomopathogenic fungi as biological control agents largely depend on the selection of high efficiency isolates. VESTERGAARD et al. (1995) reported that among 5

isolates of *M. anisopliae* and 5 isolates of *V. lecanii*, *M. anisopliae* isolate 275 was the most virulent isolate against *F. occidentalis*. Additionally, among 711 isolates of entomopathogenic fungi, only *Metarhizium* FRM515 was the most virulent isolate against *Plautia stali* SCOTT (Het., Pentatomidae) (IHARA et al. 2001). Secondly, understanding which stage is the most susceptible to fungal infection is important for the development of management tactics. Because crops are often infested with several different stages of the pest simultaneously, the potential use of an entomopathogenic fungus is greatly influenced by its ability to initiate infection on the thrips' specific stage and the susceptibility of insect's various life stages to the fungi. VESTERGAARD et al. (1995) reported that adult stage of *F. occidentalis* was the most susceptible to infection by *M. anisopliae*. While, the susceptibility of greenhouse whiteflies *Trialeurodes vaporariorum* (WESTWOOD), (Hom., Aleyrodidae) to *Aschersonia aleyrodis* WEBBER decreases with age; the older instars were less susceptible and the fungi seldom infected adults (FRANSEN et al. 1987). Thirdly, the biological and ecological characteristics of a fungus could play an important role in determining efficacy of entomopathogenic fungi. Abiotic factors, (such as temperature, relative humidity and ultraviolet radiation) have been identified as a significant factor affects the fungal physiology with respect to germination, growth, sporulation and survival. Moreover, those factors influence the host's susceptibility and resistance to the fungus and the progress of the infection within the host pest (OUEDRAOGO et al. 1997, THOMAS and JENKINS 1997). HELEN et al. (2003) stated that conidial germination and colony growth of *B. bassiana*, *M. anisopliae*, *P. fumosoroseus* and *V. lecanii* was slower at 10 and 15°C than at 20 and 25°C. Similarly, those were more pathogenic against *Aphis fabae* SCHPOLI and *Myzus perisicae* SULZER (Hom., Aphididae) at 20 and 25°C than 10 and 15°C. Optimal temperatures for growth, sporulation and infection of entomopathogenic fungi often range between 20-30◦C, but variation among geographic origin and isolate also can be significant (VIDAL et al. 1997). Thus, for successful development as biological control agents, the entomopathogenic fungi must be adapted to the environmental conditions in the area where they are to be employed (MCCOY 1990). Fourthly, tritrophic interactions among entomopathogen, insect host and host plant must be closely considered in the evaluation, as each element is known to affect many of the interactions involved in biological control. Many plants produce secondary compounds or allelochemicals, which may have antimicrobial activity against entomopathogens, such as catechol, salicyclic acid or tannic acid were found to be inhibited *P. fumosoroseus* (VEGA et al. 1997). The host plant of phytophagous insects can significantly affect their susceptibility to pathogenicity, through either

dietary stress or direct antimicrobial activity of the plant. (TANADA and KAYA 1993). Likewise, the effect of host plants on growth of *B. bassiana*, *M. anisopliae*, *Nomuraea rileyi* (FARLOW) SAMSON, *P. fumosoroseus* and *Paecilomyces lilacinus* (THOM) SAMSON has been documented (GALLARDO et al. 1990, GUIRARD et al. 1995, INYANG et al. 1999). Insects sequestering high concentrations of allelochemicals may be protected from certain pathogens (LACEY and MERCADIER 1998; VAGA et al. 1997). *Bemisia argentifolii* (BELLOWS and PERRING) (Hom., Aleyrodidae) reared on cotton were consistently significantly less susceptible to infection by *B. bassiana* and *P. fumosoroseus* than reared on melon (POPRAWSKI and WALKER 2000). Fifthly, knowledge on infection process of entomopathogenic fungi will provide a rational basis for selection of virulent isolates, improvement and explain fungal virulence or host resistance as well as may help in the production of more efficient biological control agent. ALTRE et al. (1999) reported that the infection process among isolates of *P. fumosoroseus* differed in their ability to infect *Plutella xylostella* L. (Lep., Plutellidae), avirulent isolate showed poorly attach, germinated slowly and reduced ability to penetrated on *P. xylostella* larval cuticle. Fast conidia germination has been shown to increase pathogenicity to insect (FARGUES et al. 1994). Sixthly, the safety of entomopathogenic fungi to non-target organisms is clearly an important criterion for consideration. The success of fungal entomopathogens as biological control agents depends not only on high efficiency against agricultural pests, but also on low side effects on arthropod natural enemies. Eleven isolates of *M. anisopliae* and one isolate of *B. bassiana* that development to control of grasshoppers and locusts, had no negative effect to non-target *Pimelia senegalensis* (OLIVIER) and *Trachyderma hispida* (FORSKÅL) (Col., Tenebrionidae) (DANFA and VAN der VALK 1999). Moreover, TOUNOU et al. (2003) revealed that *M. anisopliae* strain Ma.43 and *P. fumosoroseus* strain Pfr.12 caused 97 and 100% mortality on *Empoasca decipiens* PAOLI (Hom., Cicadellidae), while had no influence on egg parasitoid, *Anagrus atomus* L. (Hym., Mymaridae). Seventhly, the ability of an entomopathogenic fungus to persist in the habitat of its host is vital for its effectiveness. A major difficulty in development of entomopathogenic fungi as biological control agents is their relatively short persistence on leaf surfaces. Host and conidia may meet in two different ways, direct contacted, when conidia were sprayed upon the insect host or indirect contacted when hatching or moulting settle on conidia presented on leaf surface from earlier treatment. Hence, long persistence of conidia on leaf surfaces was important for effective control of insects. Conidia of *A. aleyrodis* could survive on leaf surfaces of cucumber for at least 3 weeks and were

still able to infect nymphs of greenhouse whitefly and also *M. anisopliae* was able to persist at least for 2 weeks on onion crops in the field (FRANSEN 1995, MANIANIA et al. 2001). Eighthly, it is highly important to assess the efficiencies of the entomopathogenic fungi potential in laboratory condition under more natural conditions, such as greenhouse. Many entomopathogenic fungi have been used successfully to control thrips in greenhouse. GILLESPIE (1986) reported that *V. lecanii* were active against *T. tabaci* on cucumber, while *N. parvispora* caused up to 60% mortality and reduced population density of *F. occidentalis* (VACANTE et al. 1994).

Recently, SENGONCA et al. (2006) reported that some entomopathogenic fungi isolated from Thailand had potential to control *F. occidentalis*. In addition, these fungal isolates have been proved to have possibilities for biocontrol of *T. tabaci* (THUNGRABEAB et al. 2005). Thus, before considering the entomopathogenic fungi isolates from Thailand as biological control agents, it is necessary to investigate these key criteria, in which such sufficient knowledge is still lacking in the literature.

Therefore, the present study aimed to selected promising entomopathogenic fungal isolates obtained from Thailand as biological control agents against *F. occidentalis* and *T. tabaci*. In laboratory study, pathogenicities of 41 isolates were assessed against 1^{st} instar larvae of these two thrips species. The isolates that showed high pathogenicity were tested to screen the virulent isolates. Further experiments were devoted to the isolates with high virulence by investigating their efficiency against various developmental stages. After that, experiments were focused on the isolates identified as superior ones, according to the important criteria for selection of biological control agents. Firstly, their biological and ecological characteristics were studied. To obtain better insight into the infection process of fungi on thrips, light microscope were carried out. The efficiencies under different abiotic and biotic factors were investigated. The side effects on non-target arthropods were also determined. Finally, with a goal to use the selected fungi isolates as biological control agent against thrips, their persistence and efficacy for controlling the thrips were tested under greenhouse conditions.

2 MATERIAL AND METHODS

2.1 Laboratory experiments

A laboratory assay to demonstrate pathogenicity, determine the relative virulence and select the efficiency isolates is generally the first step in the evaluation and selection process of entomopathogenic fungi as biological control agents.

2.1.1 Rearing of the insects

2.1.1.1 Rearing of *Frankliniella occidentalis* and *Thrips tabaci*

2.1.1.1.1 Stock culture

Frankliniella occidentalis (PERGANDE) and *Thrips tabaci* LINDEMAN (Thys., Thripidae) were initiated with few individuals, obtained from the original stock culture available at Institute of Crop Science and Resource Conservation, University of Bonn. A stock culture of *F. occidentalis* was established on bean plants, *Phaseolus vulgaris* L., cv. Marona. The bean plants were usually planted in trays (60×40 cm) under greenhouse conditions before transferring to climate room. The fresh ones were replaced the heavily infested bean plants weekly. The stock culture of *T. tabaci* was maintained on leek plants, *Allium porrum* L. or onion plants, *Allium cepa* L. in the climate room. The leek plants or onion plants were grown in small pots (10 cm diameter and 8 cm height) within a greenhouse. The fresh plants were added biweekly and the old plants were removed when the insects had moved to new plants. The rearing of the both thrips species took place in a climatically controlled room at 25±1°C temperature, 60±10% RH and 16:8 h (L:D) photoperiod with an artificial light intensity of about 4000 Lux.

2.1.1.1.2 Obtaining of individuals in the desired stage

The uniformly aged *F. occidentalis* individuals were obtained by using round Plexiglas cages (5.5 cm in diameter) with a meshed hole in the lid to allow air exchange. The round Plexiglas cages were filled with a 0.5 cm-thick-layer of 0.7% water agar, and then freshly excised bean leaf discs (4.5 cm in diameter) were placed upside down onto the water agar (Fig. 1a). In each round Plexiglas cage, 10 female adults of *F. occidentalis* picked up from stock culture were transferred on the bean leaf discs for egg laying and removed after 24 h to a newly prepared cage. Afterwards, the round Plexiglas cages were kept in climatically controlled chambers at

25±1°C temperature, 60±10% RH and 16:8 h (L:D) photoperiod. The eggs obtained in the old cages were reared further until the thrips reached the age desired for the experiments. First larval instars, pupae and one-day old adults were used in the following experiments.

To obtain the uniformly aged *T. tabaci* individuals, the same procedure as for *F. occidentalis* was used, except the bean leaves were replaced with leek leaves (Fig. 1b).

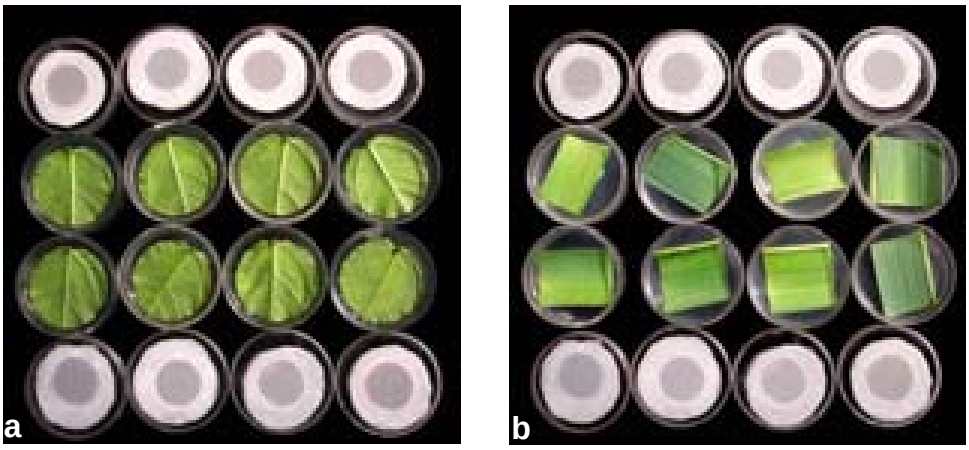


Fig. 1: Round Plexiglas for obtaining the uniformly aged and bioassay of *Frankliniella occidentalis* (a) and *Thrips tabaci* (b)

2.1.1.2 Rearing of collembolan and natural enemies

To determine side effects of entomopathogenic fungi on non-target arthropods, several beneficial arthropods were reared and tested. There were in addition to collembolan, *Heteromurus nitidus* TEMPLETON (Collembola: Entomobryidae), *Dicyphus tamaninii* WAGNER (Het., Miridae) *Chrysoperla carnea* (STEPHENS) (Neur., Chrysopidae), *Coccinella septempunctata* LINNAEUS (Col., Coccinellidae) and *Phytoseiulus persimilis* ATHIAS-HENRIOT (Acari: Phytoseiidae). All non-target arthropods had been obtained from the original stock cultures available at Institute of Crop Science and Resource Conservation, University of Bonn. The stock culture of *H. nitidus* was reared in plastic cups (9-cm diameter, 7-cm high) containing peat substrate as rearing substrate. Cups were covered to maintain approximately 100% RH and were kept at temperature room. Commercial nutritional yeast was use as the standard diet. *D. tamaninii* was reared in cages (60×60×40 cm) sealed with gauze from four sides in order to allow aeration, and the broad bean leaves, *Vicia fabae* L. cv. Scirocco infested with pea aphid, *Acyrthosiphon pisum* (HARRIS) (Hom., Aphididae) were used for rearing. The cases were kept in climatic room under the same condition used for rearing the thrips. The stock culture of *C. carnea* was held on eggs of grain

moth, *Sitotroga cerealella* (OLIVIER) (Lep; Gelechiidae) in small Plexiglas cages (3.5 cm in diameter and 1 cm in height). The small Plexiglas cages were kept in climatically controlled chambers at 25±1°C temperature, 60±10% RH and 16:8 h (L:D) photoperiod. *C. septempunctata* was maintained on broad bean leaves in round Plexiglas cages (11 cm diameter and 3 cm height), which had been infested with pea aphid, *A. pisum* as prey. The round Plexiglas cages were kept in climatic room at 25±1°C temperature, 60±10% RH and 16:8 h (L:D) photoperiod. Rearing of predatory mites, *P. persimilis* took place in climatically controlled chamber as mentioned above. The predatory mites were fed on the red spider mite *Tetranychus urticae* KOCH (Acari: Tetranychidae), which were previously infested on bean leaves.

2.1.2 Cultivation entomopathogenic fungi

Forty-one isolates of different entomopathogenic fungi originated from different hosts and belonging to 25 species from 11 genera, i.e. *Akanthomyces*, *Aschersonia*, *Beauveria*, *Cordyceps*, *Hirsutella*, *Hypocrella*, *Hymenostilbe*, *Metarhizium*, *Paecilomyces*, *Torrubiella* and *Verticillium* were obtained from the culture collection of the National Centre for Genetic Engineering and Biotechnology, Thailand (Tab. 1). Stock cultures of the isolates were stored at–80°C. The fungi were cultured on malt extract peptone agar (MEA, Merck, Darmstadt, Germany) and incubated at 25±1°C temperature under continuous light.

2.1.3 Procedures of the experiments

2.1.3.1 Screening efficiency isolates of different entomopathogenic fungi against thrips species, *Frankliniella occidentalis* and *Thrips tabaci*

In these experiments, firstly, pathogenicities of all isolates were assessed. Secondly, those isolates with high pathogenicity were determined for their virulence. Finally, the efficiencies of the isolates with superior virulence were investigated. The experiments on *F. occidentalis* and *T. tabaci* were conducted by separate tests in two sets, using bean leaves and leek leaves, respectively.

Bioassay procedures, conidia of the fungi were harvested from 1-to 3-week-old surface cultures by flooding the plates with sterile 0.05% Tween 80 water solution. The concentration of conidia was determined using an improved haemocytometer and adjusted to required level with sterile 0.05% Tween 80 water solution. Thirty uniformly aged individuals of thrips were carefully transferred to each round Plexiglas cage with leaf disc prepared as described previously.

Tab. 1: Entomopathogenic fungi isolates tested to assess pathogenicity against *Frankliniella occidentalis* and *Thrips tabaci*

Species: Isolates number	Original host	Location	Time of isolation
Akanthomyces sp.:			
AK.3497	Hemiptera - Pentotomidae	Ranong	24-Sep-97
AK.3582	Hemiptera - Pentotomidae	Ranong	11-Sep-97
Aschersonia badia:			
Ab.917	Homoptera - Coccidae	Nakhon Ratchasima	25-Nov-97
Aschersonia samoensis:			
As.4335	Homoptera - Coccidae	Kanchanaburi	18-Sep-97
As.4593	Homoptera - Coccidae	Chanthaburi	18-Nov-97
Aschersonia tamurai:			
At.5673	Homoptera - Coccidae	Phetchabun	04-Dec-98
At 6373	Homoptera - Coccidae	Chanthaburi	25-Sep-98
Beauveria bassiana:			
Bb.4591	Coleoptera - Curculionidae	Chanthaburi	08-Jun-97
Bb.5082	Hymenoptera - bee	Phetchabun	28-Aug-99
Bb.5335	Hymenoptera - ant	Phetchaburi	28-Nov-97
Bb.6243	Homoptera - cicada	Nakhon Ratchasima	28-Nov-97
Bb.7772	Host unknown	Chiangmai	28-Jun-99
Beauveria sp.:			
B.6739	Dicotyledonous leaf	Suratthani	08-Sep-98
B.6988	Host unknown	Suratthani	08-Sep-98
B.7683	Host unknown	Tak	21-Jan-99
Cordycep pseudomilitaris:			
Cp.951	Lepidoptera/larva	Saraburi	11-Jun-97
Cordyceps sp.*:*			
CO.5598	Lepidoptera-Limacodae-pupa	Chiangmai	21-Oct-98
Hirsutella citriformis:			
Hic.7679	Host unknown	Tak	21-Jan-99
Hirsutella formicarum:			
Hif.7731	Host unknown	Narathiwat	06-Jan-99
Hypocrella discoidea:			
Hd.4385	Homoptera - scale insect	Kanchanaburi	18-Nov-97
Hymenostilbe sp.:			
HY.1294	Isoptera - termite	Nakhon Ratchasima	13-Mar-97
Metarhizium anisopliae:			
Ma.5035	Homoptera - Cicadellidae	Phetchabun	28-Aug-97
Ma.6098	Homoptera	Ranong	28-Jul-98
Ma.6171	Leaf litter	Nakhon Ratchasima	04-Dec-98
Ma.7965	Homoptera	Nakhon Ratchasima	16-Dec-99
Ma.6079	Homoptera	Ranong	26-Jun-97
Metarhizium flavoviride:			
Mf.5744	Hemiptera	Kamphaengphet	30-Sep-97
Mf.1164	Soil	Lampang	01-Dec-00
Metarhizium sp.:			
M.7527	Host unknow	Prajeanburi	04-Sep-98
Paecilomyces farinosus:			
Pfa.3517	Araneae- spider	Ranong	09-Oct-98
Paecilomyces fumosoroseus:			
Pfu.5338	Bupressidae/leaf litter	Phetchaburi	5-Dec-98
Pfu.2507	Soil	Lampang	01-Dec-00
Paecilomyces javanicus:			
Pj.5870	Araneae - spider	Trat	29-Sep-98
Paecilomyces lilacinus:			
Pl.5066	Hemiptera - Cydnidae	Phetchabun	29-Sep-99
Paecilomyces tenuipes:			
Pt.6718	Lepidoptera - larva	Nakhon Ratchasima	08-Sep-98
Pt.7646	Host unknown	Suratthani	04-Dec-98
Torrubiella petchii:			
Tp.6200	Homoptera - scale insect	Phetchaburi	03-Nov-97
Torrubiella tenuis:			
Tt.345	Homoptera - scale insect	Nakhon Ratchasima	05-Aug-98
Verticillium hemipterigenum:			
Vh.6076	Homoptera	Ranong	23-Aug-98
Verticillium lecanii:			
Vl.3087	Homoptera - scale insect	Nakhon Ratchasima	29-Nov-97
Vl.2321	Host unknown	Lampang	01-Dec-00

Afterwards, the conidial suspension (1 ml) of the respective concentration was directly sprayed (Eco spray; Labo chimic France) onto the thrips (Fig. 2).

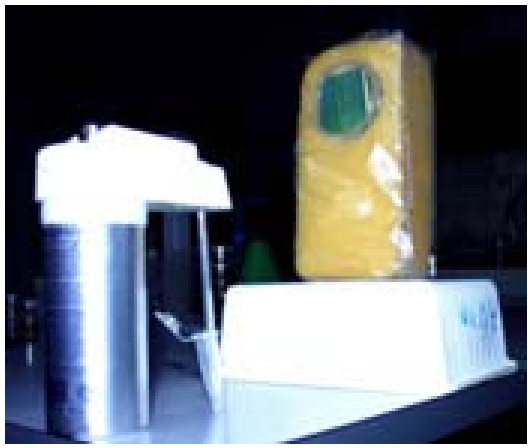


Fig. 2: Sprayer using for applied conidial suspensions

The control was sprayed only with 0.05% Tween 80 water solution. The round Plexiglas cages were covered with lids, sealed with transparent tape and kept in climatically controlled chambers at 25±1°C temperature, 60±10% RH and 16:8 h (L:D) photoperiod. The mortality caused by the fungi was confirmed by microscopic examination of hyphae and conidia on the surface of the thrips, and recorded daily for 7 days. Each treatment was replicated three times with 30 thrips individual per replicate, and the entire bioassay was usually repeated twice for each treatment.

2.1.3.1.1 Preliminary screening according to pathogenicity

Forty-one isolates of entomopathogenic fungi were screened to determine their pathogenicities with single concentration of 1×10^8 conidia/ml against 1st instar larvae of *F. occidentalis* and *T. tabaci*. The bioassay described above in capital 2.1.3.1 was performed. Mortality was recorded daily for 7 days. Percentage mortality was corrected for natural control mortality (ABBOTT 1925). Pathogenicity was determined by calculating percentage mortality.

2.1.3.1.2 Secondary screening according to virulence degree

Based on the results of preliminary screening, the 16 isolates that had showed high pathogenicity were selected for the second series assay. The virulence of these isolates was assessed using multiple concentrations. Five conidial concentrations, 1×10^2, 1×10^4, 1×10^6, 1×10^7 and 1×10^8 conidia/ml, were used. The experiments were also carried out on 1st instar larvae of *F.*

occidentalis and *T. tabaci* in a similar procedure set up as above. Mortality was recorded daily for 7 days. For each isolate, the final proportion of dead thrips at each concentration was determined by calculating the lethal concentration causing 50% mortality (LC_{50}). LC_{50} was estimated by probit analysis as described by FINNEY (1971). Virulent isolate was determined according to LC_{50} value.

2.1.3.1.3 Tertiary screening according to effectiveness

In order to investigate the efficiency of 5 highly virulent isolates, the experiments were determined susceptibility on various life stages of thrips. The highly virulent isolates were test with 1st larval, pupal and adult instars of the both thrips species. Five concentrations as mentioned above were tested for LC_{50} evaluation with the same procedure as described in capital 2.1.3.1. Based on results of the second screening, the lethal time causing 50% mortality (LT_{50}) experiments were conducted at a concentration of 1×10^7 conidia/ml. LC_{50} and LT_{50} values on various life stages were calculated.

2.1.3.2 Biological and ecological characteristics of the selected high efficiency *Beauveria bassiana* Bb.5335 and *Metarhizium anisopliae* Ma.7965

B. bassiana Bb.5335 and *M. anisopliae* Ma.7965 were the most promising isolate selected from results of the series of experiments. Therefore, they are ideal candidates for biological control of *F. occidentalis* and *T. tabaci.* However, in order to use these two fungi isolates as biological control agents against thrips, it is important to identify a range of their biological characteristics, because they could play an important role in determining efficiency and survival of the fungal isolates.

2.1.3.2.1 Morphological characteristics

For recording colony characteristics, the methods showed in figure 3 were used. The color, type aspect, and formation of synemmata and oosporein were recorded as well as the images of the observed specimens were photographed in the examination.

In order to determine conidia characteristics, three hundred conidia of each isolate, from 14-day old cultures on MEA medium, were examined for conidia shape and size at X100 magnification using Leica DMRB photomicroscope.

Conidial suspension containing 1×10^6 conidia/ml

100 µl of conidial suspension was spread evenly with a Drigalsky spatula on water agar (WA, 15 g agar, in 1000 ml H_2O)

Incubate at 25±1°C for 3 days under light condition

Mycelial mats were cut from culture plate into round agar plug using a 7-mm-diameter cork borer →

Each agar plug was then transferred onto the center of MEA media

Plate was sealed with Parafilm membrane and incubate at 25±1°C under light condition

Fig. 3: Methods for obtained mycelial mats and agar plug

2.1.3.2.2 Physiological characteristics

Studying of physiological characteristics was focused on evaluation of conidia viability, germination, conidiation and colony growth under different environmental factors.

2.1.3.2.2.1 Evaluation of conidia viability

To investigate conidia viability, the experiments were conducted for 12 months. MEA media were dispensed in 90 mm diameter petri dishes plate and after solidification were centrally inoculated with 7 mm agar plugs from 3 day-old-cultures of each *B. bassiana* and *M. anisopliae* (to obtained agar plug as the procedures described in figure 3). For every month, six plates were prepared for each isolates. The experiment consisted of 24 treatments (2 isolates × 12 months), each treatment was replicated 6 plates each plate was served as a replication and a total of 144 plates (2 isolates × 12 months × 6 plates/month). The plates were incubated at 25±1^{o}C temperature under complete darkness. Every month six plates of each isolate were removing and conidia viability was determined.

Conidia viability was determined by germination of conidia, the conidia harvested by flooding the plates with sterile 0.05% Tween 80 water solution. A 100 µl of conidial suspension containing 1×10^{6} conidia/ml was spread onto WA in 90 mm diameter petri dish plate, similar to the one described previously and incubated for 24 h under continuous light. To determine percentage germination, a drop of lactophenol cotton blue stain was put into the plate. Germination and non-germination conidia in three separated (1×1 cm^{2}) squares of agar cut from WA were counted under the microscope at X40 magnification, by using microscope slides through moving the field of view of microscope in order to cover a large area of agar square. One hundred conidia per square of agar were counted; three counts were made for each replicate and total of 1,800 conidia for every month of each isolate were counted. Percentage germination was calculated to determined conidia viability.

2.1.3.2.2.2 Germination at different environmental factors

In order to determine the germination of *B. bassiana* and *M. anisopliae*, the experiments were conducted under 4 different temperatures and 4 relative humidities levels. Furthermore, influence of 3 different intensity of light and influence of ultraviolet light was also study.

2.1.3.2.2.2.1 Temperature

The experiments were carried out under 4 temperature levels of 18±1, 25±1, 30±1 and 35±1^{o}C.

B. bassiana and *M. anisopliae* from 14-day old cultures were harvested by flooding the plates with sterile 0.05% Tween 80 water solution. Conidial suspension (100 µl of 1×10^6 conidia/ml) of each isolate was spread on WA plates as described above. Plates were then incubated individually at each temperatures of 18±1, 25±1, 30±1 and 35±1^oC, 60% RH, continuous light. Every temperature, three plates were prepared for each isolate. The experiment consisted of 8 treatments (2 isolates × 4 temperatures), each treatment was replicated 3 times and a total of 24 replicates (8 treatments × 3 plates, each plate served as a replicate). The entire experiment was repeated twice.

At 24 h after incubation, germination was halted by transferring 1 ml of 0.5% formaldehyde onto each plate. Afterwards, the procedure described in capital 2.1.3.2.2.1 for determine conidia germination was used.

2.1.3.2.2.2.2 Relative humidity

The experiments were conducted on 4 relative humidity levels of 32, 62, 85 and 100%.
Germination under different relative humidities was studied by using saturated salt solutions, ranging from 32 to 100% RH. To obtain the specific humidity; 32% RH was adjusted by dissolving $CaCl_2$ 740 g in 1000 ml H_2O, 62% RH by dissolving NH_4NO_3 in 1000 ml H_2O, while 85% RH by dissolving 175 g NaCl in 1000 ml H_2O. Deionized water was used for 100% RH. The suspensions were kept in closed chamber and were supported 2-3 cm directly above the solution on a sieve mesh. The petri dishes prepared as procedure described previously (seen in figure 3) were incubated in those chambers. The chambers were kept in incubator at 25±1°C temperature under continuous light. Every relative humidity, three plates were prepared for each isolate. The experiment consisted of 8 treatments (2 isolates × 4 relative humidities), each treatment was replicated 3 times and a total of 24 replicates (8 treatments × 3 plates, each plate served as a replicate). The entire experiment was repeated twice. The procedure for determining percentage germination similar to the one described previously.

2.1.3.2.2.2.3 Light-dark conditions

The influence of 3 different intensities of light i.e. continuous light, complete darkness and 16:8 h (L:D) photoperiod were examined.

The procedure and replication described above in capital 2.1.3.2.2.2.1 was performed, except that the petri dish were separately incubated under continuous light, in complete darkness and 16

light and 6 h dark, in climatically control chamber at 25±1°C temperature, 60±10% RH. The experiment consisted of 6 treatments (2 isolates × 3 conditions), each condition was replicated 3 times and a total of 18 replicates (6 treatments × 3 plates, each plate served as a replicate). The entire experiment was repeated twice.

Percentage germination was determined similar to the one described previously.

2.1.3.2.2.2.4 Ultraviolet light

For experiment of influence of ultraviolet light on germination, conidial suspensions (1,000 µl of 1×10^6 conidia/ml) prepared as described above were placed in 60 mm diameter petri dish plate and irradiated. The source of ultraviolet light rays was Philips TLD36W/08. The distance between exposed suspensions and UV source was 0.25 m. Exposure duration was a 3 h interval up to 24 h. At each interval time, 100 µl of irradiated suspension was spread on WA plate, 3 plates were made for every interval time of each isolates. The experiment consisted of 16 treatments (2 isolates × 8 time intervals: 3, 6, 9, 12, 15, 18, 21, 24 h), each treatment was replicated 3 times and a total of 48 replicates (16 treatments × 3 plates, each plate served as a replicate). The plates were then incubated in climatic control chamber at 25±1^{o}C temperature, 60±10% RH under continuous light. Germination of conidia was examined as the same procedure as mention above. The entire experiment was repeated twice. Percentage inhibition by ultraviolet light was examined compared to the control.

For assessing speed of germination, the procedure, condition and replication described for germination was used, except that germination of conidia was examined every 2 h, starting at 6 h up to 24 h of incubation. For each replicate of every environmental factor, the percentage of germination over time was analyzed using generalized linear model with binomial error and probit. The time taken in hours for 50% conidia to germinate (GT_{50}) was calculated according to methods as described by FINNEY (1971).

2.1.3.2.2.3 Conidiation at different environmental factors

In order to evaluate conidiation experiments were carried out under different environmental factors such as temperatures, relative humidity, light-dark conditions and ultraviolet light.

2.1.3.2.2.3.1 Temperature

The influence of temperature on conidiation was investigated at 18±1, 25±1, 30±1 and 35±1^{o}C.

The suspension from 14-day old surface cultures at a concentration of 1×10^6 conidia/ml was spread evenly with a Drigalsky spatula on the MEA medium surface in 90 mm diameter petri dish plate. For each isolate at each temperature level, three sterile petri dishes were prepared. The experiment consisted of 8 treatments (2 isolates × 4 temperatures), each treatment was replicated 3 times and a total of 24 replicates (8 treatments × 3 plates, each plate served as a replicate). The plates were incubated at each temperature mention above, 60±10% RH under continuous light condition in climatic control chamber. The whole experiment was repeated twice.

Measurement of conidiation was started at 3 days after incubation and was repeated at 5^{th}, 7^{th}, 10^{th}, 14^{th} and 21^{st} days of incubation period. Three random samples were taken from each petri dish with 12-mm-diameter cork borer and individually put into tubes filled with 5 ml of 0.1% Tween 80 water solution (72 tube per sample time: 2 isolates × 4 temperatures × 3 plates × 3 samples per plates). Fungal tissues (conidia and mycelia) were washed off by vortexing them. Then, the concentration of conidial suspension was determined by using an improved haemocytometer. For every tube, four haemocytometer counts were made. The conidiation rate was calculated as the number of conidia per square centimetre of MEA plate.

2.1.3.2.2.3.2 Relative humidity

The conidiation experiments were conducted on 4 relative humidity levels of 32, 62, 85 and 100%.

Conidiation at different relative humidities were studied by using saturated salt solutions as described in capital 2.1.3.2.2.2.2, but salt solutions were changed every week. For each isolate at each relative humidity level, three sterile petri dishes were prepared as described in capital 2.1.3.2.2.3. The experiment consisted of 8 treatments (2 isolates × 4 relative humidities), each treatment was replicated 3 times and a total of 24 replicates (8 treatments × 3 plates per relative humidity, each plate served as a replicate). The plates were incubated at each relative humidity mention above, 25±1°C temperatures under continuous light condition in incubator. The whole experiment was repeated twice. Then, measurement of conidiation was assessed as described for conidiation at different temperatures.

2.1.3.2.2.3.3 Light-dark conditions

For the experiments of conidiation under light-dark conditions were carried out under continuous light, complete darkness and 16 h of light 8 h of dark.

The procedure described above for conidiation at different temperatures was performed. For each isolate at each condition, three sterile petri dishes were prepared. The experiment consisted of 6 treatments (2 isolates × 3 conditions), each treatment was replicated 3 plates and a total of 18 replicates (6 treatments × 3 plates, each plate served as a replicate). The plates were incubated at each condition mention above, at 25±1°C temperature, 60±10% RH in climatic control chamber. The whole experiment was repeated twice. Continuous light and 16 h light was used artificial light at an intensity of about 4000 lux. The measurement of conidiation was assessed as described in capital 2.1.3.2.2.3.1.

2.1.3.2.2.3.4 Ultraviolet light

The plates were prepared as described in capital 2.1.3.2.2.3.1. This experiment was 2 treatments, treatment 1, the plates were incubated under ultraviolet light of Philips TLD36W/08 with distance between exposed petri dishes and UV source of 0.25 m. Treatment 2, served as control, the plates were incubated under continuous light, 25±1°C temperatures. For each isolate, six sterile petri dishes were prepared and total of 12 plates (2 isolates × 2 treatment × 3 plates/ treatment). Similarly, conidiation measurement was determined as mention above. Percentage inhibition by ultraviolet light was examined compared to the control. The whole experiment was repeated twice.

2.1.3.2.2.4 Colony growth at different environmental factors

The colony growth was assessing at different environmental factors such as temperature, relative humidity, light-dark conditions and ultraviolet light.

2.1.3.2.2.4.1 Temperature

The experiments were carried out under 4 temperature levels of 18±1, 25±1, 30±1 and 35±1^{o}C.

The agar plug described in figure 3 was transferred singly onto the center of a fresh MEA media petri dishes plate of 90 mm diameter. Petri dishes were sealed with Parafilm membrane and incubated individually for each temperature of 18±1, 25±1, 30±1 and 35±1^{o}C at 60±10% RH under continuous light. The experiments were carried out 8 treatments (2 isolates × 4 temperatures) each treatment was replicated 3 plates and a total of 24 replicates (8 treatments × 3 plates, each plate served as a replicate). The whole experiment was repeated twice.

Colony growth was recorded daily for 21 days by using two cardinal diameters, through two orthogonal axes previously draw on the bottom of each petri dish to serve as a reference. The diameter of the colonies was estimated by calculating the mean of two perpendicular measurements.

2.1.3.2.2.4.2 Relatives humidity

The colony growths at different relative humidities were conducted to 32, 62, 85, and 100% RH.

The plates prepared as describes in figure 3 were performed. The experiments consisted of 8 treatments (2 isolates × 4 relative humidities) each treatment was replicated 3 plates and a total of 24 replicates (8 treatments × 3 plates, each plate served as a replicate). Every six plates were separately in each chamber containing saturated salt solution for specific humidity (32, 62, 85, and 100%) prepared as procedure described in capital 2.1.3.2.2.2.2. The chambers were then incubated at 25±1°C temperature, under continuous light in incubator. Similarly, the colony growth was determined as mentioned above. The whole experiment was repeated twice.

2.1.3.2.2.4.3 Light-dark conditions

For the experiments of colony growth under light–dark conditions were carried out under continuous light, complete darkness and 16 h of light 8 h of dark.

The experiment was carried out 6 treatments (2 isolates × 3 conditions) each treatment was replicated 3 plates. Each plate, the procedure described in figure 3 was used and total 18 plates were prepared. All plates were separately in 3 groups, each group were incubated under continuous light, complete darkness and 16:8 h (L:D). The colony growth as described previously was performed. The whole experiment was repeated twice.

2.1.3.2.2.4.4 Ultraviolet light

The experiment of colony growth under ultraviolet light, the procedure and replication described above in capital 2.1.3.2.2.3.4 for conidiation under ultraviolet light were performed, except that plate were prepared as procedure described previously in figure 3. Colony growth was measured daily for 21 days using two cardinal diameters, through two orthogonal axes previously draw on the bottom of each petri dish to serve as a reference. The diameter of the colonies was estimated by calculating the mean of two perpendicular measurements. The whole experiment was repeated twice.

2.1.3.3 Mode of infection of *Beauveria bassiana* Bb.5335 and *Metarhizium anisopliae* Ma.7965 to *Frankliniella occidentalis*

To know the infection process of entomopathogenic fungi differences in susceptibility on the various life stages and if the difference temperatures had significant effects, light microscopic investigations were carried out on larval, pupal and adult stages under different temperatures.

The experiments mode of infection of *B. bassiana* and *M. anisopliae* were carried out on 1st larval, pupal and 1 day adult stages of *F. occidentalis* under 4 temperature levels of 18±1, 25±1, 30±1 and 35±1°C. There were 24 treatments (2 isolates × 3 life stages × 4 temperatures).

Larva, pupa and adult inoculated with *B. bassiana* or *M. anisopliae* at a concentration of 1×10^7 conidia/ml with the same procedure as described in capital 2.1.3.1 and then incubated at either temperature 18±1, 25±1, 30±1 and 35±1°C with 60±10% RH, 16:8 (L:D) photoperiod. Afterwards, sample of each treatment was taken at 12 h intervals up to 72 h (12, 24, 36, 48, 60, 72 h) after inoculation and were then fixed in chloral hydrate fixing solution (250 g/100 ml H_2O). These samples (consisted of 144 samples: 2 isolates × 3 life stages × 4 temperatures × 6 time intervals) were used in the following experiments.

2.1.3.3.1 Attachment of conidia

To study the attachment abilities of *B. bassiana* and *M. anisopliae* conidia on the cuticle of *F. occidentalis*, the experiments were conducted on the samples that incubated at 25±1°C, took at 24 h after inoculation, and those were 18 treatments (2 isolates × 3 life stages × 3 body regions).

The specimens were normally placed ventrally on glass microscope slide in a drop of 0.05% w/v Calcofluor White M2R (Sigma-Aldrich, Steinheim, Germany). Calcofluor was bound to carbohydrate with a β-linkage and could not penetrate the insect cuticle (NICHOLAS et al. 1994); therefore, it stained the cell wall of the fungi on the insect surface. Consequently, the conidia, germ tube and appressorium of the entomopathogenic fungi on the insect surface might fluoresce under UV-light. The specimen was observed by using BP 340-380/FT 400/LP 430 filter combination at Leitz DMR photomicroscope (Leica) with an incident light fluorescence mode and objective lenses of X1000 magnification. The numbers of conidia on larvae and pupae were tallied from a 25-μm^2 area of cuticles at abdominal segments, legs and head, respectively, while adults were observed on abdomen segments, legs and wings. Five specimens were examined for each treatment.

Fluorescence images of observed specimens were photographed with a fitted digital camera and could be observed on connected screen. The images were saved using the program "Discus" (Technisches Büro Hilgers, Koenigswinter, Germany).

2.1.3.3.2 Germination and evaluation of germ tube on cuticle

To determine the germination on cuticle, the methods used were similar to those described above for attachment of conidia, but the germination was examined at a 12 and 24 h after inoculation. There were 48 treatments (2 isolates × 3life stages × 4 temperatures × 2 interval times).

To evaluate germination on the thrips cuticle, germinated (observed with a germ tube) and non-germinated conidia were observed at least 100 conidia on each part of the body region (similar in capital 2.1.3.3.1), five specimens were tallied for each treatment. Average percentage germination was compared for every life stages among different temperatures. Further, germinated conidia was measured the germ tube and the penetration peg were recorded.

2.1.3.3.3 Penetration of fungal into the cuticle

To investigate the penetration process of *B. bassiana* and *M. anisopliae* into the *F. occidentalis*, the samples of inoculated thrips were taken at 36 and 48 h after inoculation. There were 24 treatments (2 isolates × 3 life stages × 4 temperatures × 2 time intervals). The specimens were fixed and discolored with chloral hydrate for at least 3 days (adult required longer). After thrips were fully cleared off, they were stained with 0.01% fuchsin acid (Merck, Darmstadt, Germany) in lacto phenol. Fuchsin acid was a stain for light and fluorescence microscopy, which could bind to carbohydrates. It stained the cell contents of fungi, and then the fungal structures pinked. The specimens were evaluated by the interference contrast in Leitz DMR photomicroscope (Leica) under X1000 magnification. Twenty-five microscopic fields (each 25 μm^2) were examined for each treatment. Germinated conidia penetrated or no penetrated were records after that the data at 36 h after inoculation were examined for percentage penetration of each treatment. Five specimens were examined for each treatment.

The images of observed specimens were photographed with a fitted digital camera and could be observed on connected screen. The images were saved using the program "Discus"

2.1.3.3.4 Colonization

The methods described above in capital 2.1.3.3.3 were performed for examined colonization of *B. bassiana* and *M. anisopliae* into *F. occidentalis*. The samples of inoculated thrips were taken at 60 and 72 h after inoculation. The images of observed specimens were photographed with a fitted digital camera and could be observed on connected screen. The images were saved using the program "Discus"

2.1.3.4 Efficiency of *Beauveria bassiana* Bb.5335 and *Metarhizium anisopliae* Ma.7965 against *Frankliniella occidentalis* and *Thrips tabaci*

The efficacy of entomopathogenic fungi to host insect is subject to a number of abiotic and biotic factors. These experiments were set up to study the influence of three important abiotic and biotic factors, i.e. temperature, relative humidity and host plant on efficacy of *B. bassiana* Bb.5335 and *M. anisopliae* Ma.7965 against different life stages of *F. occidentalis* and *T. tabaci*.

2.1.3.4.1 Influence of abiotic factors

Abiotic factors has been identified as a significant factor that can affect the physiology of fungus on rate of germination, conidiation, growth, and survival as well as its ability to infect the host insect. This part was focused on influence of abiotic factor i.e. temperature and relative humidity on efficiency of *B. bassiana* Bb.5335 and *M. anisopliae* Ma.7965 against different life stages of *F. occidentalis* and *T. tabaci*

The experiments were conducted on 1^{st} instars larval, pupal and 1-day adult stage of *F. occidentalis* and *T. tabaci*. Five conidial concentrations, 1×10^2, 1×10^4, 1×10^6 1×10^7 and 1×10^8 were used.

To determined influence of temperature, experiments were carried out at 4 different temperatures levels of 18±1, 25±1, 30±1 and 35±1^{o}C with relative humidity of 60±10% and 16:8h (L:D) photoperiod. There were 24 treatments (2 fungi isolates × 3 life stages ×4 temperatures). Each treatment was replicated three times with 30 thrips individual per replicate and the entire bioassay was usually repeated twice for each treatment. Bioassay procedures described in capital 2.1.3.1 were performed, except those Plexiglas cases were separately incubated at each temperature mentioned above.

To investigate the influence of relative humidity on efficiency of both fungi, experiments were set up at 40±10, 60±10, 85±10 % RH under 25±1°C temperature and 16:8h (L:D) photoperiod. There were 18 treatments (2 fungi isolates × 3 life stages × 3 relative humidities) bioassay procedures and replications described in capital 2.1.3.1 were used, except those Plexiglas cases were separately incubated at each relative humidity mentioned above.

2.1.3.4.2 Influence of host plants

Four crop plant species, namely bean, leek, cucumber (*Cucumis sativus* L.) (Cucurbitales: Cucubitaceae), cotton (*Gossypium hirsutum* L.) (Malvales: Malvaceae), as well as two ornamental plants species, namely swan river daisy (*Brachycome iberidifolia* BENTH) (Asterales: Asteraceae) and saintpaulia (*Saintpaulia brevipilosa* BURTT) (Lamiales: Gesneriaceae) were selected in the experiments.

Based on several individuals obtained from stock cultures of *F. occidentalis* on bean and *T. tabaci* on leek, separate rearing was conducted with each of the six host plants in order to adapt the insects to the new host plant in climate room under similar conditions as mentioned before for thrips rearing. The test population on each host plant was reared for 3-4 generations before being used for the experiments.

Freshly excised leaf discs of bean, leek, cucumber and cotton were prepared in round Plexiglas cages as described previously, while swan river daisy and saintpaulia were prepared with excised flowers. Three flowers of swan river daisy were excised from the plant and arranged in the round Plexiglas cages filled with 0.5 cm-thick-layer of 0.7% water agar. The same procedure was used with saintpaulia, but with three flowers (Fig. 4). To obtain uniformly aged individuals the method provided in capital 2.1.1.1.2 were used.

Five concentrations of conidia, 1×10^2, 1×10^4, 1×10^6, 1×10^7 and 1×10^8 conidia/ml were used against 1st instars larvae with the same procedure as mentioned before. There were 12 treatments (2 fungi isolates × 6 host plants). Each treatment was replicated three times with 30 thrips individual per replicate and the entire bioassay was usually repeated twice for each treatment. The experiments were carried under the same climatic conditions as noticed above. The efficacies of *B. bassiana* and *M. anisopliae* on different host plants against both thrips species were performed by calculating the LC_{50} values.

Fig. 4: Round Plexiglas cases used for the experiments on influence of host plant on efficiency

2.1.3.5 Side effects on non-target arthropods

B. bassiana Bb. 5335 and *M. anisopliae* Ma.7965 are promising as biological control agents against *F. occidentalis* and *T. tabaci*, but it is essential to prove that they are no risk to non-target organisms, especially arthropods natural enemies.

The experiments of side effects of *B. bassiana* and *M. anisopliae* on non-target were carried out on five orders of beneficial arthropods, such as Collembola, Heteroptera, Neuroptera, Coleoptera, and Acari. The conidial suspensions of both fungi used in all the experiments were carried out at a concentration of 1×10^8 conidial/ml, and applied by using a sprayer as it mentioned in figure 2.

Collembolan, *H. nitidus* was performed by mixing 3 ml of the conidial suspensions into 5 g of rearing substrate in each plastic cup (Fig. 5a). After that, 30 collembolans were added into individual cup and dried yeast was supplied as food source. The cups were closed with lid and incubated for 14 days at room temperature. The control was treated in a similar way, except that substrate was treated with 0.05% Tween 80 water solution. Mortality was recorded daily for 14 days and then dead collembolans were transferred to plates with moist filter paper in order to allow fungi sporulate. Each treatment was repeated three times with 30 collembolans per replicate, and the whole experiment was repeated twice.

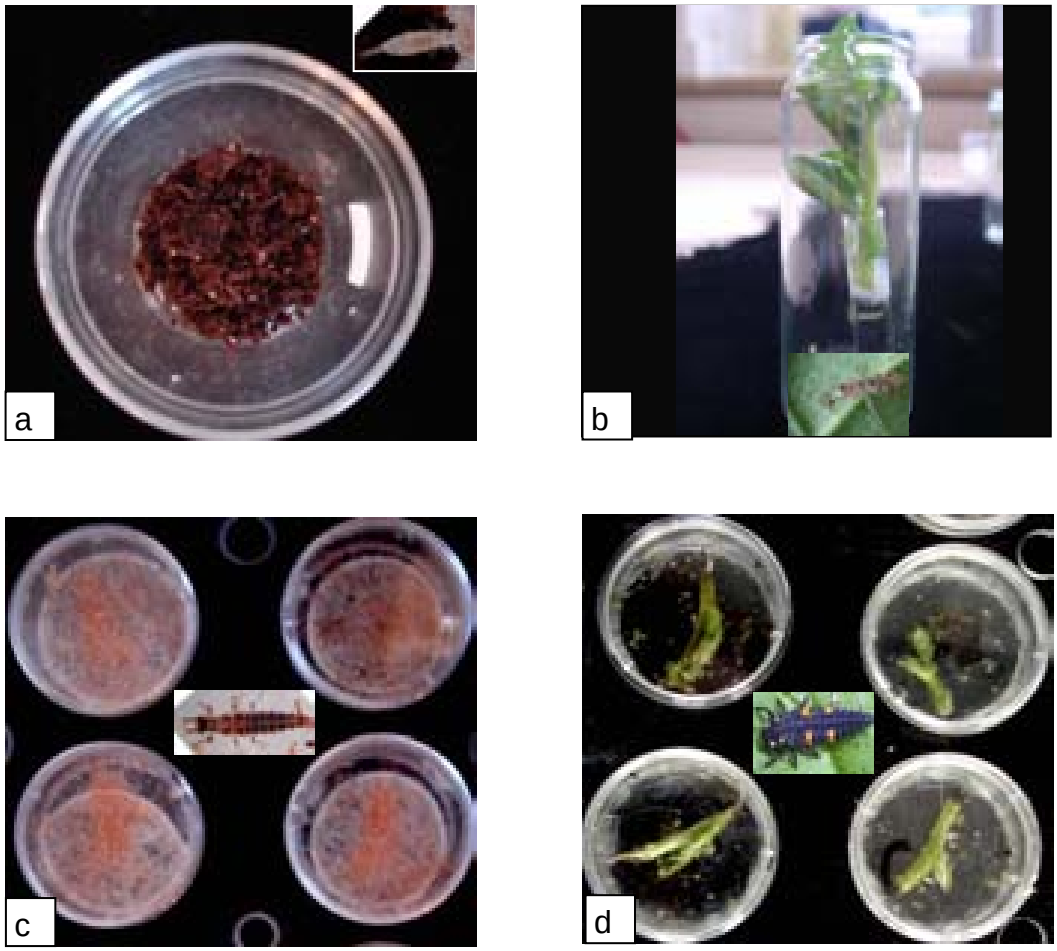


Fig.5: Experiments conducted for side effects of *Beauveria bassiana* and *Metarhizium anisopliae* on non-target arthropods. Plastic cup used for *Heteromurus nitidus* (a), Vial used for *Dicyphus tamaninii* (b), Round Plexiglas cages used for *Chrysoperla carnea* (c), Round Plexiglas cages used for *Coccinella septempunctata* (d)

Adult stage of *D. tamaninii* was conducted to determine side effects of *B. bassiana* and *M. anisopliae* on Heteroptera. Broad bean leaves infested with *A. pisum* were sprayed with 1 ml of *B. bassiana* or *M. anisopliae* conidial suspension. Leaves were allowed to air dry and then placed individually into a vial 2.5 cm in diameter and 7.5 cm in height (Fig. 5b). *D. tamaninii* were sprayed with 1 ml conidial suspensions through mesh sheet and then transferred individual into each vial. The vials were covered with a mesh-hole in the lid and kept in climatically controlled chamber at a temperature of 25±1°C, relative humidity of 60±10% and 16:8h (L:D) photoperiod. The control was processed as previously described, except conidial suspension was replaced by 0.05% Tween 80 water solution. Mortality was recorded daily until next generation. Each

treatment was replicated three times, each time with 30 *D. tamaninii* per replicate and the whole experiments repeated twice.

To determine potential side effects on *C. carnea*, experiment was carried out on 1st larval instars. For each replicate, thirty of 1st larval instars were transferred into 90-mm diameter petri dish with Whatman no. 1 filter paper, and then 1 ml of conidial suspensions of each *B. bassiana* or *M. anisopliae* were sprayed onto the larvae. The treated larvae were transferred singly to small round Plexiglas cages, 3.5 cm in diameter and 1 cm in height (Fig. 5c). The larvae were fed with egg of grain moth as prey. Control was sprayed with 0.05% Tween 80 water solution. Mortality was recorded daily until the next generation and cadavers were conducted similarly as the test collembolans. Experiment was conducted in climatic control chamber at 25±1°C temperature, 60±10% RH and 16:8 h (L:D) photoperiod. Each treatment was replicated three times, each time with 30 *C. carnea* and the whole assay was usually repeated twice.

The above procedure was then repeated with 1st larval instars of *C. septempunctata* to investigate the side effects on Coleoptera, expect small round Plexiglas were contained broad bean leaf infested with *A. pisum* as prey sprayed with conidial suspensions earlier (Fig. 5d). *C. septempunctata* were fortnightly offered fresh prey. In addition, mortality was recorded daily till the next generation. The cadavers were transferred to plates with moist filter paper in order to allow fungi sporulate. Each treatment consisted of three replicates with 30 *C. septempunctata* larvae per replicate and the whole experiment was repeated twice.

To investigate influence of *B. bassiana* and *M. anisopliae* on predatory mite, *P. persimilis* adults were used for the experiments. Bean leaves infested with *T. urticae* were prepared in round Plexiglas case as previously described for bioassay of thrips. Thereafter, 1 ml of each conidial suspension was sprayed onto the leaves. Thirty *P. persimilis* adults were transferred into each round Plexiglas case. The round Plexiglas cases were kept in a climatically controlled chamber as mentioned above. Fresh preys were transferred to round Plexiglas cases, whenever more prey was needed. Test mites were checked daily for mortality after inoculation and held until the next generation. Each treatment was replicated three times; each time with 30 *P. persimilis* per replicate and the entire assay was repeated two times.

2.2 Experiments under greenhouse conditions

To enhance the chance for using *B. bassiana* Bb.5335 and *M. anisopliae* Ma.7965 as biological control agents, it was worthy to investigate the efficiency of these two fungi under greenhouse conditions. Therefore, persistence of *B. bassiana* and *M. anisopliae* on different host plants and efficiency for controlling in greenhouse were studied.

2.2.1 Persistence of *Beauveria bassiana* Bb.5335 and *Metarhizium anisopliae* Ma.7965 on different host plants

Persistence of fungi on host plant can improve efficiency to control insect. Likewise, on different host plants the same fungal isolate may show difference in persistence, as a consequence of chemical and/or morphological difference between plants. Because the influence extent of a host plant on the survival of entomopathogenic fungal conidia is still unknown. This experiment was set up to assess the persistence of *B. bassiana* Bb.5335 and *M. anisopliae* Ma.7965 on several host plants by determined the viability of conidia and the mortality of *F. occidentalis* and *T. tabaci* caused by conidia after prolonged exposure on leaf/flower surfaces.

Four crop plants; bean, leek, cucumber and cotton and 2 ornamental plants (swan river daisy and saintpaulia) were planted in pots (13-cm diameter and 10-cm height) in greenhouse. Conidial suspensions of each *B. bassiana* and *M. anisopliae* at 1×10^8 conidia/ml were evenly sprayed on the abaxial and adaxial leaf/flower surface by using hand spray tower. Treated plants were kept in the greenhouse under temperature, relative humidity fluctuated daily depending on photoperiod, which may occur in a greenhouse. After application, leaves of treated crop plants (bean, leek, cucumber and cotton) as well as flowers of treated ornamental plants (swan river daisy and saintpaulia) were excised immediately and repeated at every 3 days for 30 days after application. There were 80 treatments of crop plants (2 isolates × 4-host plants × 10 time intervals; 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 days) and 40 treatments of ornamental plants (2 isolates × 2-host plants × 10 time intervals) each treatment consisted of 3 replications. Every treatment was used in the following experiments: assessing the conidia viability and efficiency of conidia after prolonged exposure by determining percentage mortality of *F. occidentalis* and *T. tabaci*. All the 6 host plants tested were run concurrently.

2.2.1.1 Conidia viability after prolonged exposure

To examine conidia viability, method modified from FIRSTENCEL et al. (1990) by using parallel vital fluorochrome staining was used. Fluorescein diacetate (FDA) indicated viability conidia, while propidium (PI) indicated non-viability conidia.

Approximately 20 µl of each FDA (Sigma-Aldrich, Steinheim, Germany) and PI (Sigma-Aldrich, Steinheim, Germany) was mixed on the treated leaflets of bean, leek, cucumber and cotton as well as treated flowers of swan river daisy and saintpaulia (based on described in capital 2.2.1). Afterward, those were examined immediately under fluorescence microscopy with the BP 355-425/FT 455/LP 460 filter combination at the Leitz-microscope with the incident light fluorescence mode. When conidia fluoresced yellow-green indicated that conidia were viable but when conidia fluoresced red indicated that conidia non-viability (Fig. 6). For each replicates was examining 3 times each time 200 conidia were observed and total 600 conidia/replicate were counted. Percentage conidia viability was calculated.

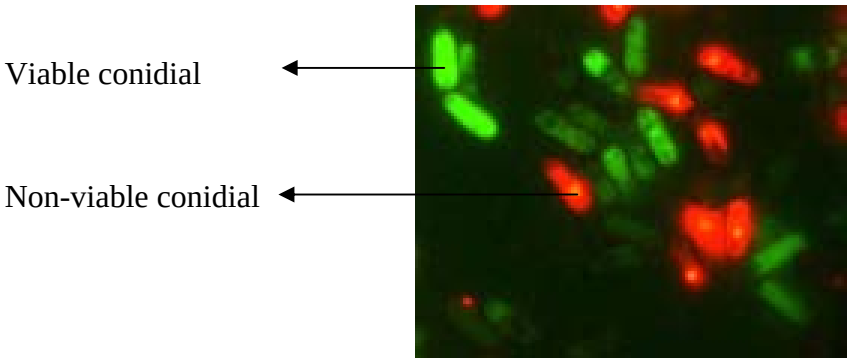


Fig. 6: *Metarhizium anisopliae* conidia on the leaflet with fluorochrome staining indicated viability of conidia

2.2.1.2 Efficiency of conidia after prolonged exposure

Based on experiments described in capital 2.2.1, excised treated leaves of bean, leek, cucumber and cotton as well as treated flowers of swan river daisy and saintpaulia samples from every time interval after inoculation were used to determine efficiency of conidia. The procedures seen in capital 2.1.3.1 were performed. The leaf discs from untreated leaves or flowers were used as control. The experiments were conducted on *F. occidentalis* and *T. tabaci.* The efficiency of conidia after prolonged exposure was determined by calculating percentages mortality and LT_{50}.

2.2.2 Efficiency of *Beauveria bassiana* Bb.5335 and *Metarhizium anisopliae* Ma.7965 for controlling *Frankliniella occidentalis* in greenhouse

From the series of experiments indicated that *B. bassiana* Bb.5335 and *M. anisopliae* Ma.7965 were promising isolates as biological control agents against *F. occidentalis* and *T. tabaci*, because of their high tolerances to temperature and relative humidity, good efficiencies on different host plants and long persistence on leaf surfaces under greenhouse conditions. Therefore, studies efficiency for controlling target pests in greenhouse were needed to confirm whether the laboratory results reflected an isolate performance in the greenhouse.

Two experiments in crop scale were done. Experiment 1 was conducted from December 2005 to January 2006 and experiment 2 was from March to April 2006. Both of the experiments were carried out on cucumber plants grown in greenhouse. There were three treatments, *B. bassiana*, *M. anisopliae* and control (0.05% Tween 80 water solution), set up in three separated glass cabins (3 m in length and 2 m in width). The cabins were completely sealed to prevent immigration and emigration of insects. The cucumber plants used were potted with 3 plants/pot, each plant was with 2 fully developed true leaves. Thirty plots were placed in individually cabin, three rows of 10 pots/row. Approximately 10 female adults of *F. occidentalis* were released on every cucumber leaf. After 7 days the thrips released, the conidial suspensions of each fungal isolate at a concentration of 1×10^8 conidial/ml (prepared as described in capital 2.1.3.1) were applied at volumes by using hand held sprayer, which gave a thorough coverage of upper and lower leaf surfaces. Every 7 days after application, three pots in each treatment were transferred to the laboratory in order to determine the numbers of thrips (larvae + adults). Percentage reduction population caused by fungi were determined by calculating number of thrips on treatment compared to the control.

2.3 Statistical analysis

In all the experiment, analysis of variance (ANOVA) was used for statistic evaluation. The general linear models of analysis of variance were used to analyze the experiments in one-way comparison of mean. To perform multiple comparison, analysis of variance appropriate for factorials in completely randomize design were used. One-or multifactor-analysis of variance was conducted to detect differences among means. In case differences among means were detected, the second step was then to determine the significant differences among the means at a probability level of ≤ 0.05. In which, among several means the Duncan's multiple rang test was

used (GOMEZ and GOMEZ 1984). In case of comparing between two means only, T-test was conducted. Small and capital letters represent significant difference parameters in the figures and tables.

Percentage of mortality was transformed by arcsine square root to normalize the mean percentage (GOMEZ and GOMEZ 1984) after considering natural mortality (ABBOTT 1925) and subjected to analysis of variance (ANOVA) appropriate for a completely randomize design.

To classify pathogenicity level, cluster analysis was introduced into grouping percentage mortality.

The lethal concentration causing 50% mortality (LC_{50}), the lethal time causing 50% mortality (LT_{50}) and the time causing 50% conidia to germinate (GT_{50}) were estimated by probit analysis as described by FINNEY (1971) using microcomputer statistical program.

To classify the degrees of virulence, the LC_{50} values were analyzed based on the ANOVA and were combined with the normal distribution curve, and then divided into three groups according to the mean (x) and standard deviation (SD) as shown in figure 7. The isolates, with LC_{50} values more than 0.5 SD above the mean were classified as low virulence, those in the range of 0.5 SD above to 0.5 SD below the mean as moderately virulent, and those 0.5 SD below the mean as possessing a high virulence.

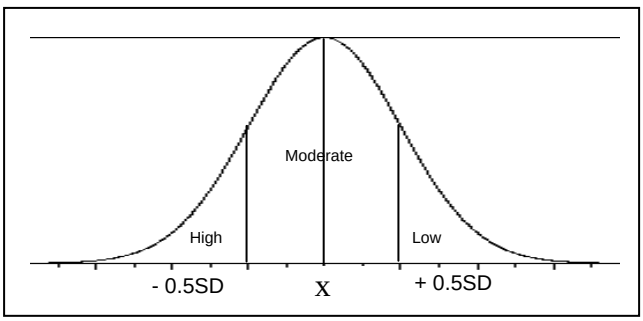


Fig. 7: Normal distribution curve for classify virulence degree

Susceptibilities of thrips on various developmental stages, under different abiotic factors and on different host plant species were performed by calculating LC_{50} and subjected to analysis of variance appropriate for factorial in completely randomize design.

Statistical analyses were performed using the Statistical Analysis System software (SAS) (ANONYMOUS2002)

3 RESULTS

3.1 Laboratory experiments

The laboratory evaluation of effectiveness of potential biological control agent is the first step in development a biological control program. Thus, this part of the study deals with the results of screening efficient isolates, their biological and ecological characteristics of the selected highly efficient isolates, their efficiencies under different abiotic and biotic factors, mode of infection and side effects on non target arthropods.

3.1.1 Screening efficiency isolates of different entomopathogenic fungi against thrips species*, Frankliniella occidentalis* and *Thrips tabaci*

One of the first steps in development of an entomopathogenic fungus as a biological control agent is choosing appropriate isolates, with high efficiency against the target pest. Thus, this part in the study deals with the results of screening efficient isolates, which have been examined through the following processes. Firstly to assess the pathogenicities of 41 the isolates; secondly to determine virulent isolates, thereafter the selected highly virulent isolates were investigated on their efficiencies against various life stages of two thrips species, *F. occidentalis* and *T. tabaci*. Finally, the isolates displayed superiors were present.

3.1.1.1 Preliminary screening according to pathogenicity

The results pathogenicities of 41 isolates of entomopathogenic fungi against *F. occidentalis* and *T. tabaci* are presented in table 2. Thirty-seven out of 41 isolates belonging to *Akanthomyces* sp., *Aschersonia* spp., *Beauveria* spp., *Cordyceps* sp., *Hirsutella* spp., *Metarhizium* spp., *Paecilomyces* spp., *Torrubiella petchii* (HYWEL-JONES) and *Verticillium* spp. were found to be pathogenic to *F. occidentalis* (Fig. 8). The mortalities were ranged from 4.54 to 100% at 7 days after treatment, while the control mortality was 5.10%. In the case of pathogenicity against *T. tabaci*, those isolates caused similar pathogenicities, except *T. petchii* (Tp.6200). Among the 36 isolates, the mortalities ranged from 4.54 to 97.33% after 7 days, while the mortality in control was 4.52%. *Hymenostilbe* sp., *Torrubiella tenuis* (PETCH), *Hypocrella discoidea* (BERK and BROOME) and *Cordyceps pseudomilitaris* (HYWEL-JONES) were found to be non-pathogenic against both thrips species. Highly significant differences ($P \leq 0.05$) were found among the isolates. In addition, these different levels of pathogenicity were detected in the bioassay within genera, species and isolates of fungi as well as thrips species. For example, mortalities caused by

Tab. 2: Percentages of corrected mortalities of *Frankliniella occidentalis* and *Thrips tabaci* larvae treated with different entomopathogenic fungi at a concentration of 1×10^8 conidia/ml at 25±1°C temperature in climatic chambers

Isolate no.	Corrected mortality (%±SE)		Isolate no.	Corrected mortality (%±SE)	
	Frankliniella occidentalis	*Thrips tabaci*		*Frankliniella occidentalis*	*Thrips tabaci*
AK.3497	49.28±7.41 f-iA	42.87±4.48 e-jA	Ma.5035	53.96±3.01 d-hA	49.94±15.00 d-iA
AK.3582	42.51±14.62 g-jA	37.37±4.04 f-jA	Ma.6098	20.85±5.00 lmA	23.52±5.96 h-jA
Ab.917	52.19±11.45 e-iA	12.45±9.81 i-kB	Ma.6171	75.57±11.67 bcA	80.00±5.06 a-cA
As.4335	11.76±4.40 mnA	7.64±0.45 jkA	Mf.5744	47.61±9.53 f-jA	33.33±3.33 f-jA
As.4593	52.83±6.23 e-iA	15.74±9.64 ijB	Mf.1164	8.76±4.71 mnB	43.33±9.62 e-jA
At.5673	24.02±1.35 k-mA	4.54±0.11 jkB	Ma.6079	87.10±3.27 abA	96.84±1.80 aA
At.6373	52.43±1.66 e-iA	20.63±4.41 h-jB	M. 7527	86.67±1.93 abA	90.37±6.58 abA
Bb.4591	98.57±1.43 aA	96.66±3.91 aA	Ma.7965	100.00±0.00 aA	97.33±2.22 aA
Bb.5082	68.91±17.98 c-eA	65.08±9.54 a-fA	Pfa.3517	69.04±12.99 c-eA	54.56±8.06 c-hA
Bb.5335	100.00±0.00 aA	95.52±2.93 aA	Pfu.5338	95.42±1.05 aA	87.76±2.23 a-cA
Bb.6243	90.32±1.31 abA	91.18±2.26 abA	Pfu.2507	89.87±4.49 abA	76.11±0.56 a-eA
Bb.7772	70.12±11.54 cdA	72.62±2.57 a-eA	Pj.5870	8.29±4.07 nA	11.11±1.50 jkA
B. 6739	62.73±4.48 c-fA	35.10±4.35 f-jB	Pl.5066	62.84±4.62 c-fA	73.69±0.68 a-eA
B. 6988	98.76±1.24 aA	92.15±0.45 abA	Pt.6718	5.09±3.04 nA	15.27±2.37 ijkA
B. 7683	43.53±4.08 g-jB	72.60±0.96 a-dA	Pt.7646	59.12±9.98 c-gA	36.11±6.59 ijB
Cp. 951	00.00±0.66 nA	00.00±0.00 kA	Tp.6200	4.54±4.54 nA	00.00±0.00 kA
CO.5598	68.59±8.37 c-eA	66.66±9.62 a-fA	Tt. 345	00.00±0.00 nA	00.00±0.00 kA
Hic.7679	54.07±11.12 d-hA	9.47±1.49 jkB	Vh.6076	38.72±16.76 h-kA	20.15±0.53 h-jA
Hif.7731	7.64±7.63 mnB	28.89±8.01 g-jA	Vl.3087	31.97±5.49 j-lA	11.52±2.57 jkB
Hd.4385	00.00±0.00 nA	00.00±0.00 kA	Vl.2321	36.07±7.59 i-lA	8.76±0.50 jkB
HY.1294	00.00±0.00 nA	00.00±0.00 kA			

Means in columns followed by different small letters are significantly different among different isolates of entomopathogenic fungi within the same thrips species (one-way ANOVA, P≤0.05, Duncan's multiple rang test). Means in rows with different capital letters are significantly different between different thrips species within the same isolate (P≤0.05, T-test).

Beauveria spp. ranged from 43.53 to 100%, and 35.10 to 96.67 % on *F. occidentalis* and *T. tabaci*, respectively, whereas *Metarhizium* spp. ranged from 8.76 to 100% and 23.52 to 97.33% on the two thrips species as mentioned above. Among the five isolates tested, *B. bassiana* showed the highest and the lowest mortalities of 100% and 68% on *F. occidentalis* and 96 to 65% on *T. tabaci*, respectively. Furthermore, the results herein indicated that the pathogenicity of

entomopathogenic fungi differed between thrips species. Almost entomopathogenic fungi were more pathogenic to *F. occidentalis* than *T. tabaci*, but there was no significant difference mostly.

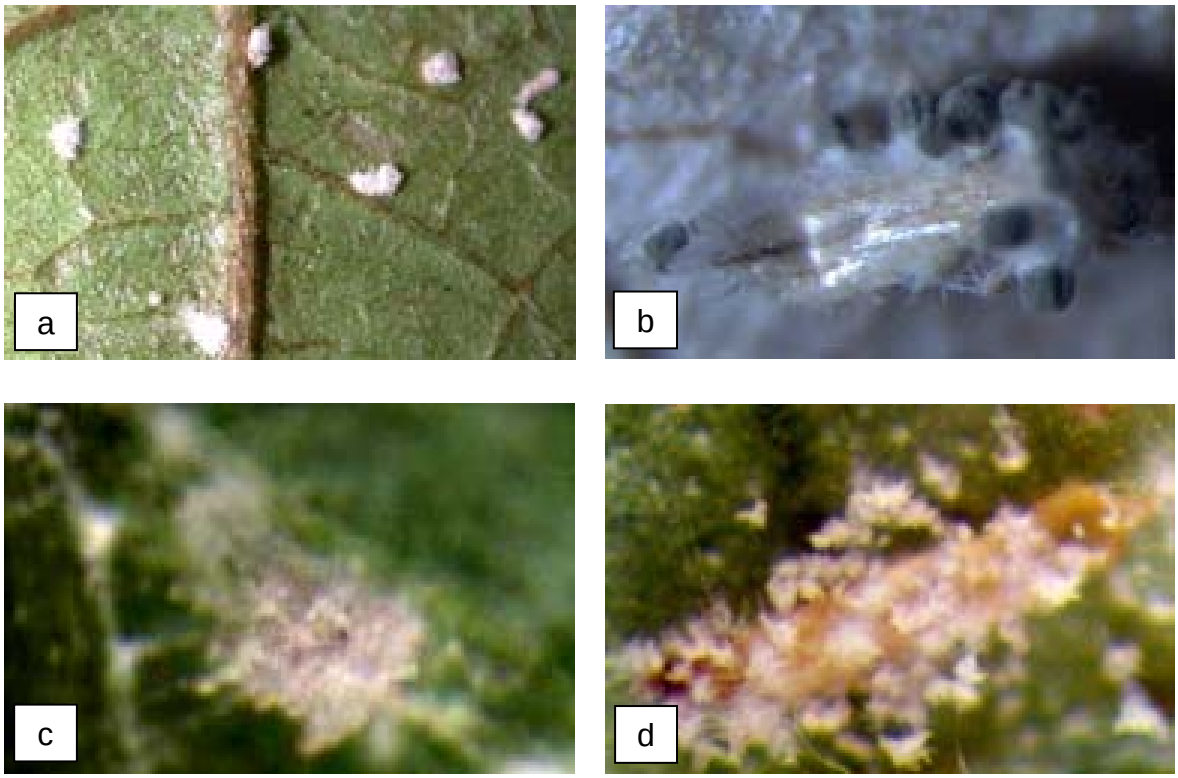


Fig. 8: *Frankliniella occidentalis* infected with *Beauveria bassiana* (a), *Metarhizium anisopliae* (b), *Paecilomyces fumosoroseus* (c) and *Cordyceps* sp. (d)

The cluster analysis was introduced to group the isolates into 3 pathogenicity groups with a distance of D=33.96, based on the mortalities of *F. occidentalis* and *T. tabaci* showed at table 2. The results are demonstrated in figure 9. The mortality rates in the 1st group were greater than 64%, while from 30 to 64% in the 2nd group and it was less than 30 % in the 3rd group. The 1st group contained 16 isolates, e.g. 7 isolates of *Beauveria*, 4 isolates of *Metarhizium*, 4 isolates of *Paecilomyces* and 1 isolate of *Cordyceps*, which were then identified as highly pathogenic isolates and were used in the secondary bioassay.

3.1.1.2 Secondary screening according to virulence degree

The estimated LC_{50} values of 16 isolates are showed in table 3. Highly significant differences ($P \leq 0.05$) were found among the isolates. For example, the LC_{50} values of *Beauveria* spp. to *F. occidentalis* ranged from 2.39×10^4 to 5.89×10^6 conidia/ml, whereas those of *Metarhizium* spp.

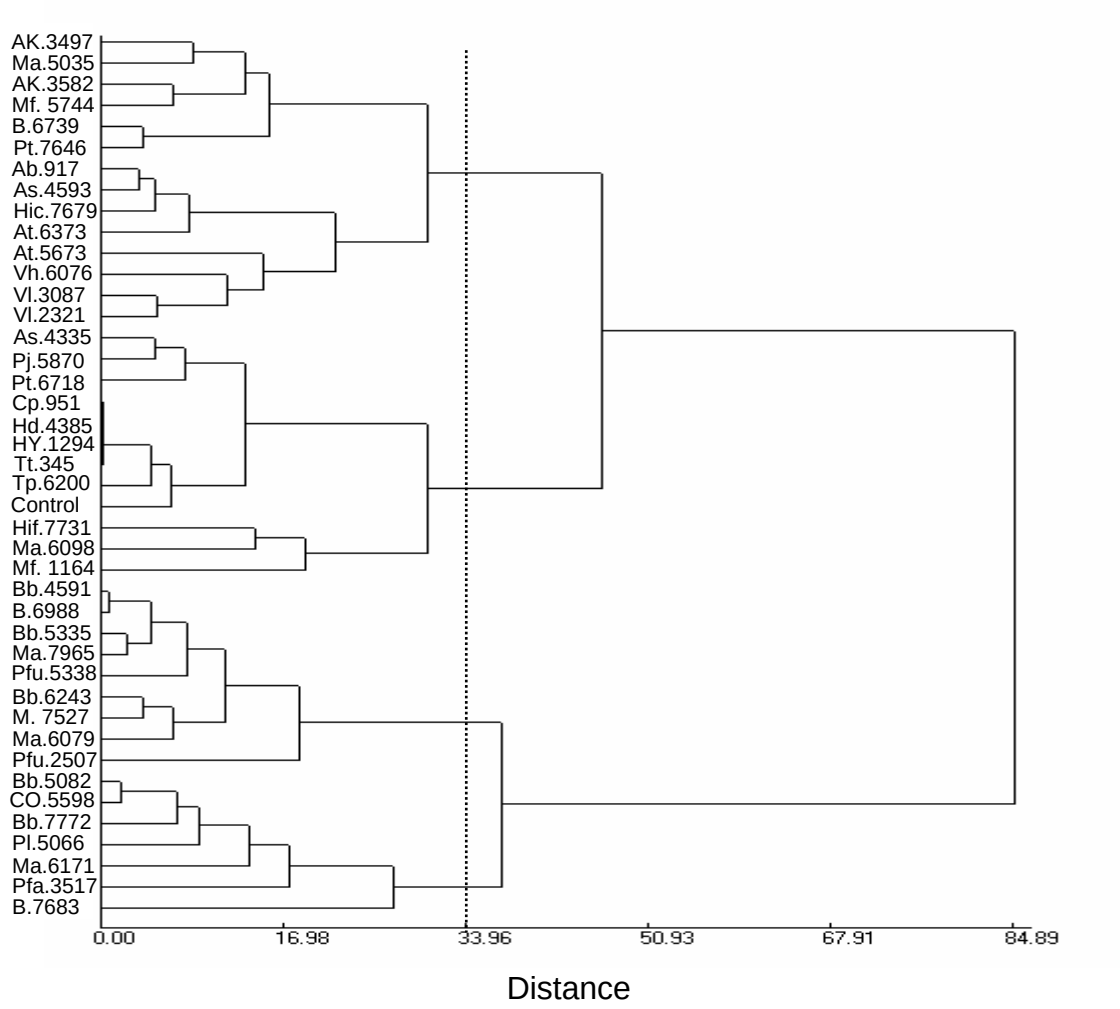


Fig. 9: Cluster analysis of pathogenicity group on different entomopathogenic fungi against *Frankliniella occidentalis* and *Thrips tabaci* larvae based on the mortalities of both thrips species with a distance of D = 33.96.

ranged from 2.00×10^4 to 5.00×10^5 conidia/ml. The LC_{50} values of *Paecilomyces* spp. ranged from 3.89×10^4 to 5.49×10^6 conidia/ml, while that of *Cordyceps* were 4.67×10^5 conidia/ml. Slopes of regression equations were all significantly different from zero. However, different degrees of virulence were detected in the bioassays within genera, species and isolates.

Sixteen fungi isolates were classified into different degrees of virulence according to the LC_{50} values (Tab. 3). Five isolates, (2 isolates of *B. bassiana*, i.e. Bb.4591 and Bb.5335, 2 isolates of *M. anisopliae*, i.e. Ma.6079 and Ma.7965 as well as 1 isolate of *P. fumosoroseus*, Pfu.5338) were higher virulent than all the other isolates against both thrips species. The 5 isolates were further for their efficiencies against various life stages of both thrips species.

Tab. 3: LC_{50} of entomopathogenic fungal isolates with high pathogenicity against *Frankliniella occidentalis* and *Thrips tabaci* larvae at 25±1°C temperature

Isolate no.	*Frankliniella occidentalis*		*Thrips tabaci*		Virulence degree
	LC_{50}±SE (conidia/ml)	Slope±SE[a]	LC_{50}±SE (conidia/ml)	Slope±SE[a]	
Ma.7965	$(2.00±1.38)×10^4$ f	0.50±0.07**	$(1.82±1.75)×10^4$ f	0.40±0.13*	High virulence
Bb.5335	$(2.39±0.09)×10^4$ f	0.55±0.12**	$(2.57±1.22)×10^4$ f	0.61±0.10**	
Pfu.5338	$(3.89±0.24)×10^4$ ef	0.55±0.04***	$(3.98±1.04)×10^4$ ef	0.43±0.06**	
Bb.4591	$(3.98±1.47)×10^4$ ef	0.42±0.07***	$(8.91±2.39)×10^4$ ef	0.70±0.03***	
Ma.6079	$(6.61±1.35)×10^4$ def	0.52±0.09**	$(4.26±1.21)×10^4$ ef	0.59±0.09**	
Pfu.2507	$(1.00±1.82)×10^5$ cdef	0.59±0.03***	$(1.58±2.33)×10^5$ de	0.59±0.02***	Moderate-virulence
B.6988	$(1.15±1.73)×10^5$ cdef	0.48±0.08**	$(1.83±2.03)×10^5$ de	0.45±0.07**	
M.7527	$(2.95±2.00)×10^5$ cde	0.55±0.05***	$(1.86±1.86)×10^5$ de	0.30±0.09*	
CO.5598	$(4.67±1.72)×10^5$ cd	0.66±0.07***	$(4.67±1.10)×10^5$ cd	0.46±0.08***	
Ma.6171	$(5.00±2.08)×10^5$ cd	0.50±0.03***	$(8.70±1.73)×10^5$ cd	0.52±0.11**	
Bb.6243	$(6.31±1.94)×10^5$ bc	0.76±0.15**	$(1.02±1.61)×10^6$ bc	0.60±0.10**	Low virulence
B.7683	$(1.45±4.91)×10^7$ a	0.70±0.10**	$(2.95±1.32)×10^6$ ab	0.60±0.04***	
Bb.7772	$(4.26±0.08)×10^6$ ab	0.63±0.05***	$(6.45±1.27)×10^6$ a	0.59±0.09**	
Pfa.3517	$(5.49±2.11)×10^6$ a	0.50±0.11**	$(7.94±1.72)×10^6$ a	0.52±0.12**	
Bb.5082	$(5.89±2.20)×10^6$ a	0.54±0.05**	$(6.76±1.89)×10^6$ a	0.63±0.09**	
Pl.5066	$(1.78±4.20)×10^7$ a	0.48±0.03**	$(1.29±1.05)×10^7$ a	0.41±0.03***	

Means in columns with different small letters indicate significant differences among different isolates of entomopathogenic fungi (one way ANOVA, P≤0.05; Duncan's multiple rang test).
[a] T test on slope of regression equation.
*** Significantly different from zero (P≤0.001).
** Significantly different from zero (P≤0.01).
* Significantly different from zero (P≤0.05).

3.1.1.3 Tertiary screening according to effectiveness

Table 4 shows the efficiencies of 5 selected fungi isolates against various life stages of *F. occidentalis*. All isolates tested had effectiveness to all the stages of *F. occidentalis*, but the efficiency varied with the life stage. There were significant differences (P≤0.05) among different life stages. In all the isolates assayed, the efficiencies of fungi decreased with proceeding development, i.e. from larva over pupal to adult stages. Highly significant differences (P≤0.05) were found among different isolates. For instance, *M. anisopliae* Ma.7965 had the lowest LC_{50} value at all the life stages with $1.12×10^4$, $2.95×10^4$ and $6.92×10^4$ conidia/ml to larval, pupal and

Tab. 4: LC_{50} values of 5 selected fungi isolates against various life stages of *Frankliniella occidentalis* and *Thrips tabaci* at 25±1°C

Isolate no.	LC_{50} (conidia/ml)			
	Frankliniella occidentalis		*Thrips tabaci*	
	LC_{50} ±SE	Slope±SE[a]	LC_{50} ±SE	Slope±SE[a]
Larval stage				
Bb.4591	$(3.55±0.81)×10^4$ a	0.48±0.09**	$(5.37±1.41)×10^4$ a	0.62±0.02***
Bb.5335	$(1.55±0.25)×10^4$ b	0.50±0.18*	$(2.51±1.35)×10^4$ a	0.54±0.07***
Ma.6079	$(4.17±1.31)×10^4$ a	0.46±0.08**	$(3.98±1.37)×10^4$ a	0.58±0.09***
Ma.7965	$(1.12±0.31)×10^4$ b	0.36±0.04***	$(7.94±1.33)×10^3$ b	0.48±0.04***
Pfu.5338	$(1.62±0.31)×10^4$ b	0.33±0.02***	$(2.40±1.24)×10^4$ a	0.5±0.12**
Pupal stage				
Bb.4591	$(1.07±0.67)×10^6$ a	0.64±0.22*	$(2.04±1.39)×10^6$ a	0.83±0.10***
Bb.5335	$(7.41±0.72)×10^4$ b	0.76±0.24*	$(1.57±1.05)×10^5$ c	0.58±0.01***
Ma.6079	$(3.63±0.12)×10^5$ ab	0.58±0.12**	$(1.10±1.16)×10^5$ c	0.49±0.03***
Ma.7965	$(2.95±0.43)×10^4$ b	0.43±0.13*	$(2.51±1.80)×10^4$ d	0.67±0.25***
Pfu.5338	$(1.48±1.30)×10^5$ ab	0.81±0.17**	$(4.07±1.32)×10^5$ b	0.57±0.24[ns]
Adult stage				
Bb.4591	$(1.32±1.26)×10^6$ a	0.69±0.12*	$(1.07±2.94)×10^7$ a	0.55±0.06**
Bb.5335	$(1.62±0.46)×10^5$ bc	0.67±0.11**	$(1.82±1.16)×10^5$ bc	0.54±0.06***
Ma.6079	$(3.98±1.54)×10^5$ ab	0.59±0.04*	$(6.03±1.87)×10^5$ b	0.58±0.09***
Ma.7965	$(6.92±0.28)×10^4$ c	0.47±0.04***	$(5.37±2.07)×10^4$ c	0.48±0.04***
Pfu.5338	$(2.75±0.86)×10^5$ b	0.51±0.04***	$(6.46±1.68)×10^5$ b	0.54±0.12**

Means in columns with different small letters indicate significant differences among different isolates of entomopathogenic fungi within the same stages (one way ANOVA, P≤0.05; Duncan's multiple rang test).
[a] T test on slope of regression equation
*** Significantly different from zero (P≤0.001)
** Significantly different from zero (P≤0.01)
ns Non significantly

adult stages, respectively; whereas *M. anisopliae* Ma.6079 had the highest LC_{50} values of $4.17×10^4$, $3.63×10^5$ and $3.98×10^5$ conidia/ml against larval, pupal and adult stages, respectively. The slopes of regression equations were significantly different from zero and ranged from 0.36-0.47 within *M. anisopliae* Ma.7965 and from 0.46-0.58 within *M. anisopliae* Ma.6079 (Tab. 4). Similar efficiency trend was found with LT_{50} values, where Ma.7965 had the shorter LT_{50} than Ma.6079. The LT_{50} of Ma.7965 with a concentration of $1×10^7$ against larval, pupal and adult

stages were 3.37, 3.87 and 4.40 days, while those of Ma.6079 were 4.06, 4.28 and 5.79 days, respectively (Tab. 5).

Tab. 5: LT_{50} values (days) with a concentration of 1×10^7 conidia/ml at the various life stages of *Frankliniella occidentalis* and *Thrips tabaci* at 25±1°C

Isolate no.	LT_{50} values (days)			
	Frankliniella occidentalis		*Thrips tabaci*	
	LT_{50} ±SE	Slope±SE[a]	LT_{50} ±SE	Slope±SE[a]
Larval stage				
Bb.4591	4.19±0.4 abB	5.93±0.59**	4.49±0.4 abB	5.57±0.64***
Bb.5335	3.77±0.2 bcB	5.97±0.74**	4.07±0.1 bcB	6.10±0.811**
Ma.6079	4.06±0.1 aB	6.86±0.17***	4.36±0.4 aB	5.85±0.86**
Ma.7965	3.37±0.2 cB	6.90±0.28***	3.67±0.6 cB	5.86±0.85**
Pfu.5338	4.43±0.2 aB	6.50±0.47***	4.73±0.3 aB	6.13±0.75**
Pupal stage				
Bb.4591	4.27±0.5 abB	5.27±0.65**	5.17±0.1 abB	5.59±0.52***
Bb.5335	3.87±0.1 bcB	6.37±0.40***	4.30±0.3 bcB	7.04±0.18***
Ma.6079	4.28±0.1 aB	6.64±0.40***	5.58±0.5 aB	5.59±0.87**
Ma.7965	3.87±0.2 cB	6.09±0.58***	4.25±0.2 cB	5.59±0.48***
Pfu.5338	4.44±0.2 aB	5.98±0.80**	5.14± 0.1 aB	7.67±1.53**
Adult stage				
Bb.4591	5.27±0.3 abA	5.53±0.67***	5.57±0.1 abA	6.00±0.83**
Bb.5335	4.88±0.3 bcA	5.15±0.40***	5.18±0.1 bcA	6.50±1.09**
Ma.6079	5.79±0.2 aA	4.67±0.62***	6.09±0.1 aA	4.61±0-63**
Ma.7965	4.40±0.1 cA	5.57±0.64***	4.65±0.6 cA	6.22±1.22**
Pfu.5338	5.14±0.4 aA	7.55±0.52***	5.44±0.4 aA	7.55±0.53***

Means in the same column followed by different small letters indicate significant differences among different isolates of entomopathogenic fungi within the same stage. Means in the same row followed by the different capital letters indicate significant differences among different developmental stages within the same isolate (two factors ANOVA, $P\leq0.05$; Duncan's multiple rang test)

[a] T test on slope of regression equation

*** Significantly different from zero ($P\leq0.001$)

** Significantly different from zero ($P\leq0.01$)

Five isolates had also effectiveness against all the stages of *T. tabaci*, which varied with isolate and life stage (Tab. 4). For example, *B. bassiana* Bb.5335 had LC_{50} values of 2.51×10^4, 1.57×10^5 and 1.82×10^5 conidia/ml against larval, pupal and adult stages, respectively, while *M. anisopliae* Ma.7965 had LC_{50} values of 7.94×10^3 conidia/ml for larval, 2.51×10^4 conidia/ml for pupal and

5.37×10^4 conidia/ml for adult stages (Tab. 4). The LT_{50} values differed significantly among the fungal isolates when applied at a concentration of 1×10^7 conidia/ml on the various life stages. The LT_{50} values showed the same tendency as LC_{50}, as the larval stages had the shortest LT_{50} and the adult stages had the longest LT_{50}. The slopes of regression equations of all isolates on the various life stages were significantly different from zero (Tab. 5). Among five isolates tested, *M. anisopliae* Ma.7965 and *B. bassiana* Bb.5335 displayed considerably better efficiencies than the other three fungal isolates. These two fungi isolates were then selected for further experiments.

3.1.2 Biological and ecological characteristics of the selected high efficiency *Beauveria bassiana* Bb.5335 and *Metarhizium anisopliae* Ma.7965

Successful use of entomopathogenic fungi as biological control agents, it is important to identify a range of characteristics favourable for survival. Therefore, this part of the study deals with the results of morphological and physiological characteristics of *B. bassiana* Bb.5335 and *M. anisopliae* Ma.7965 that have showed the highest efficiencies through the following order of screening experiments, under different temperatures, relative humidity, light-dark conditions and ultraviolet light.

3.1.2.1. Morphological characteristics

Figure 10a-e shows the morphology of *B. bassiana*. The colony of *B. bassiana* appeared white at immatural stage and became pale-yellow at mature stage. Its conidiophores consisted of whorls and dense clusters of conidiogenous cells, which were hyaline and smooth-walled. The conidogenous cells were flask-shaped with an apical denticulate rachis giving a distinctly zig-zag, rachiform, proliferating and sympodially, and often aggregated into sporodochia or synnemata. The conidia were one-celled, hyaline, thin-walled and globose to ellipsoidal in shape. Conidia shape that determined on 14-day-old surface cultures of MEA medium ranged from 2.16 - 3.26 µm diameters.

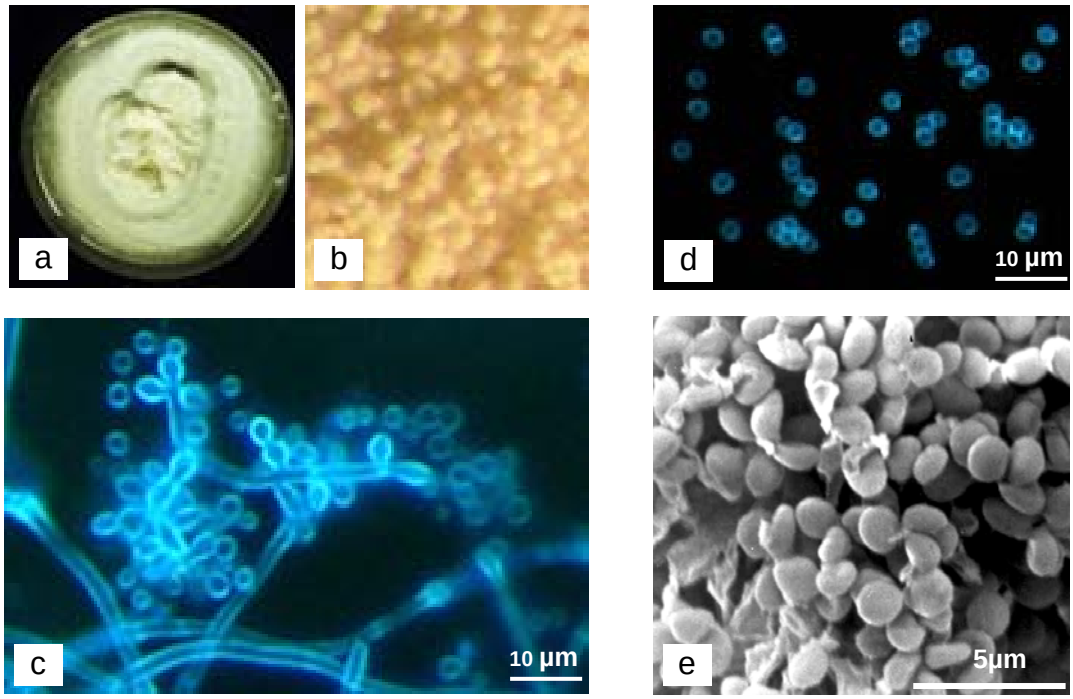


Fig. 10: Morphology of *Beauveria bassiana* showing, colony (a), balls of conidia (b), conidiophores stained with 0.05% diethanol, under fluorescence light microscope (c), conidia stained with 0.05% diethanol, under fluorescence light microscope (d) and conidia under scanning electron microscope (e)

The morphology of *M. anisopliae* is showed in Figure 11a-f. The colony of *M. anisopliae* appeared white when young, but the color turned to green as the conidia matured. The mycelium is white. *M. anisopliae* produced green conidia (phialospores) from closely packed and parallelly oriented conidiogenous cells born upon sporodochium-like mass hyphae. The conidiogenous cells are considered as phialides. Phialides are cylindrical, ~ 15 µm long. The conidia are one-celled, cylindrical with rounded ends, smooth-walled and hyaline to slightly coloured, forming long chains and often aggregating into prismatic columns. The conidiophores are branched, and the initial conidia were produced by simple abstriction at the distal end of the conidiophore. The basipetally produced chains adhered into distinct cylindrical columns with conidiogenous cells at column bases. A chain of conidia is formed on each conidiophore with the youngest conidium being adjacent to the conidiophore. The mass of conidia chains became so dense and cohered with each other to produce prismatic masses of columns of conidia chains. The fragile chains of conidia were held together by slight thickenings of wall material at the apex of each conidial initial, which bound to the lamellate, basal septum of previously formed conidia. The conidia size that determined on 14-day-old surface cultures of MEA medium ranged from 6-6.8 µm long and 2-2.7 µm wide.

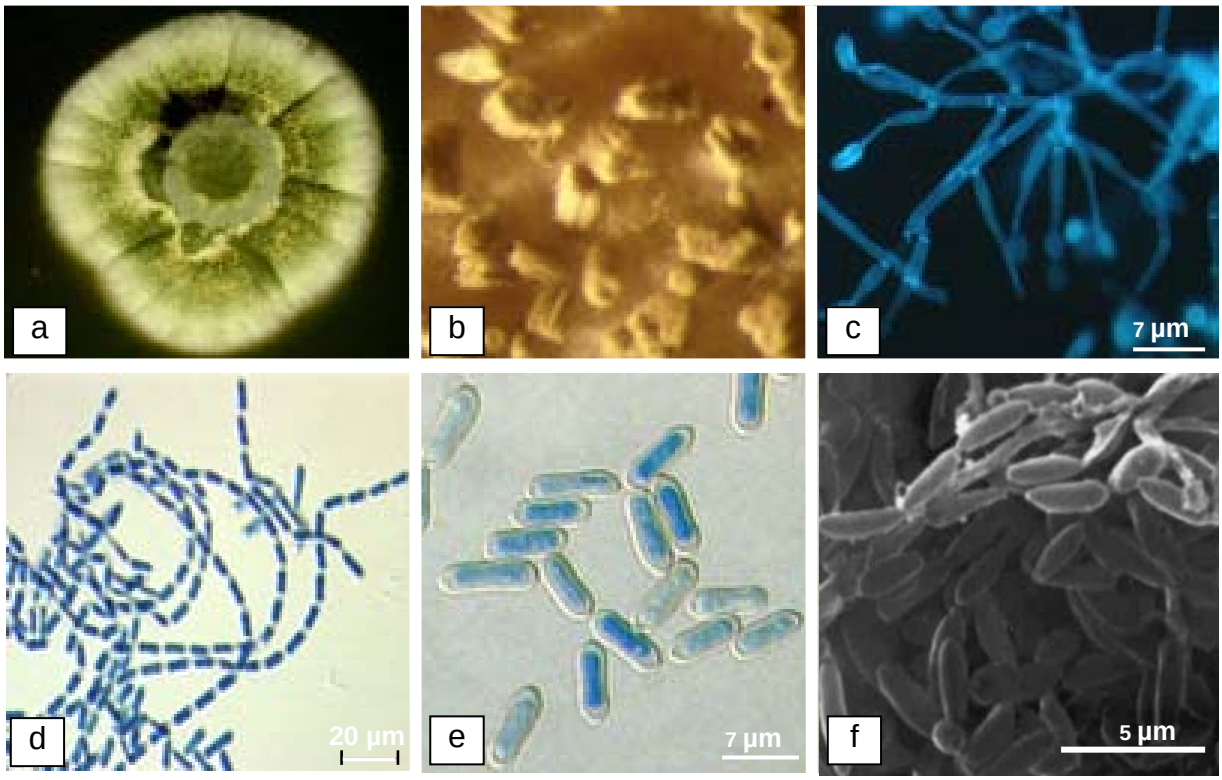


Fig. 11: Morphology of *Metarhizium anisopliae* showing, colony (a), columns of conidia (b), conidiophores stained with 0.05% diethanol, under fluorescence light microscope (c), chain of conidia stained with lectophenol cotton blue, under light microscope (d), conidia stained with lectophenol cotton blue, under light microscope (e), conidia, under scanning electron microscope (f)

3.1.2.2 Physiological characteristics

Physiological characteristics of entomopathogenic fungi are related to their efficiencies. Conidia viability, conidia production, speed of germination, and colony growth are responding to environmental factors, such as temperature, relative humidity, light conditions and ultraviolet light. These characteristics influenced the efficacy of fungal isolates as biological control agents. Therefore, this study's part deals with the results of the experiments, which had been conducted to determine physiological characteristics under different environment factors.

3.1.2.2.1 Evaluation of conidia viability

B. bassiana and *M. anisopliae*, which cultured on MEA medium, were taken every month for one-year to determined percentage germination of conidia. The results indicated that based on the percentage of germination, *M. anisopliae* conidia were viable for at least one year while no

conidia viability of *B. bassiana* conidia was found after 11 months (Fig. 12). The viability of conidia decreased as time increased. The conidial viability of *B. bassiana* decreased more rapidly than *M. anisopliae*.

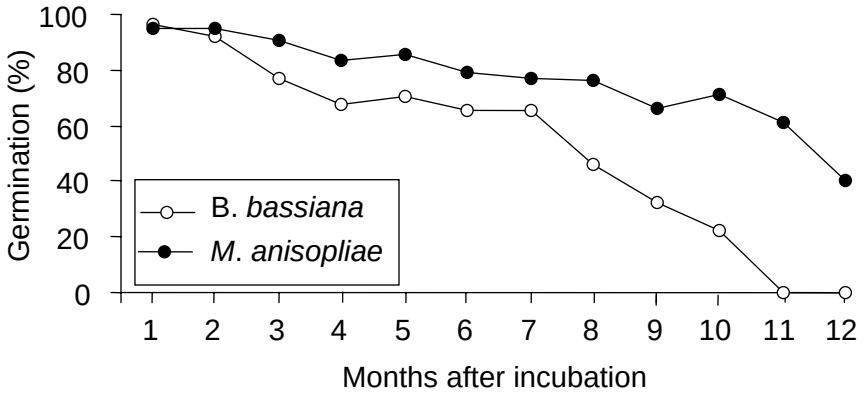


Fig. 12: Percentage of germination of *Beauveria bassiana* and *Metarhizium anisopliae* at different months after incubation in a climatic chamber at 25±1°C temperature, 40±10% RH under complete darkness

3.1.2.2.2 Germination at different environmental factors

3.1.2.2.2.1 Temperature

The germinations of *B. bassiana* and *M. anisopliae* conidia occurred at temperature from 18°C to 35°C, and the optimal ranged from 25°C to 30°C. At the extreme temperatures tested (18°C and 35°C), the germination process delayed but without significant difference ($P \leq 0.05$) among different temperatures at 24 h after incubation. In addition, no significant difference was found in germination between the fungal isolates at all the temperatures (Fig. 13A).

Based on the GT_{50} values, the speed of germination of *B. bassiana* increased from 18°C to 25-30°C (Fig. 13B). At 18°C, the first development of germ tubes occurred at 14 h after incubation and the maximum rate was reached after 20 h. At 25 to 30°C, the germination took places after 8 h and completed after only 16 h. The delay of germination was longer at 18°C than at 35°C. At 18°C, the 1st germ tubes of *M. anisopliae* appeared at 12 h of incubation, the proportion of germinated conidia increased considerably in the following two hours until 20 h of observation, by this time, 100% of conidia had germinated (Fig. 13B).

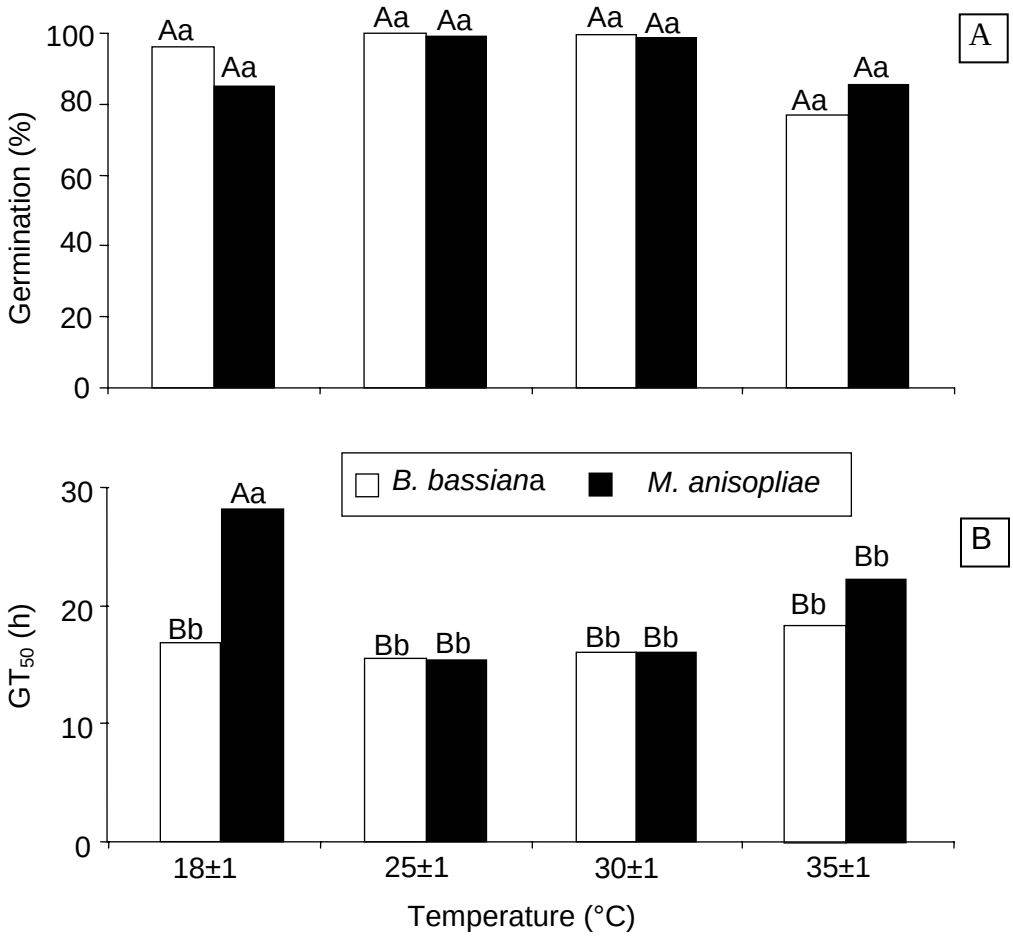


Fig. 13: Percentage germination (A) and GT_{50} (B) of *Beauveria bassiana* and *Metarhizium anisopliae* conidia at different temperatures, 60±10% RH under continuous light on WA medium. [Bars with different capital letters indicate significant differences among temperatures within the same isolate (two factors ANOVA, P≤0.05; Duncan's multiple rang test). Bars with the different small letters indicate significant difference between the two isolates within the same temperature at P≤0.05 (T-test)]

3.1.2.2.2.2 Relative humidity (RH)

The results showed that the conidia germination was relatively humidity-dependent. Percentage germination increased as relative humidity was increased. The germinations of *B. bassiana* conidia were 48, 92, 99 and 99 % at 32, 62, 85 and 100% RH, while those of *M. anisopliae* were 49, 90, 96 and 96%, respectively. Both fungal isolates had significantly (P≤0.05) higher germination at 62, 85 and 100% RH than at 32% RH. No significant difference was found among the germination at 62, 85 and 100% RH, but those at 32% were significantly lower than the three higher humidity (Fig. 14A).

Based on the GT_{50} values, similarly, low relative humidity caused a decrease of the germination speed (Fig. 14B), for example, *B. bassiana* had a germination time of 14.3 h at 100% RH and had the longest one of 32.5 h at low relative humidity of 32% RH.

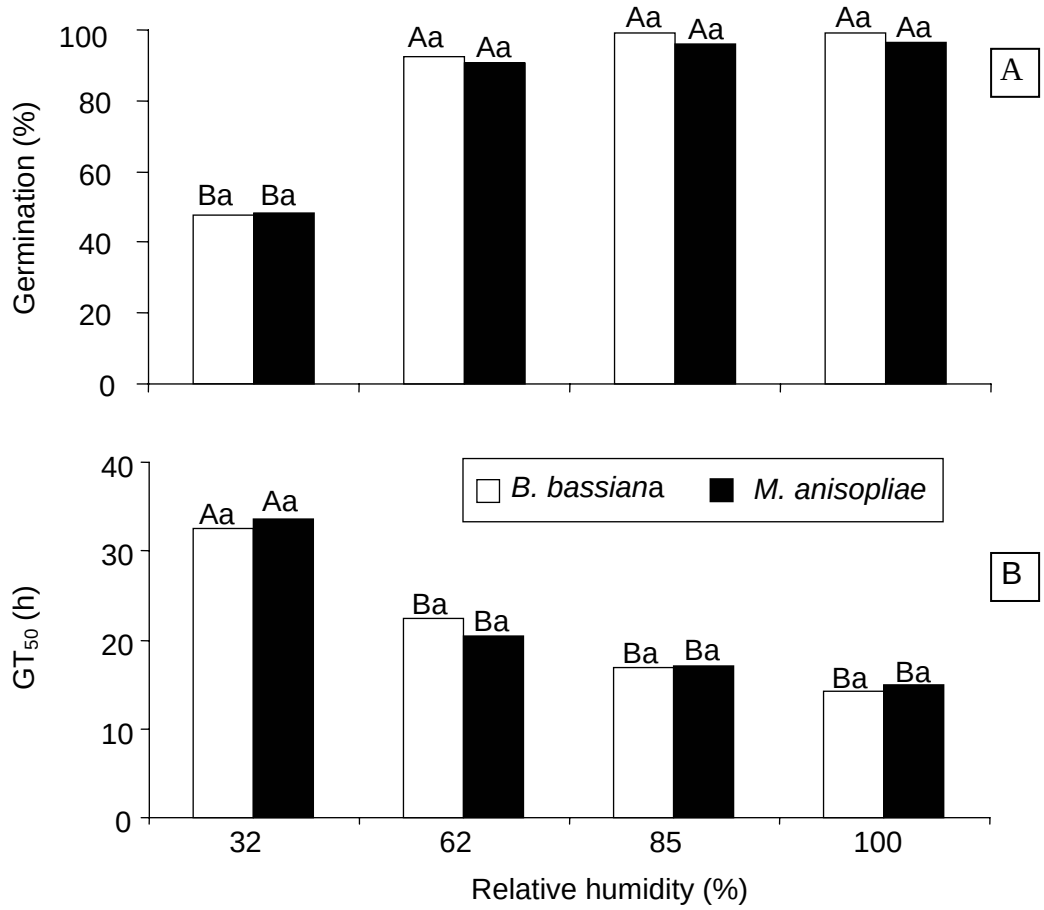


Fig. 14: Percentage germination (A) and GT_{50} (B) of *Beauveria bassiana* and *Metarhizium anisopliae* conidia at different relative humidity, at 25±1°C temperature under continuous light on WA medium. [Bars with different capital letters indicate significant differences among different RHs within the same isolate (two factors ANOVA, $P \leq 0.05$; Duncan's multiple rang test). Bars with the different small letters indicate significant difference between the two isolates within the same RH at $P \leq 0.05$ (T-test)]

3.1.2.2.3.3 Light–dark conditions

Under continuous light, complete darkness as well as 16 h light and 8 h dark, both isolates had very similar germination of 100% at 24 h after incubation (Fig. 15A). However, the germination speeds of both fungal isolates was delayed under dark conditions (Fig. 15B), but no significant difference was revealed by the analysis of variance among these conditions.

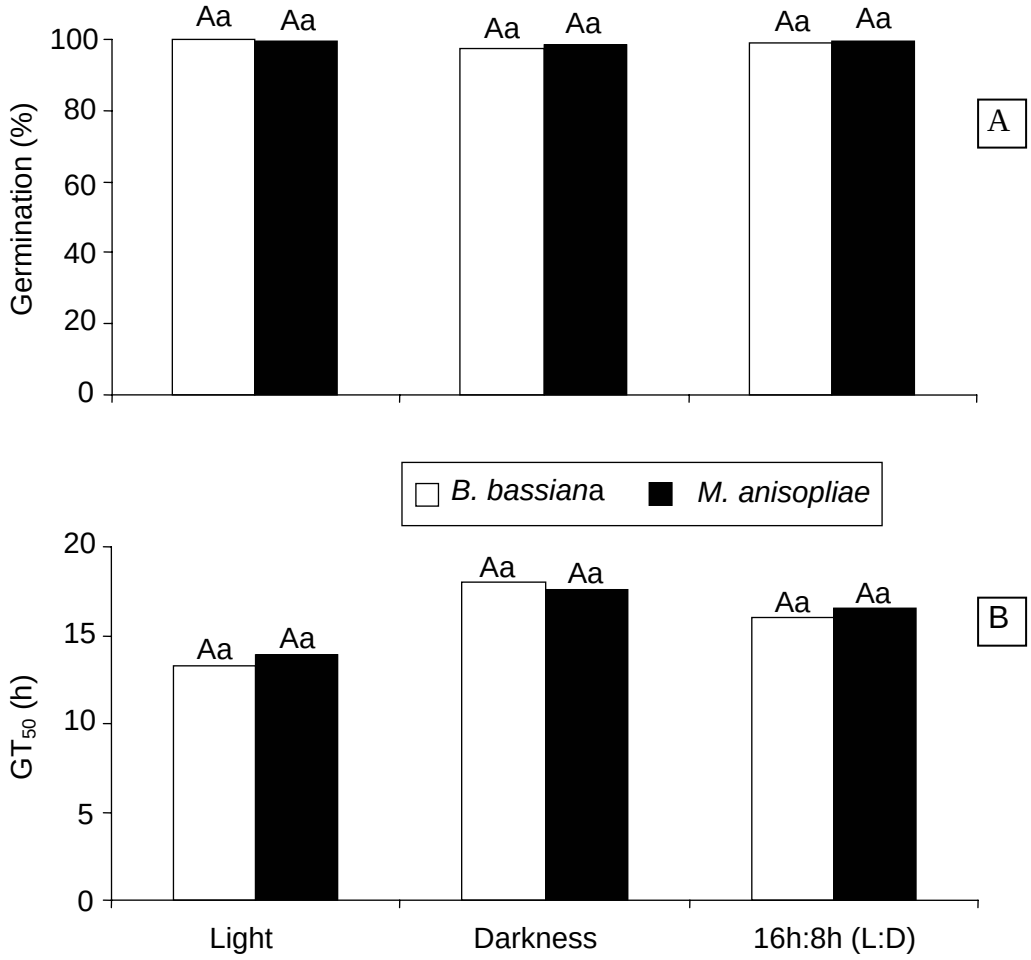


Fig. 15: Percentage germination (A) and GT_{50} (B) of *Beauveria bassiana* and *Metarhizium anisopliae* conidia under different light-dark conditions, at 25±1°C temperature on WA medium. [Bars with the same capital letters indicate no significant difference among different light conditions (two factors ANOVA, P≤0.05; Duncan's multiple rang test). Bars with the same small letters are not significantly different between the two isolates within the same light-dark condition at P≤0.05 (T-test)]

3.1.2.2.2.4 Ultraviolet light (UV)

The exposure to UV light caused a decrease of germination ability. UV radiation doses were inversely proportional to conidia survival. Higher percentages of inhibition were observed on *M. anisopliae* than *B. bassiana*, which means that *B. bassiana* conidia were less sensitive to UV light than those of *M. anisopliae* (Fig. 16A, B).

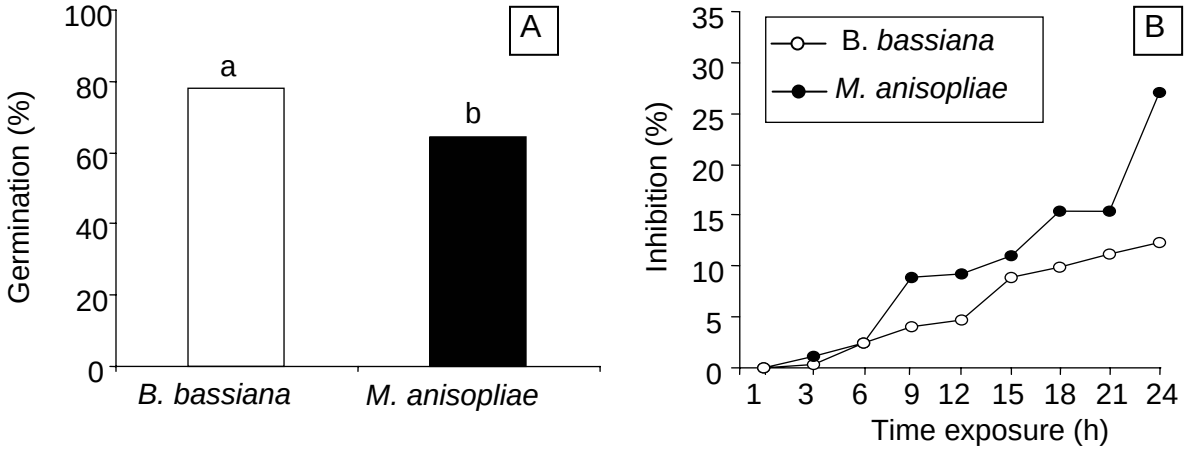


Fig. 16: Percentage of germination of *Beauveria bassiana* and *Metarhizium anisopliae* conidia on WA medium after 24 h exposure to UV light (A) and percentage of inhibition at different exposure times of UV light (B) at 25±1°C temperature. Bars with the same letters are not significantly different between the two isolates within the same light-dark conditions at P≤0.05 (T-test)]

3.1.2.2.3 Conidiation at different environmental factors

This study's part deals with the results of the experiments, which were conducted to assess the influences of temperature, relative humidity, light-dark conditions and UV light on conidiation of *B. bassiana* and *M. anisopliae.*

3.1.2.2.3.1 Temperature

The results showed that the conidiation of *B. bassiana* and *M. anisopliae* on MEA medium incubated at different temperatures, 60±10% RH under continuous light were dependent on temperature. The conidiation process of *B. bassiana* occurred faster than that of *M. anisopliae*. *B. bassiana* produced conidia on MEA medium within 3 days at 25±1, 30±1 and 35±1°C. The timing and abundance of conidia production varied among different fungi and temperatures. *B. bassiana* produced more abundant conidia than *M. anisopliae*, where the conidia optimal production mostly at 25±1°C, followed by 18±1 and 30±1°C, respectively. In contrast, *M. anisopliae* produced conidia most abundantly at 30±1°C, followed by 25±1 and 18±1°C, respectively. In view of the present results, *M. anisopliae* could produce conidia at 35±1°C, while *B. bassiana* did not produce any at this temperature (Fig. 17). For *B. bassiana*, conidia production increased with max production at 14 days whereas that of *M. anisopliae* gradually increased even at 21 days after incubation.

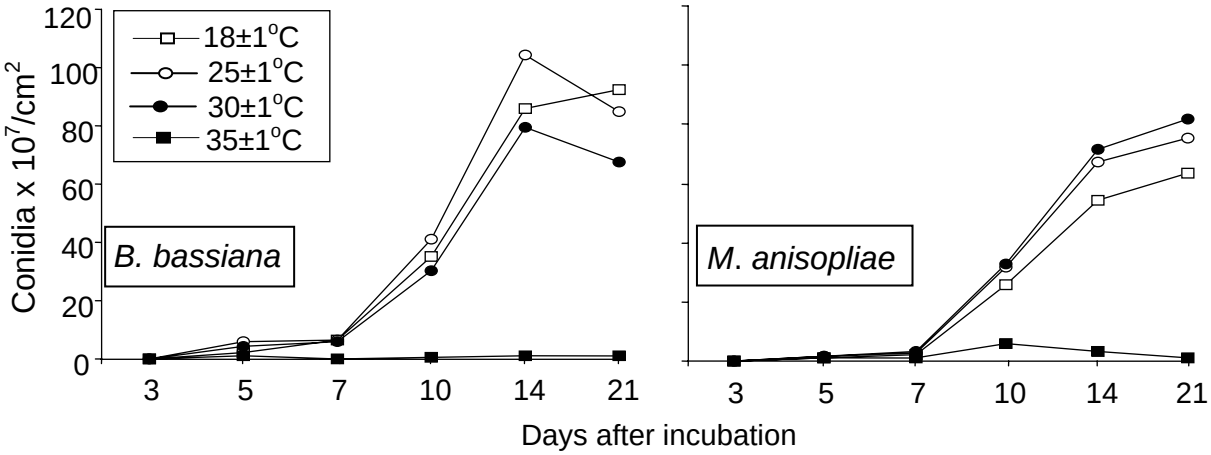


Fig. 17: Conidia production of *Beauveria bassiana* and *Metarhizium anisopliae* cultured on MEA medium and incubated at different temperatures

3.1.2.2.3.2 Relative humidity

Figure 18 shows the conidiation of *B. bassiana* and *M. anisopliae* cultured on MEA medium and incubated at different relative humidity under continuous light. *B. bassiana* started producing conidia from the third day after incubation. The timing and abundance of conidia production varied among different fungi and relative humidity. *B. bassiana* produced conidia more abundantly than *M. anisopliae* with peak production at 100% RH, followed by 85, 62 and 32% RH with 5.2×10^8, 5.9×10^8, 8.0×10^8 and 8.1×10^8 conidia/cm^2 at 14 days after incubation, respectively. *M. anisopliae* had the same tend as *B. bassiana* that the conidiation increased with increasing relative humidity, in which the conidiation for the four levels of relative humidity ranged from 3.3×10^8 to 4.3×10^8 conidia/cm^2 after 14 days incubation.

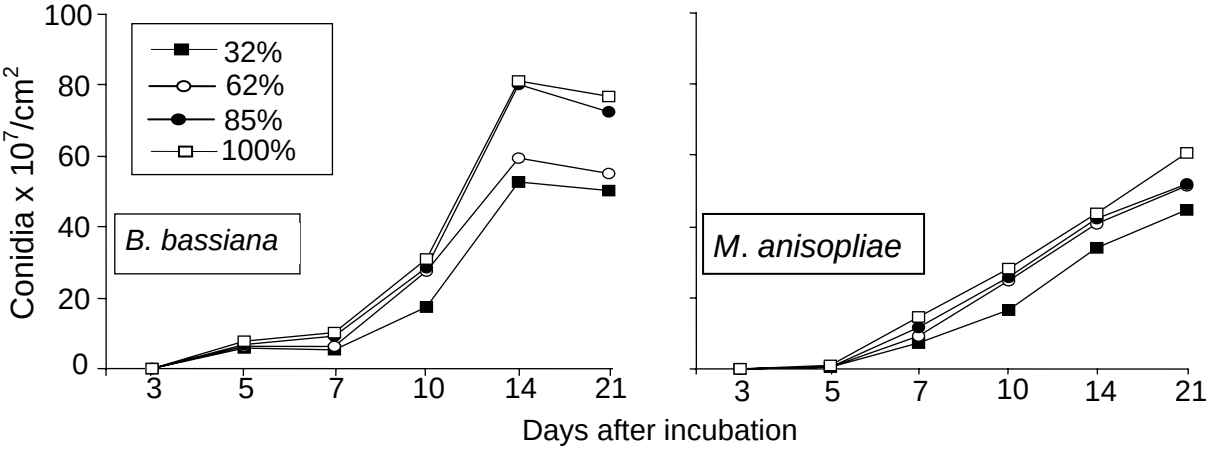


Fig. 18: Conidia production of *Beauveria bassiana* and *Metarhizium anisopliae* cultured on MEA medium and incubated at different relative humidity, at 25±1°C temperature

3.1.2.2.3.3 Light-dark conditions

The experiments of *B. bassiana* and *M. anisopliae* under different light conditions showed that, both of fungal isolates produced more conidia under continuous light than the others. The conidiation of *B. bassiana* and *M. anisopliae* under continuous light were 1.0×10^9 and 8.9×10^8 conidia/cm^2 after 14 days incubations, respectively. The lowest conidiation was found under complete darkness, with 6.6×10^8 conidia/cm^2 for *B. bassiana* and 3.5×10^8 conidia/cm^2 for *M. anisopliae* after 14 days incubation (Fig. 19). Peak conidiation rates were observed at day 14 for *B. bassiana*, whereas *M. anisopliae* still produced conidia after 21 days of incubation. Furthermore, light-dark conditions more affected the conidiation of *M. anisopliae* than *B. bassiana*.

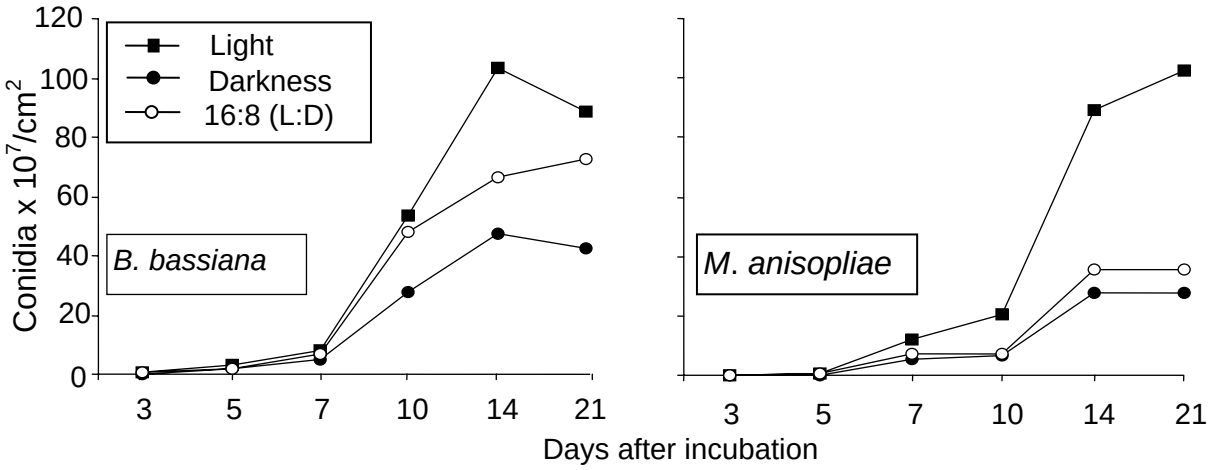


Fig. 19: Conidia production of *Beauveria bassiana* and *Metarhizium anisopliae* cultured on MEA medium and incubated under different light conditions, at 25±1°C temperature

3.1.2.2.3.4 Ultraviolet light (UV)

Ultraviolet light affected the conidiation of both fungi. *M. anisopliae* had greater response to UV light than *B. bassiana*. After 14 days of exposure to UV light, conidia productions were 6×10^6 and 2.7×10^6 conidia/cm^2 for *B. bassiana* and *M. anisopliae*, respectively (Fig. 20 A). Ultraviolet light inhibited the conidiation of *M. anisopliae* more than *B. bassiana* (Fig. 20 B).

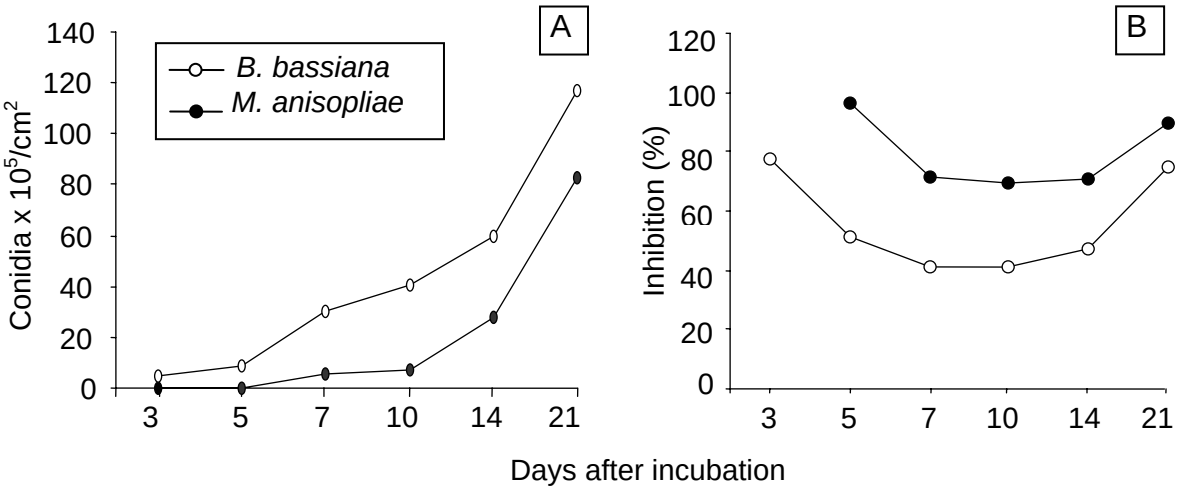


Fig. 20: Conidia production (A) and percentage inhibition (B) of *Beauveria bassiana* and *Metarhizium anisopliae* cultured on MEA medium under ultraviolet light, at 25±1°C

3.1.2.2.4 Colony growth at different environmental factors

3.1.2.2.4.1 Temperature

The colony growths of *B. bassiana* and *M. anisopliae* occurred at all the temperatures, but they were slower at 18±1 and 35±1°C as compared to 25±1 and 30±1°C. The growth was significantly affected by temperature (P≤0.05). The optimum temperature for growth of both isolates ranged between 25-30°C (Fig. 21).

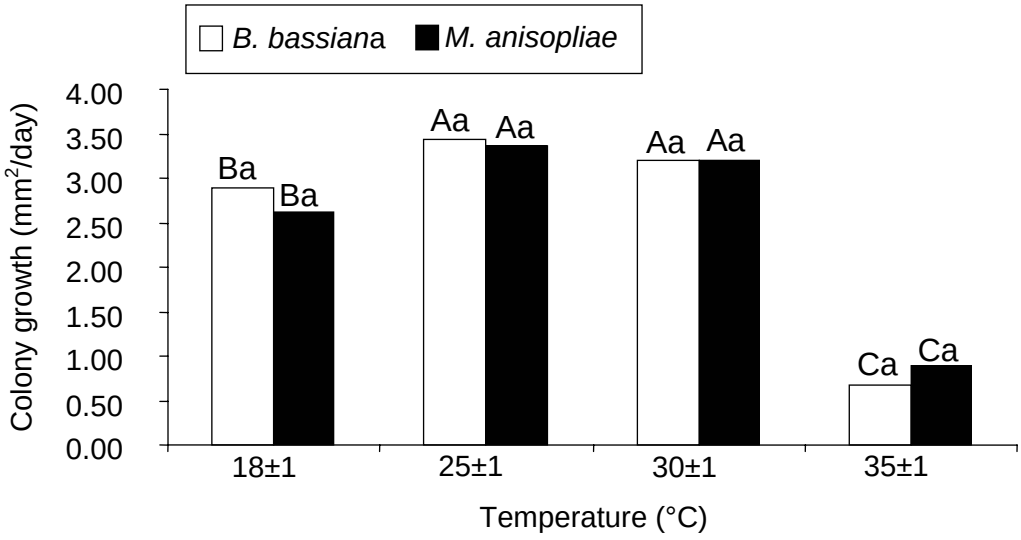


Fig. 21: Colony growth per day at different temperatures cultured on MEA medium. [Bars with different capital letters indicate significant differences among different temperatures within the same isolate (two factors ANOVA, P≤0.05; Duncan's multiple rang test). Bars with the same small letters are not significantly different between the two isolates within the same temperature at P≤0.05 (T-test)].

3.1.2.2.4.2 Relative humidity

The results effect of relative humidity on colony growth showed the same tendency as temperature, the colony growth of the fungi occurred at all relative humidity, but the rate of colony growth was significantly ($P \leq 0.05$) affected by relative humidity. The colony growth was slower at 32% RH compared to 62, 85 and 100% RH. The optimal relative humidity for colony growth of both fungi was 100% RH (Fig. 22).

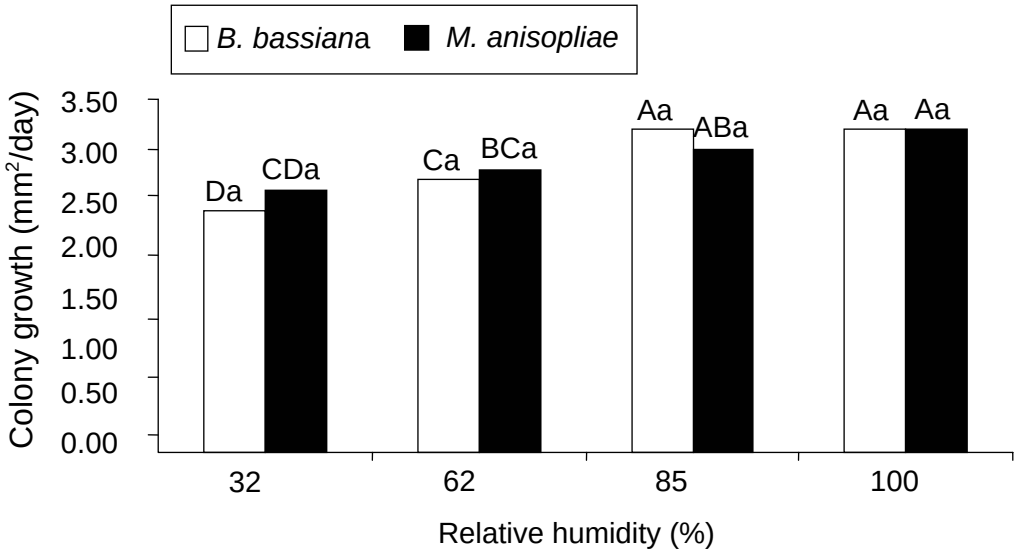


Fig. 22: Colony growth per day at different relative humidity cultured on MEA medium at 25±1°C temperature [Bars with different capital letters indicate significant differences among different relative humidity within the isolate (two factors ANOVA, $P \leq 0.05$, Duncan's multiple rang test). Bars with the small letters are not significantly different between the two isolates within the same relative humidity at $P \leq 0.05$ (T-test)]

3.1.2.2.4.3 Light-dark conditions

Figure 23 shows the colony growth under continuous light, complete darkness and 16 h of light 8 h of dark. The colony growth was faster under a continuous light than darkness and 16 h of artificial light. The colony growths of *B. bassiana* was 3.6, 2.6 and 2.5 mm^2/day under continuous light, 16 h of light and complete darkness, respectively, while colony growth of *M. anisopliae* was 3.0, 1.6 and 1.4 under conditions mentioned previously. There were significant differences ($P \leq 0.05$) in colony growth and fungal isolates between continuous light and complete darkness as well as 16 h of light.

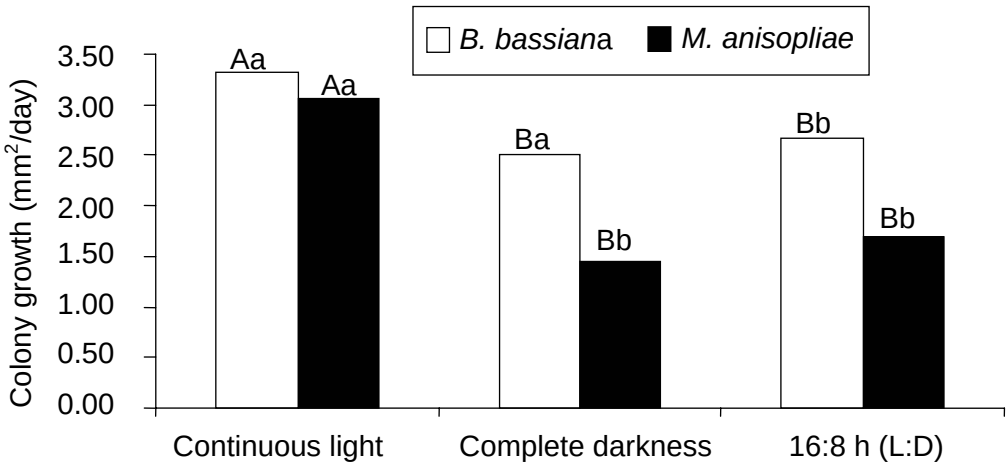


Fig. 23: Colony growth per day under light-dark conditions cultured on MEA medium at 25±1°C temperature. [Bars with different capital letters indicate significant differences between light-dark conditions within the same isolate (two factors ANOVA, P≤0.05, Duncan's multiple rang test). Bars with different small letters indicate significant differences between the isolates within the same light-dark conditions at P≤0.05% (T-test)]

3.1.2.2.4.4 Ultraviolet light (UV)

The differences between the colony growths of *B. bassiana* and *M. anisopliae* under ultraviolet light are illustrated in figure 24A. Ultraviolet light affected the colony growths of both fungi. The colony growth was 2.4 and 2.3 mm^2/day for *B. bassiana* and *M. anisopliae,* respectively. *B. bassiana* had more response to the ultraviolet radiation than *M. anisopliae,* but there was no significant difference between them (P≤0.05) (Fig. 24B).

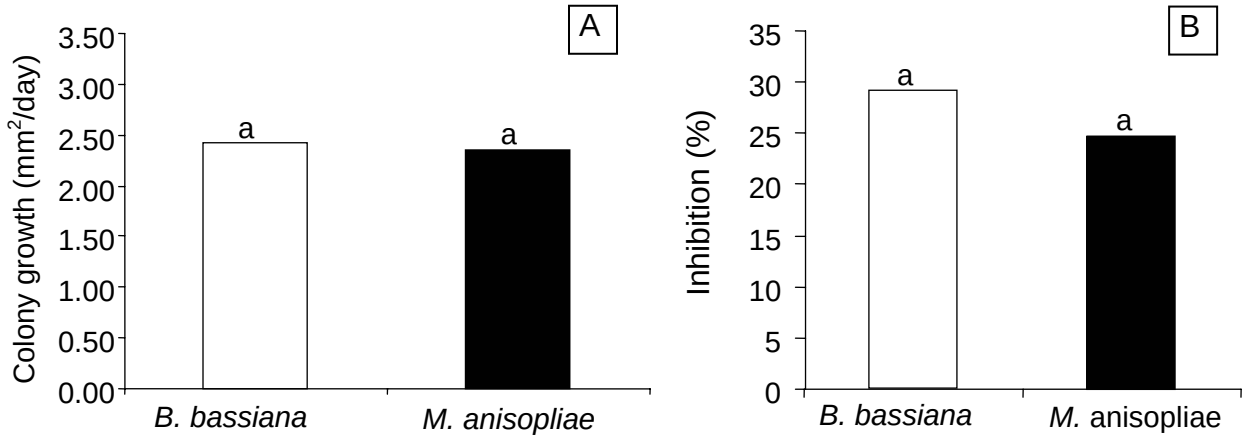


Fig. 24: Colony growth per day (A) and percentage inhibition (B) under exposure to ultraviolet light cultured on MEA medium. [Bars with the same letters are not significantly different between *Beauveria bassiana* and *Metarhizium anisopliae* at P≤0.05% (T-test)]

3.1.3 Mode of infection of *Beauveria bassiana* Bb.5335 and *Metarhizium anisopliae* Ma.7965 to *Frankliniella occidentalis*

This part of study deals with the results of the experiments, which were conducted to examine the infection process of *B. bassiana* and *M. anisopliae* on thrips, involving the followed experiments: attachment of conidia on the thrips cuticle, their germination, germ tube formation, penetration and internal colonization culminating in the host death.

3.1.3.1 Attachment of conidia

The conidia of either fungus was capable of binding to any site on the cuticle of thrips， but was frequently trapped by the setae that were particularly numerous on the wings and legs. Significantly, ($P \leq 0.05$) higher conidia number was observed on abdomen, followed by the legs and head/wings (Fig. 25). The conidia of *B. bassiana* and *M. anisopliae* randomly distributed over all the body regions of *F. occidentalis* cuticle after inoculation (Fig. 26), such as intersegmental folds, legs as well as antennae.

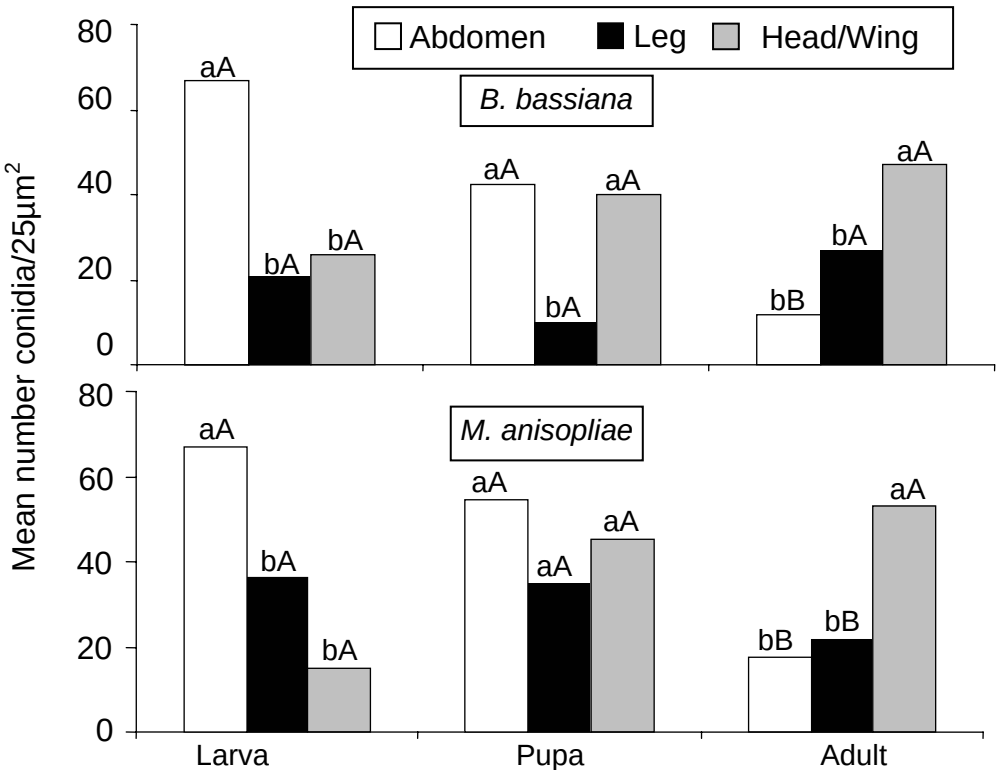


Fig. 25: Mean conidia number of *Beauveria bassiana* and *Metarhizium anisopliae* conidia on 25µm² cuticle of various life stages of *Frankliniella occidentalis* at 24 h after inoculation. [Bars with different small letters indicate significant differences among different body regions. Bars with capital letters indicate significant differences among different life stages (one way ANOVA, $P \leq 0.05$; Duncan's multiple rang test)]

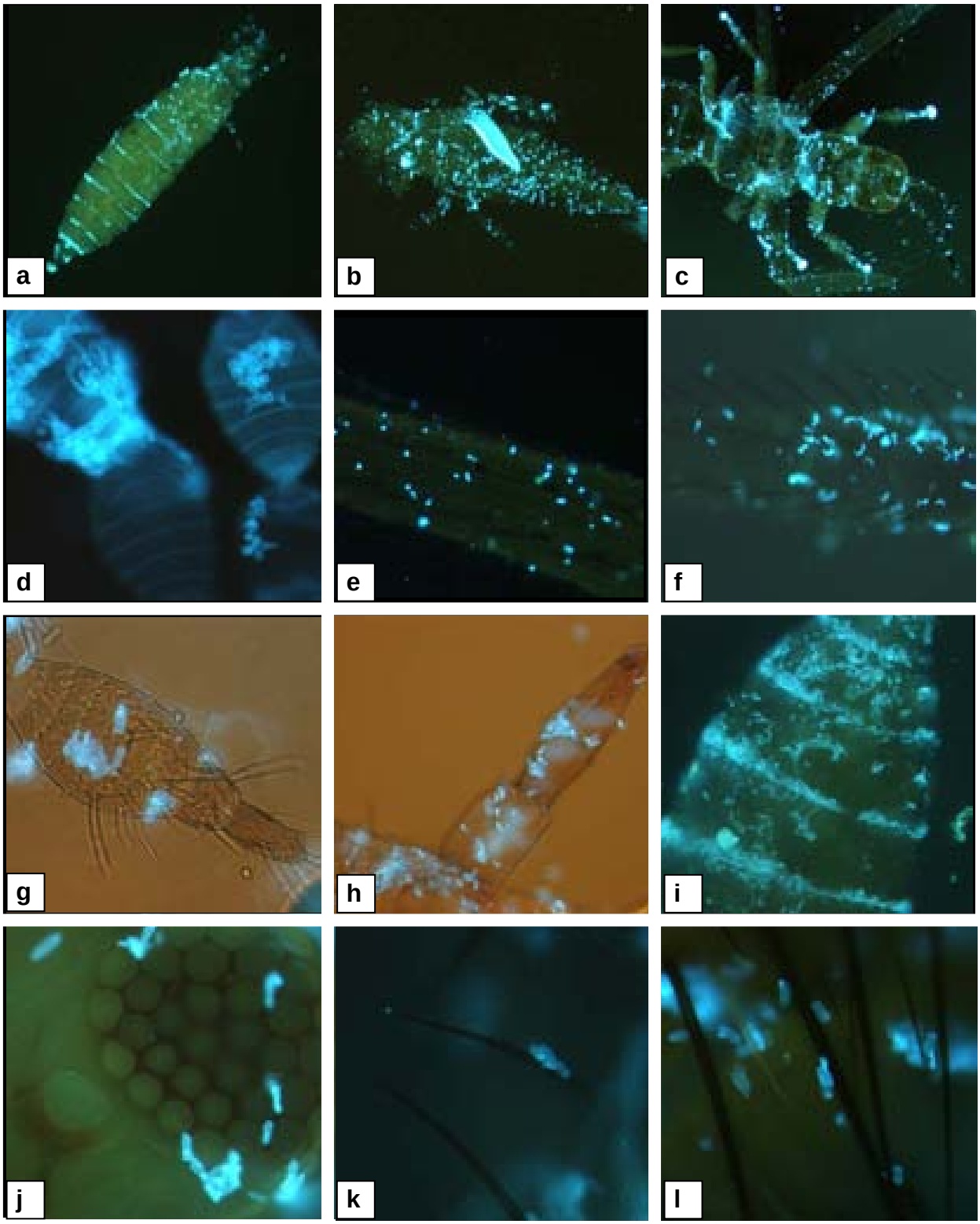


Fig. 26: *Beauveria bassiana* and *Metarhizium anisopliae* conidia attached on body region of *Frankliniella occidentalis* under florescence light microscope. On larva (a), on pupa (b), on adult (c), *B. bassiana* conidia attached on antenna (d), on wing (e), *M. anisopliae* conidia attached on wing (f), on antenna (g), on leg, (h), intersegmental fold abdomen of larva (i), on eye, (j) on setae (k), on caudal (l)

3.1.3.2 Germination and evaluation of germ tube on cuticle

Table 6 represents percentages of germination of *B. bassiana* and *M. anisopliae* conidia on various life stage of *F. occidentalis* cuticle at different temperature. Both fungi germinated on cuticle of all life stages started at 12 h after inoculation, and continued up to 24 h after inoculation. Significant differences ($P \leq 0.05$) were found among life stages and temperatures. At 12 h after inoculation, *B. bassiana* germinated on cuticle of larva, pupa and adult of 67.48, 39.23 and 14.28% at 18°C, 98.33, 56.33 and 26.64% at 25°C while at 30°C of 98.67, 64.48 and 27.33% and of 99.33, 45.21 and 26.67% at 35°C, respectively. While, those of *M. anisopliae* were 56.98, 99.33, 99.77 and 100%, respectively, on larva, on pupa were 22.67, 33.33, 45.54 and 51.26%, and on adult were 15.15, 22.45, 23.31 and 25.15% at 18, 25, 30 and 35°C respectively. The time required for conidia germination on larval cuticle almost occurred after 12 h at all temperature treatments, while 24 h was necessary for germination on both pupal and adult stages, and at 18°C the germination of conidia was longer than other temperature tests. The fungal conidia could germinate on all regions of insect body surface without any apparent preference.

At all temperature tests, the first germ tube appeared at 12 h after inoculation. The evaluation of germ tube consisted of elongation and shape of germ tube was temperature and life stage-dependent (Tab. 6 and Fig. 27, 28). There were significant differences ($P \leq 0.05$) in elongation of germ tube between temperatures and life stages. For example, the germ tubes of *B. bassiana* at 24 h after inoculation was 6.1, 3.0 and 2.2 µm on larva pupa and adult stages at 18°C, while at 25°C were 13.0, 6.8, and 4.1 µm on life stages as mentioned above (Tab. 6). The elongation of germ tube of *M. anisopliae* showed the same tendency as *B. bassiana* that elongation of germ tube varied with temperatures and life stages. The longest germ tubes of *M. anisopliae* were found on larva at 30°C while, the shortest on adult at 18°C were observed.

Furthermore, there were differences in shape of germ tube between temperatures on *M. anisopliae* were observed while *B. bassiana* did not (Fig. 27, 28). The results also showed that conidia of *B. bassiana* and *M. anisopliae* could produce germ tubes on all the places where they had attached.

Tab. 6: Percentage germination and elongation of germ tube of *Beauveria bassiana* and *Metarhizium anisopliae* on *Frankliniella occidentalis* cuticle on various life stages and different temperatures, 12 and 24 h after inoculation

Time (h)	Temperature (°C)	Germination and elongation of conidia at different life stages					
		Larva		Pupa		Adult	
		Germination (%±SE)	Elongation (µm±SE)	Germination (%±SE)	Elongation (µm±SE)	Germination (%±SE)	Elongation (µm±SE)
Beauveria bassiana							
12	18±1	67.48±3.15 bA	-	39.23±3.89 bB	-	14.28±5.00 aC	-
	25±1	98.33±1.26 aA	-	64.48±3.78 aB	-	26.64±1.13 aC	-
	30±1	98.67±1.18 aA	-	56.33±3.78 aB	-	27.33±5.67 aC	-
	35±1	99.33±0.05 aA	-	45.21±2.21 abB	-	26.67±2.12 aC	-
24	18±1	98.89±0.89	6.1±1.2 bA	100.00±0.00	3.0±3.3aB	89.67±1.08	2.2±2.3 aB
	25±1	100.00±0.00	13.0±2.3 aA	99.87±0.08	6.8±4.3aB	90.36±1.58	4.1±3.6 aB
	30±1	100.00±0.0	11.3±1.4 aA	100.00±0.00	5.6±1.9aB	96.67±1.63	3.7±2.6 aB
	35±1	99.33±0.55	9.4±2.3 aA	98.99±0.05	4.9±2.3aB	98.29±2.00	4.2±4.3 aA
Metarhizium anisopliae							
12	18±1	56.98±3.15 bA	-	22.67±1.11 bB	-	15.15±6.30 aB	-
	25±1	99.33±0.09 aA	-	33.33±3.58 abB	-	22.45±5.15 aB	-
	30±1	99.77±0.03 aA	-	45.54±2.25 aB	-	23.31±1.31 aC	-
	35±1	100.00±0.00 aA	-	51.26±5.18 aB	-	25.15±1.51 aC	-
24	18±1	99.83±0.02	4.9±1.3 aA	100.00±0.00	4.9±2.3 aA	98.89±0.09	1.2±1.3 aB
	25±1	100.00±0.00	8.8±2.3 aA	99.33±0.59	6.3±3.5 aA	97.56±2.14	3.4±2.2 aB
	30±1	100.00±0.00	9.4±3.4A a	99.67±0.19	6.1±1.3 aA	100.00±0.00	3.5±1.9 aB
	35±1	100.00±0.00	8.9±4.3 aA	100.00±0.00	5.0±2.8 aB	100.00±0.00	2.3±2.3 aB

Means in columns with different small letters indicate significant differences among different temperatures within the same life stage. Means in row with different capital letters indicate significant differences among different life stages within the same temperature (two factors ANOVA, $P \leq 0.05$; Duncan's multiple rang test).

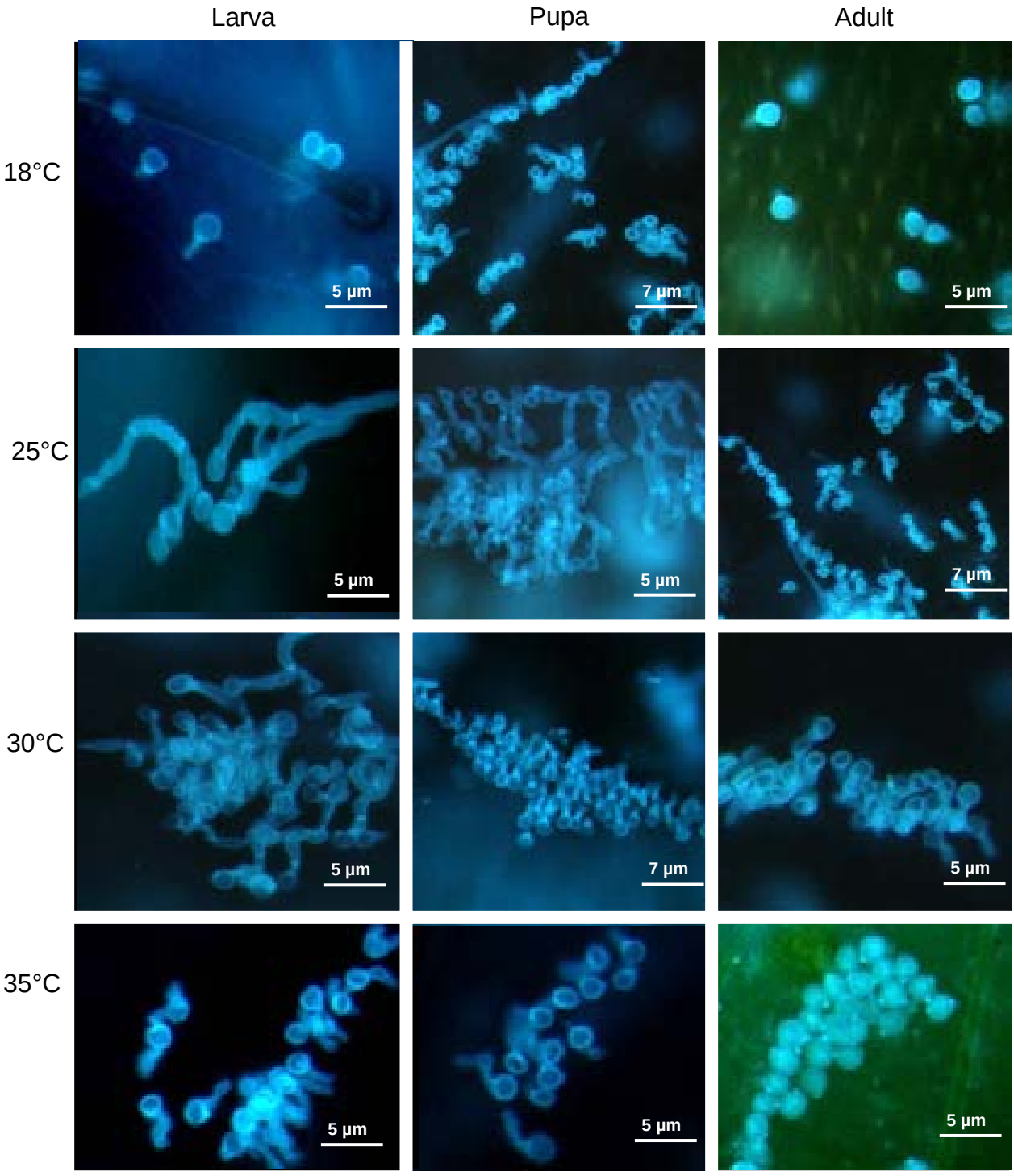


Fig. 27: The germinated conidia of *Beauveria bassiana* at different temperatures on various life stages of *Frankliniella occidentalis* cuticle, 24 h after inoculation under fluorescence light microscope

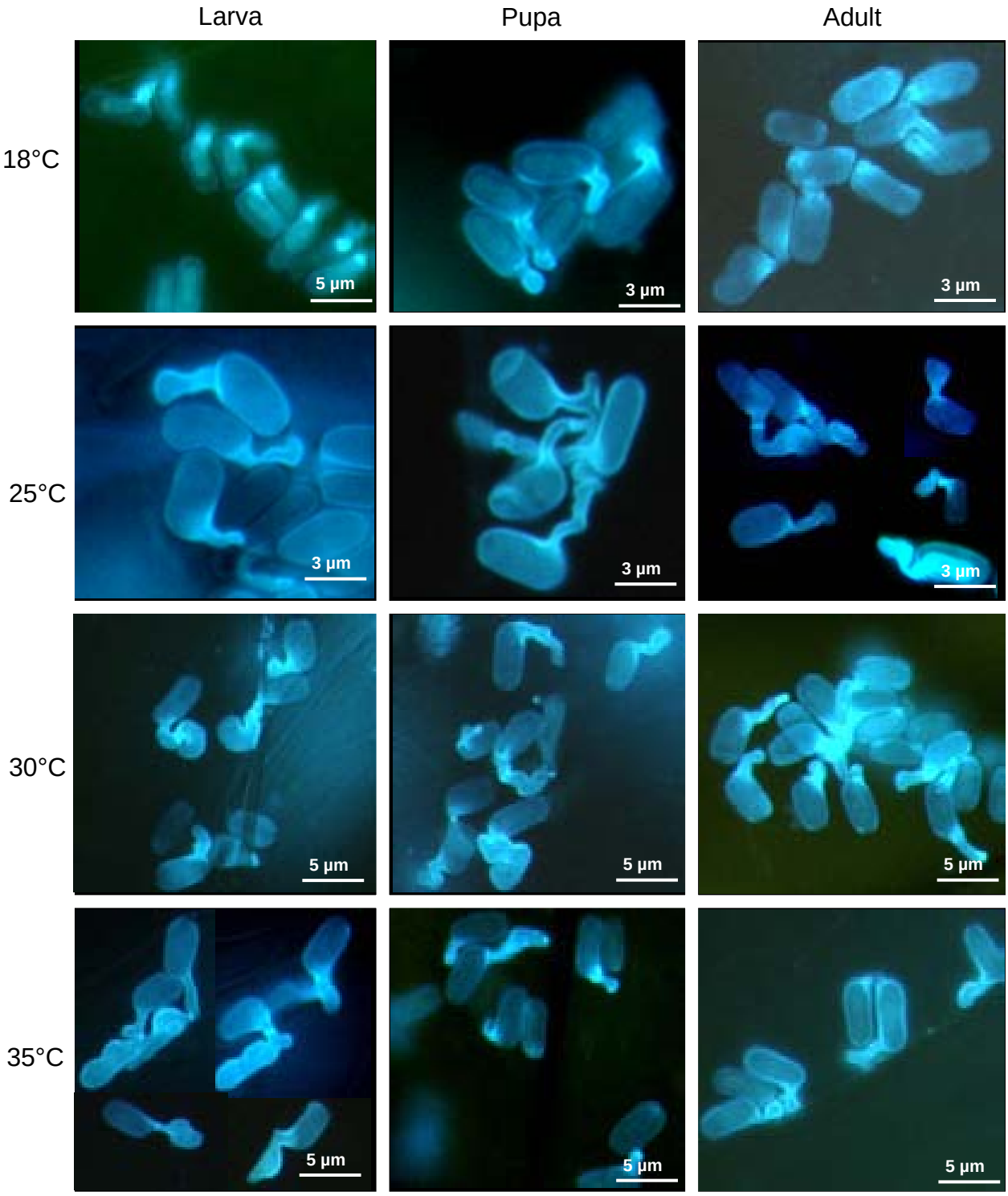
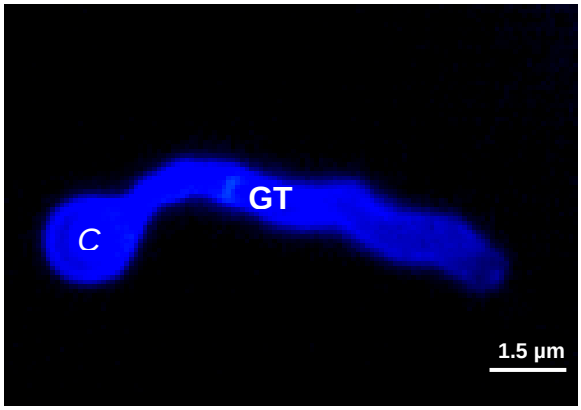


Fig. 28: The germinated conidia of *Metarhizium anisopliae* at different temperatures on various life stages of *Frankliniella occidentalis* cuticle, 24 h after inoculation under fluorescence light microscope

3.1.3.3 Penetration of the fungal into the cuticle

The results on fungal penetration into cuticle of *F. occidentalis* showed that the germinated conidia of *B. bassiana* penetrated into the cuticles started at 36 h after inoculation. Germinated conidia were not formed appressoria, the fungi pierced by direct hyphal penetration (Fig. 29). The percentage and speed of penetration were dependent on the life stage and temperature (Tab. 7, Fig. 30). On Larvae, the majority of successful penetrations occurred in the abdominal region, whereas on pupae were often found on thorax and legs. In addition, the penetration sites on adults were noted on the head, thorax, abdomen and the thickest part of the wings. There were significant differences ($P \leq 0.05$) in percentage penetration among temperatures. At low temperature (18°C), the time required for penetration into thrips was longer than higher temperature (Tab. 7).



C=conidium,
GT=germ tube

Fig. 29: The germinated conidium of *Beauveria bassiana* without forming appressoria on the cuticular surface of *Frankliniella occidentalis* under fluorescence light microscope

Tab. 7: Percentage penetration of *Beauveria bassiana* and *Metarhizium anisopliae* into the cuticle of *Frankliniella occidentalis* at different temperatures and various life stages, 36 h after inoculation

Temperature (°C)	Penetration (%±SE) at different life stages					
	Beauveria bassiana			*Metarhizium anisopliae*		
	Larva	Pupa	Adult	Larva	Pupa	Adult
18±1	72.57±5.56 bA	60.00±2.02 bA	9.15±2.95 bB	55.58±2.21 bA	43.25±3.13 bA	0.00±0.00 bB
25±1	98.77±1.18 aA	89.97±1.25 aA	27.23±2.56 aB	99.97±0.03 aA	79.67±2.89 aB	21.00±3.33 bC
30±1	98.23±1.15 aA	90.03±1.11 aA	30.30±1.45 aB	100.00±0.00 aA	89.98±3.36 aA	19.27±2.25 bB
35±1	99.41±0.05 aA	92.58±5.36 aA	39.67±2.28 aB	100.00±0.00 aA	89.55±4.28 aA	13.33±2.12 bB

Means in columns with different small letters indicate significant differences among different temperatures within the same life stage. Means in rows with different capital letters indicate significant differences among different life stages within the same temperature (two factors ANOVA, $P \leq 0.05$; Duncan's multiple rang test)

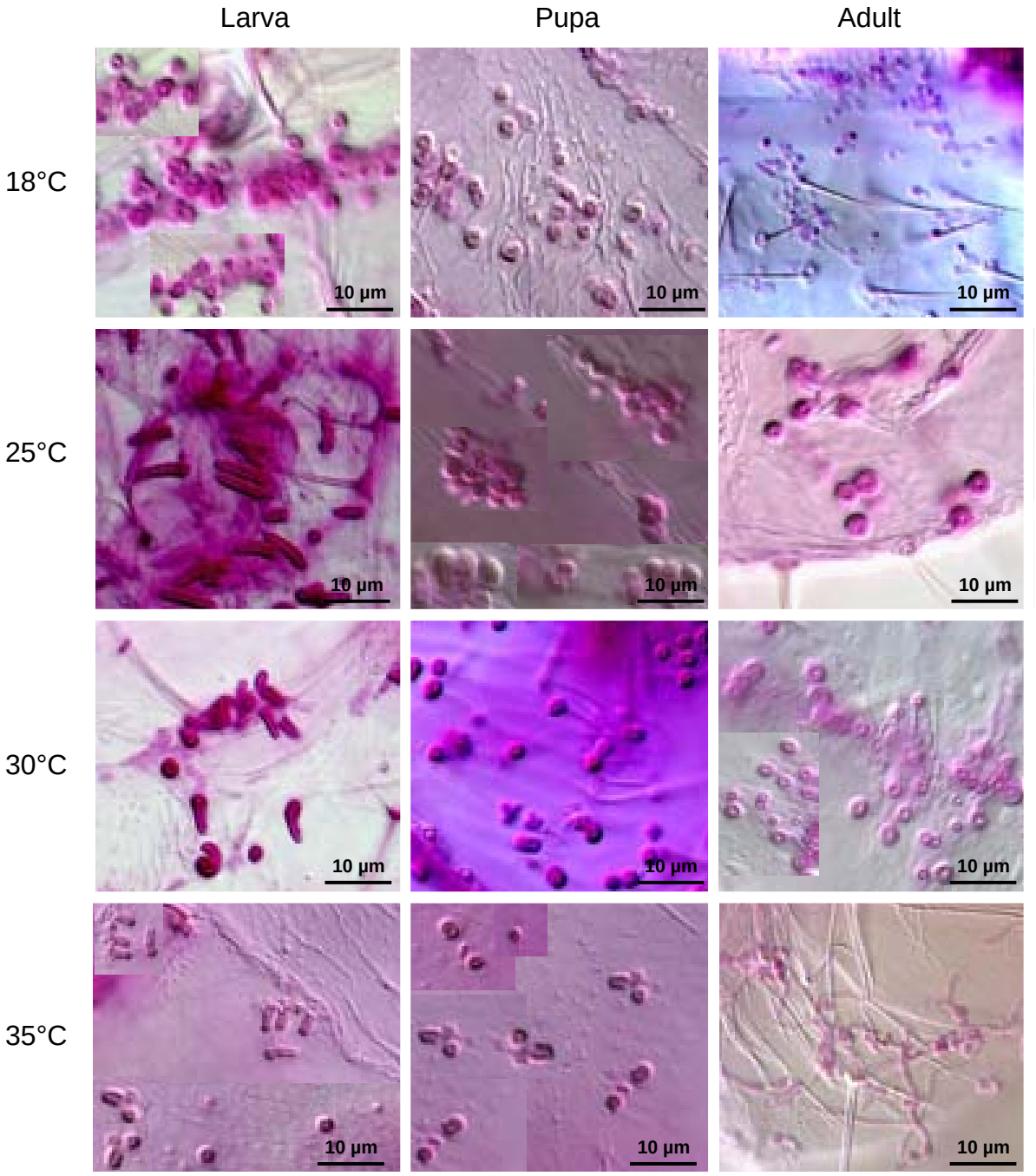


Fig. 30: *Beauveria bassiana* penetrated into the cuticle of various life stages of *Frankliniella occidentalis* under different temperatures, 36 h after inoculation under light microscope

Germinated conidia of *M. anisopliae* produced appressorium in two distinct manners: the appressorium was formed near the conidium, or the conidium firstly produced a long germ tube and then the appressorium was formed at the end of the germ tube. Appressoria were different in

shape, i.e. globose or clavate (Fig. 31a-b). Although the conidia produced appressoria all over the body regions, the formation of the appressoria appeared to be concentrating on the intersegmental areas of the abdomen. The conidia on the head, thorax or wings generally produced the second type of appressoria. These were possibly owing to the cuticle flexibility on different parts of the larva, where the head and thorax were more rigid than the intersegmental areas on the abdomen.

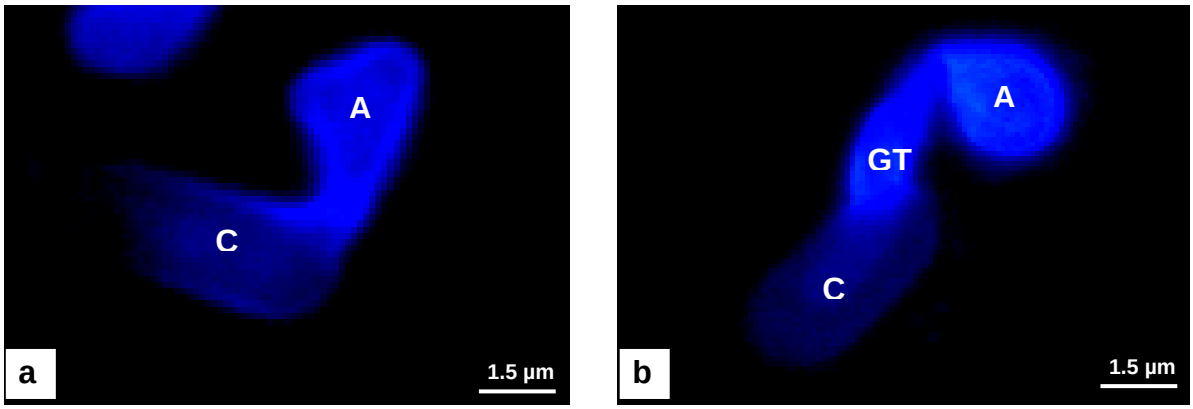


Fig. 31: The germinated conidium of *Metarhizium anisopliae* on the cuticular surface of *Frankliniella occidentalis* under fluorescence light microscope. Appressorium formed near the conidium (a), Appressorium formed at the end of germ tube (b) C=conidium, GT=germ tube, A=appressorium

M. anisopliae penetrated the cuticle by producing penetration pegs. A short penetration peg could be produced by a conidium and then penetrated the cuticle directly, but most often a narrow peg was produced by an appressorium (Fig. 32).

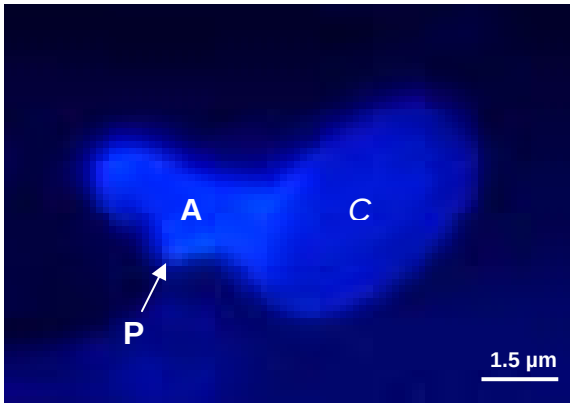


Fig. 32: Penetration peg of *Metarhizium anisopliae* on the cuticular surface of *Frankliniella occidentalis* under fluorescence light microscope
C = conidium
A = appressorium,
P = penetration peg

Almost any part of thrips body could be penetrated, particularly the base of hair sockets. However, the first penetration was found on intersegmental membrane. The penetration peg entering the *F. occidentalis* cuticle was dependent on body region and temperature. Moreover,

the percentage and speed of penetration on *M. anisopliae* were also dependent on the life stages and temperature (Tab. 7, Fig. 33).

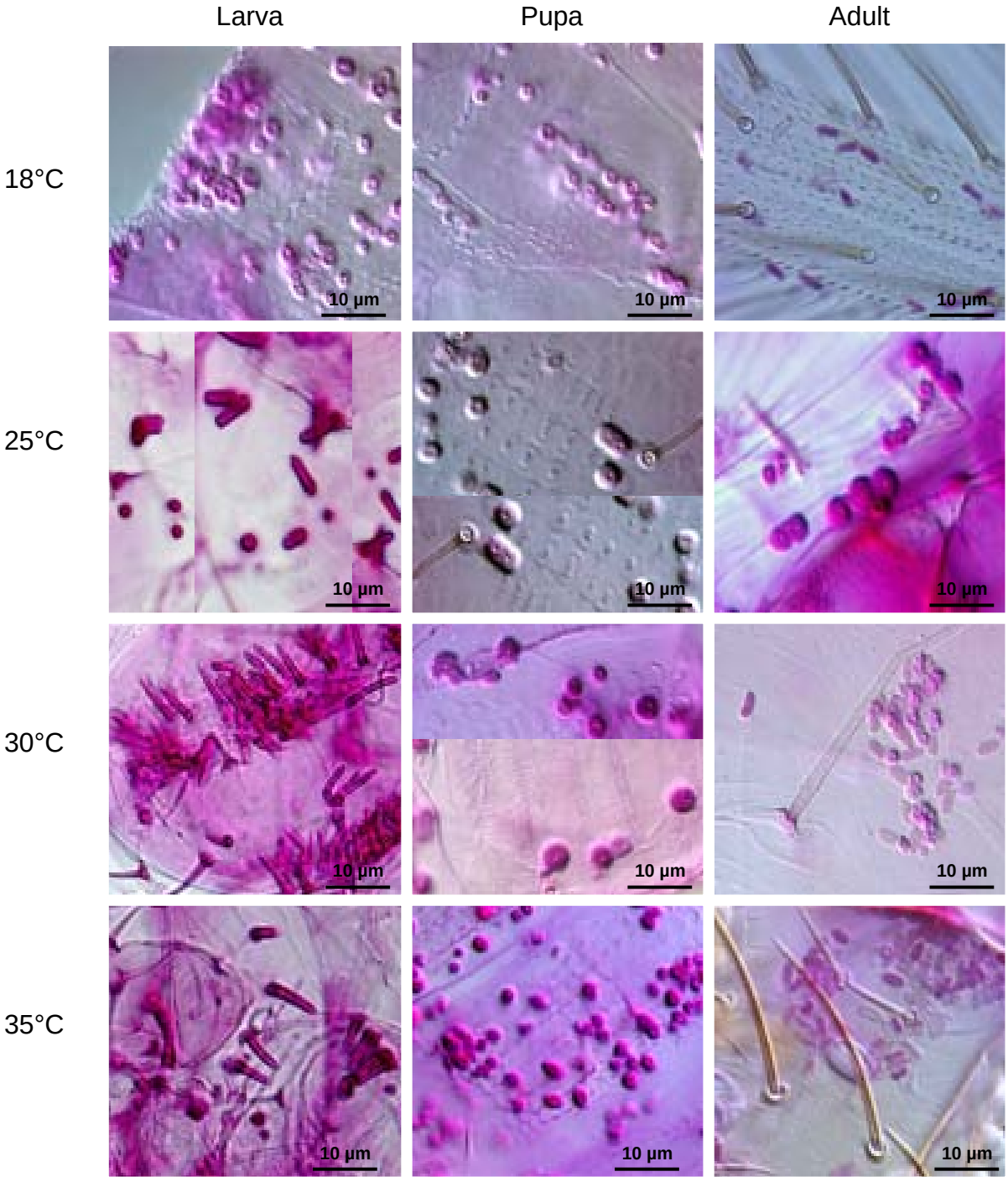


Fig. 33: *Metarhizium anisopliae* penetrated into cuticle of various life stages of *Frankliniella occidentalis* at different temperatures, 36 h after inoculation under light microscope

3.1.3.4 Colonization

Figure 34 shows development period of *B. bassiana* into *F. occidentalis* larva. The behaviour of colonization observed on *B. bassiana* and *M. anisopliae* were similar. The hyphal bodies of fungus inside the host's haemocoel were observed under a light microscope at 48 h after inoculation. At 60 h after inoculation, the hyphal bodies multiplied in the hemolymph and the hyphae from the interior through the cuticle to exterior of the thrips within 72 h. Almost, any part of thrips body the hyphae could be through (Fig. 35).

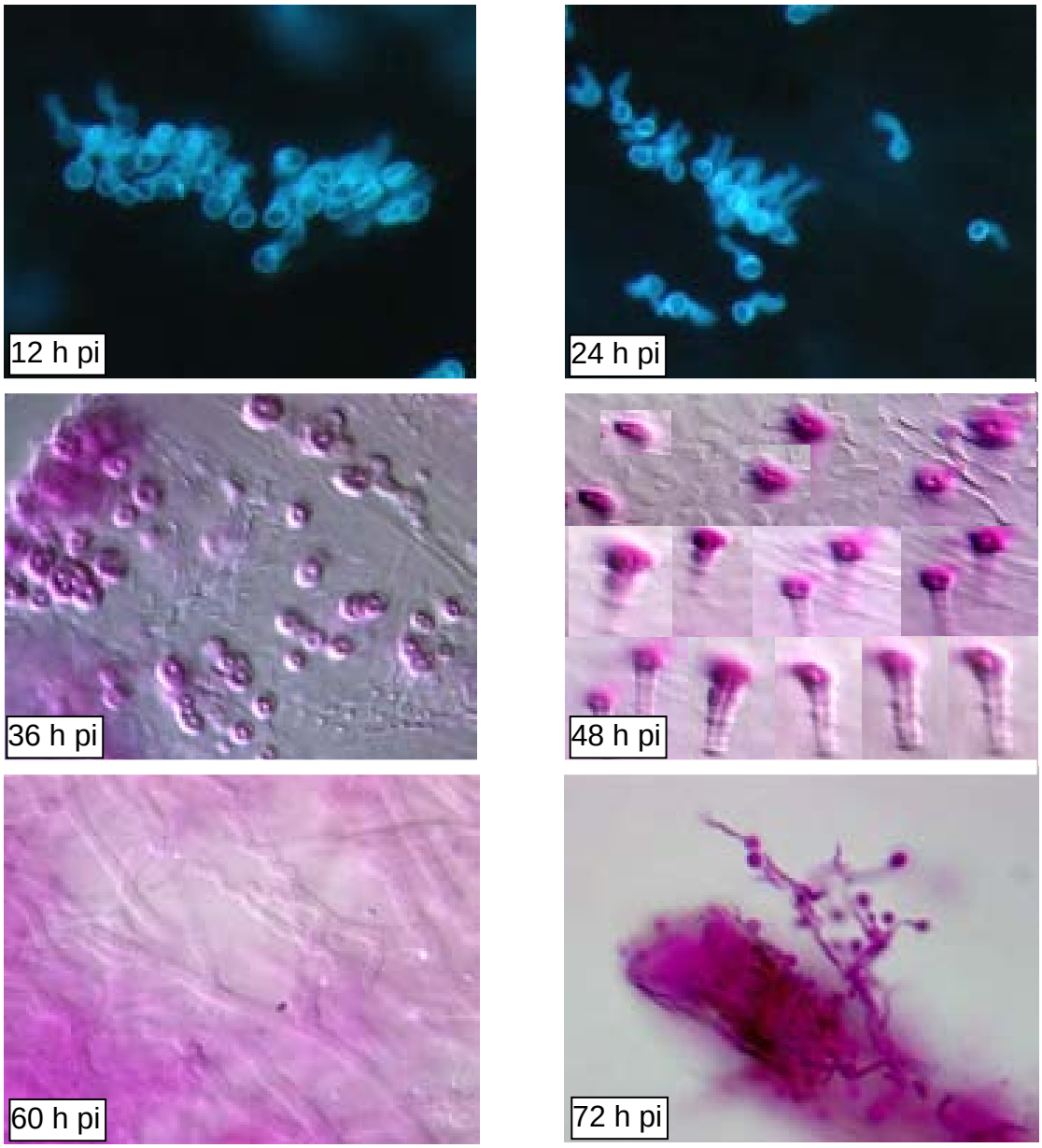


Fig. 34: Development period of *Beauveria bassiana* on *Frankliniella occidentalis* larva at different times after inoculation, under light microscope

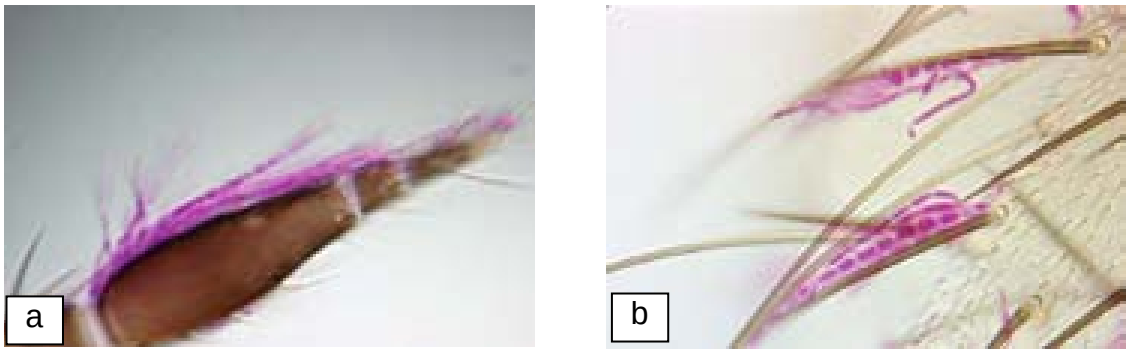


Fig. 35: The Penetration of *Beauveria bassiana* hyphae from the interior through the cuticle to exterior of *Frankliniella occidentalis* under light microscope, antenna (a), base of hair sockets (b)

From the observation, the successful of infection process of both *B. bassiana* and *M. anisopliae* on various life stages of *F. occidentalis* occurred in the abdominal region (Fig. 36).

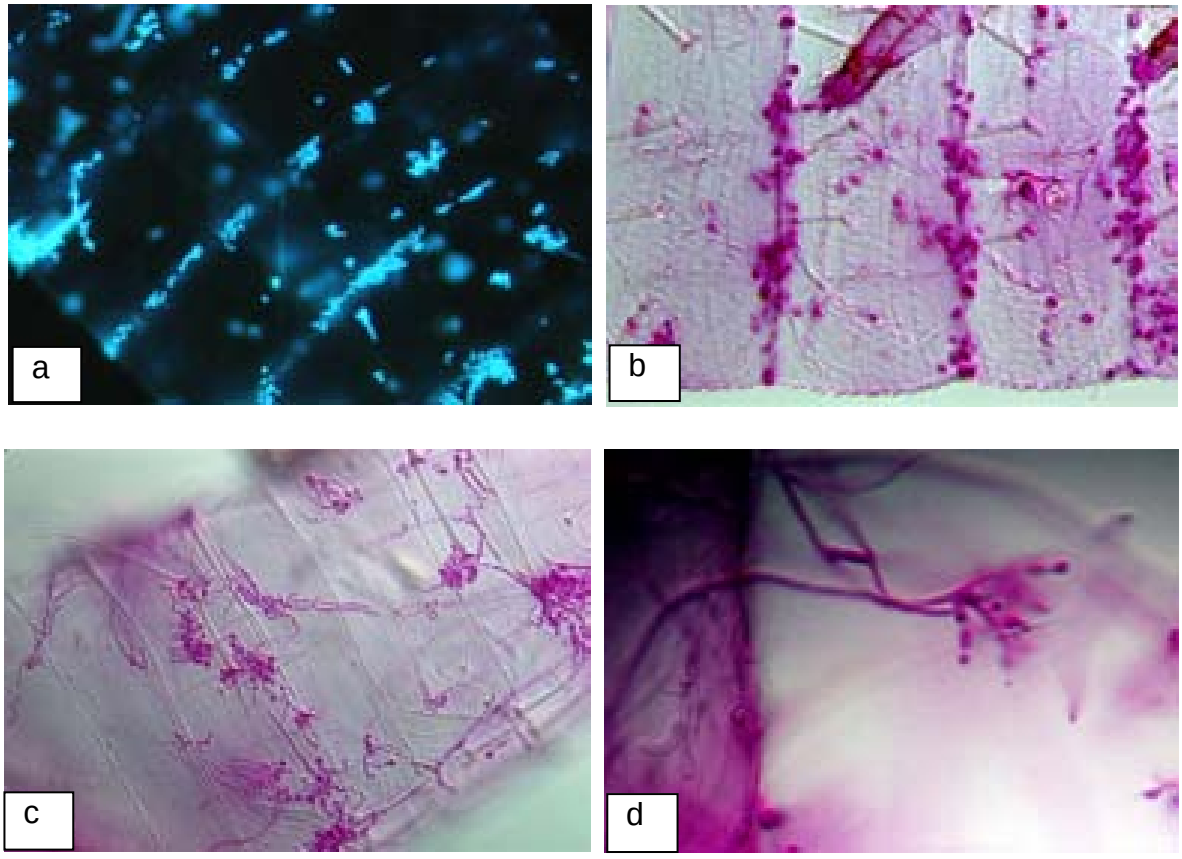


Fig. 36: Development of *Beauveria bassiana* on intersegmental folds of abdomen region of *Frankliniella occidentalis* larva, under light microscope. Germination (a), Penetration (b), Growth in the mycelial phase (c), Hyphae from the interior through cuticle to exterior of the thrips (d)

3.1.4 Efficiency of *Beauveria bassiana* Bb.5335 and *Metarhizium anisopliae* Ma.7965 against *Frankliniella occidentalis* and *Thrips tabaci*

The efficacy of entomopathogenic fungi against insect pests was dependent on abiotic e.g. temperature and relative humidity, and biotic factors, namely host plant. Thus, this part of the study deals with the results of both abiotic and biotic factors on the efficiency of *B. bassiana* and *M. anisopliae* against *F. occidentalis* and *T. tabaci.*

3.1.4.1 Influence of abiotic factors

3.1.4.1.1 Temperature

The efficiencies of *B. bassiana* and *M. anisopliae* against *F. occidentalis* on various life stages under different temperature conditions are presented in table 8. Significant differences ($P \leq 0.05$) were found among different temperatures and life stages within the same isolate. For example, *B. bassiana* had LC_{50} values of 1.73×10^5, $8.32\times10^5$and 3.16×10^6 conidia/ml against the larval, pupal and adult stage of *F. occidentalis* at 18°C, respectively. At 25°C of 1.78×10^4, 5.37×10^4 and 1.82×10^5 conidia/ml, 8.31×10^3, 3.55×10^4 and 1.23×10^5 conidia/ml at 30°C and at 35°C of 1.45×10^7, 1.41×10^8 and 3.16×10^8 conidia/ml, respectively. No significant difference was observed between LC_{50} values of the two isolates at all temperatures. No obvious variation was found between the fungal efficiencies when treated at 25 and 30°C, but both temperatures had significant effects on enhancing the efficiency of the fungi against thrips at 18 and 35°C. Slopes of regression equations of both isolates were significantly different from zero and ranged from 0.45-0.78 within *B. bassiana* and 0.42-0.62 within *M. anisopliae* (Tab. 8).

Tab. 8: LC_{50} values of *Beauveria bassiana* and *Metarhizium anisopliae* to various life stages of *Frankliniella occidentalis* at different temperatures

Stage of Thrips	Temperature (°C)	LC_{50} (conidia/ml)			
		Beauveria bassiana		***Metarhizium anisopliae***	
		LC_{50} ±SE	Slope±SE[a]	LC_{50} ±SE	Slope±SE[a]
Larval stage					
	18±1	$(1.73\pm1.33)\times10^{5}$ bA	0.52±0.03***	$(5.37\pm5.67)\times10^{5}$ bA	0.44±0.10**
	25±1	$(1.78\pm3.57)\times10^{4}$ cA	0.76±0.14**	$(1.74\pm5.00)\times10^{4}$ cA	0.42±0.05***
	30±1	$(8.31\pm1.10)\times10^{3}$ cA	0.71±0.08***	$(2.57\pm0.91)\times10^{3}$ cA	0.42±0.01***
	35±1	$(1.45\pm5.19)\times10^{7}$ aA	0.63±0.09**	$(1.26\pm5.78)\times10^{6}$ aA	0.59±0.03**
Pupal stage					
	18±1	$(8.32\pm6.26)\times10^{5}$ bA	0.58±0.03***	$(1.05\pm7.57)\times10^{6}$ bA	0.53±0.08***
	25±1	$(5.37\pm11.87)\times10^{4}$ cA	0.55±0.09**	$(3.31\pm7.08)\times10^{4}$ cA	0.53±0.13*
	30±1	$(3.55\pm1.27)\times10^{4}$ cA	0.45±0.08**	$(7.24\pm3.98)\times10^{3}$ cA	0.55±0.11***
	35±1	$(1.41\pm9.33)\times10^{8}$ aA	0.78±0.11**	$(1.15\pm1.84)\times10^{7}$ aA	0.60±0.19***
Adult stage					
	18±1	$(3.16\pm0.75)\times10^{6}$ bA	0.54±0.01***	$(7.94\pm4.72)\times10^{6}$ bA	0.62±0.09***
	25±1	$(1.82\pm1.76)\times10^{5}$ cA	0.60±0.08***	$(1.15\pm2.35)\times10^{5}$ cA	0.65±0.13***
	30±1	$(1.23\pm5.39)\times10^{5}$ cA	0.69±0.18*	$(3.98\pm1.60)\times10^{4}$ cA	0.45±0.08***
	35±1	$(3.16\pm3.69)\times10^{8}$ aA	0.59±0.08*	$(2.34\pm5.65)\times10^{8}$ aA	0.55±0.10***

Means in columns with different small letters indicate significant differences among different temperatures within the same stage (one way ANOVA, P≤0.05; Duncan's multiple rang test). Means in row with the same capital letters are not significantly different between the different isolates of entomopathogenic fungi within the same temperature (T-test, P≤0.05).

[a] T test on slope of regression equation.

*** Significantly different from zero (P≤0.001)

** Significantly different from zero (P≤0.01)

* Significantly different from zero (P≤0.05)

Similar tendency was found in the results of two fungi against *T. tabaci* under different temperature conditions (Tab. 9). Significantly higher efficiency (P≤0.05) was observed at 25 and 30°C than at 18 and 35°C. For example, the LC_{50} values of *M. anisopliae* against the larval, pupal and adult stages of *T. tabaci* at 18°C were 6.61×10^{5}, 4.07×10^{6} and 5.62×10^{6} conidia/ml; while 7.94×10^{3}, 2.51×10^{4} and 5.37×10^{4} conidia/ml at 25°C. At 30°C they were 3.98×10^{3}, 8.91×10^{3} and 2.88×10^{4} conidia/ml and at 35°C of 1.48×10^{6}, $2.14\times10^{7}$and 2.41×10^{8} conidia/ml, respectively. *T. tabaci* was less susceptible to fungal infection than *F. occidentalis*. Slopes of regression equations of both isolates were significantly different from zero (Tab. 9).

Tab. 9: LC_{50} values of *Beauveria bassiana* and *Metarhizium anisopliae* on various life stages of *Thrips tabaci* at different temperatures

Stages of thrips	Temperature (°C)	LC_{50} (conidia/ml)			
		Beauveria bassiana		*Metarhizium anisopliae*	
		LC_{50} ±SE	Slope±SE[a]	LC_{50} ±SE	Slope±SE[a]
Larval stage					
	18±1	$(5.37\pm7.09)\times10^{5}$ bA	0.48±0.04***	$(6.61\pm5.67)\times10^{5}$ bA	0.54±0.11***
	25±1	$(4.17\pm8.12)\times10^{4}$ cA	0.63±0.07***	$(7.94\pm3.85)\times10^{3}$ cA	0.44±0.05***
	30±1	$(1.38\pm0.87)\times10^{4}$ cA	0.41±0.09**	$(3.98\pm0.75)\times10^{3}$ cA	0.45±0.02***
	35±1	$(3.63\pm1.76)\times10^{7}$ aA	0.55±0.09**	$(1.48\pm3.19)\times10^{6}$ aA	0.49±0.07*
Pupal stage					
	18±1	$(2.00\pm2.94)\times10^{6}$ bA	0.46±0.06***	$(4.07\pm2.77)\times10^{6}$ bA	0.65±0.12***
	25±1	$(1.05\pm0.40)\times10^{5}$ cA	0.54±0.11***	$(2.51\pm1.98)\times10^{4}$ cA	0.51±0.14*
	30±1	$(6.76\pm5.33)\times10^{4}$ cA	0.56±0.01***	$(8.91\pm1.36)\times10^{3}$ cA	0.52±0.06***
	35±1	$(6.02\pm8.55)\times10^{8}$ aA	0.51±0.09***	$(2.14\pm1.16)\times10^{7}$ aA	0.56±0.13**
Adult stage					
	18±1	$(4.90\pm5.45)\times10^{6}$ bA	0.63±0.13***	$(5.62\pm2.57)\times10^{6}$ bA	0.64±0.08***
	25±1	$(2.51\pm1.58)\times10^{5}$ cA	0.62±0.10***	$(5.37\pm4.00)\times10^{4}$ cA	0.45±0.08***
	30±1	$(1.26\pm5.96)\times10^{5}$ cA	0.76±0.14***	$(2.88\pm0.29)\times10^{4}$ cA	0.36±0.03***
	35±1	$(7.76\pm2.81)\times10^{7}$ aA	0.70±0.12***	$(2.14\pm1.50)\times10^{8}$ aA	0.50±0.08**

Means in columns with different small letters indicate significant differences among temperatures within the same stage (one way ANOVA, P≤0.05; Duncan's multiple rang test). Means in rows with the same capital letters are not significantly different between different isolates of entomopathogenic fungi within the same temperature (T-test, P≤0.05).

[a] T test on slope of regression equation.

*** Significantly different from zero (P≤0.001)

** Significantly different from zero (P≤0.01)

* Significantly different from zero (P≤0.05)

3.1.4.1.2 Relative humidity (RH)

The results illustrated that *B. bassiana* and *M. anisopliae* were efficient at all relative humidity levels to *F. occidentalis* and *T. tabaci* (Tab. 10), but the LC_{50} values varied with relative humidity, life stage and thrips species. For instance, *B. bassiana* had LC_{50} values of 1.66×10^{5}, 2.75×10^{6} and 1.00×10^{7} conidia/ml against the larval, pupal and adult stage of *F. occidentalis* at 40% RH, respectively. At 60% RH were 2.63×10^{4}, 6.61×10^{4} and 3.47×10^{5} conidia/ml as well as 1.74×10^{4}, 7.08×10^{4} and 2.45×10^{5} conidia/ml at 85% RH, respectively. LC_{50} values of *B.*

Tab. 10: LC_{50} values of *Beauveria bassiana* and *Metarhizium anisopliae* on various life stages of *Frankliniella occidentalis* and *Thrips tabaci* at different relative humidities

Stages of thrips	Relative humidity (%)	LC_{50} (conidia/ml)			
		Beauveria bassiana		*Metarhizium anisopliae*	
		LC_{50} ±SE	Slope±SE[a]	LC_{50} ±SE	Slope±SE[a]
Frankliniella occidentalis					
Larval stage					
	40±10	$(1.66\pm1.69)\times10^{5}$ aA	0.71±0.08***	$(1.55\pm1.04)\times10^{5}$ aA	0.49±0.09***
	60±10	$(2.63\pm1.50)\times10^{4}$ bA	0.63±0.14**	$(1.29\pm0.28)\times10^{4}$ bA	0.47±0.07**
	85±10	$(1.74\pm0.79)\times10^{4}$ bA	0.55±0.14*	$(1.10\pm0.33)\times10^{4}$ bA	0.47±0.05***
Pupal stage					
	40±10	$(2.75\pm0.62)\times10^{6}$ aA	0.72±0.19***	$(1.41\pm0.76)\times10^{6}$ aA	0.75±0.20*
	60±10	$(6.61\pm2.27)\times10^{4}$ bA	0.57±0.04***	$(5.89\pm8.96)\times10^{4}$ bA	0.63±0.16*
	85±10	$(7.08\pm6.89)\times10^{4}$ bA	0.57±0.21***	$(5.37\pm2.94)\times10^{5}$ bA	0.49±0.01***
Adult stage					
	40±10	$(1.00\pm0.40)\times10^{7}$ aA	0.50±0.11**	$(6.03\pm5.25)\times10^{6}$ aA	0.58±0.07***
	60±10	$(3.47\pm0.62)\times10^{5}$ bA	0.40±0.04***	$(1.12\pm0.26)\times10^{5}$ bA	0.68±0.05***
	85±10	$(2.45\pm1.17)\times10^{5}$ bA	0.78±0.07***	$(1.51\pm4.57)\times10^{5}$ bA	0.48±0.08**
Thrips tabaci					
Larval stage					
	40±10	$(1.74\pm1.00)\times10^{5}$ aA	0.52±0.14*	$(1.55\pm0.84)\times10^{5}$ aA	0.48±0.06***
	60±10	$(2.63\pm0.50)\times10^{4}$ bA	0.62±0.09**	$(9.12\pm1.67)\times10^{3}$ bA	0.62±0.08***
	80±10	$(2.95\pm1.57)\times10^{4}$ bA	0.56±0.12**	$(7.94\pm2.04)\times10^{3}$ bA	0.47±0.09**
Pupal stage					
	40±10	$(3.47\pm5.83)\times10^{6}$ aA	0.47±0.02***	$(2.40\pm1.04)\times10^{6}$ aA	0.60±0.10***
	60±10	$(1.15\pm0.27)\times10^{5}$ bA	0.57±0.07***	$(5.89\pm3.99)\times10^{4}$ bA	0.73±0.12***
	80±10	$(2.00\pm3.10)\times10^{5}$ bA	0.53±0.08**	$(1.17\pm0.73)\times10^{4}$ bA	0.62±0.09***
Adult stage					
	40±10	$(1.15\pm0.33)\times10^{7}$ aA	0.54±0.09**	$(7.94\pm2.06)\times10^{6}$ aA	0.62±0.05***
	60±10	$(3.63\pm2.39)\times10^{5}$ bA	0.42±0.05***	$(8.71\pm3.46)\times x10^{4}$ bA	0.69±0.11***
	80±10	$(3.02\pm1.33)\times10^{5}$ bA	0.50±0.10**	$(6.17\pm4.48)\times10^{4}$ bA	0.61±0.04***

Means in columns with different small letters indicate significant differences among different relative humidity within the same stage (one way ANOVA, P≤0.05; Duncan's multiple rang test). Means in rows with the same capital letters are not significantly different between different isolates of fungi within the same relative humidity (T-test, P≤0.05).

[a] T test on slope of regression equation.

*** Significantly different from zero (P≤0.001).

** Significantly different from zero (P≤0.01).

* Significantly different from zero (P≤0.05).

bassiana against *T. tabaci* were 1.74×10^5 conidia/ml to larvae, 3.47×10^6 conidia/ml to pupae, and 1.15×10^7 conidia/ml to adults at 40% RH. The LC_{50} values were 2.63×10^4, 1.15×10^5 and 3.63×10^5 conidia/ml at 60% RH and were 2.95×10^4, 2.00×10^5 and 3.02×10^5 conidia/ml at 85% RH on life stages as mentioned above. The LC_{50} values decreased with increasing relative humidity. Both fungal isolates were more effective at 60 and 85% RH than at 40% RH at all life stages. No significant difference ($P\leq0.05$) was observed in LC_{50} values between fungal isolates. Slopes of regression equations of both isolates were significantly different from zero and ranged from 0.40-0.78 for *F. occidentalis* and 0.42-0.73 for *T. Tabaci* (Tab. 10).

3.1.4.2 Influence of host plant

Based on LC_{50} values, there was a significant effect of host plant on susceptibility of thrips to the infections by *B. bassiana* and *M. anisopliae* ($P\leq0.05$). *F. occidentalis* reared on bean, leek, cucumber and swan river daisy were highly susceptible to the infections by both fungi. The LC_{50} values of *B. bassiana* were 1.50×10^4, 2.79×10^4, 7.84×10^3 and 8.95×10^3 conidia/ml when *F. occidentalis* was reared on bean, leek, cucumber and swan river daisy, respectively, while those of *M. anisopliae* were 9.90×10^4, 2.09×10^4, 5.69×10^3 and 6.68×10^3 conidia/ml on the above mentioned host plant species (Tab. 11). *F. occidentalis* reared on cotton and saintpaulia was significantly less susceptible to the infection. LC_{50} values of *B. bassiana* were 7.15×10^4 conidia/ml on cotton and 7.29×10^4 conidia/ml on saintpaulia, while LC_{50} values of *M. anisopliae* on cotton and saintpaulia were 3.70×10^4 and 4.18×10^4 conidia/ml, respectively. Slopes of regression equations were significantly different from zero and ranged from 0.397-0.662 within *B. bassiana* and 0.483-0.621 within *M. anisopliae*. Similar virulence trends were found on *T. tabaci*, in which *T. tabaci* reared on bean, leek, cucumber and swan river daisy were more susceptible than those reared on cotton and saintpaulia (Tab. 11). LC_{50} values of *B. bassiana* against *T. tabaci* were 1.65×10^4, 1.86×10^4, 1.76×10^4, 1.06×10^4, 3.89×10^5 and 5.71×10^5 conidia/ml on bean, leek, cucumber, swan river daisy, cotton and saintpaulia, respectively. Whereas, LC_{50} values caused by *M. anisopliae* were 1.19×10^4, 1.42×10^4, 6.08×10^3, 1.46×10^3, 3.88×10^5 and 1.68×10^5 conidia/ml on host plant as noted previously. Slopes of regression equations were significantly different from zero and ranged from 0.461-0.680 within *B. bassiana* and 0.411-0.615 within *M. anisopliae.* Moreover, the present study found that there was no significant effect of host plant on the two thrips species and fungi.

Tab. 11: LC_{50} values of *Beauveria bassiana* and *Metarhizium anisopliae* against *Frankliniella occidentalis* and *Thrips tabaci* larvae on different host plants

Host plant	LC_{50} (conidia/ml)			
	Beauveria bassiana		*Metarhizium anisopliae*	
	LC_{50}±SE	Slope±SE[a]	LC_{50}±SE	Slope±SE[a]
Frankliniella occidentalis				
Bean	$(1.50\pm0.18)\times10^4$ bcA	0.527±0.088***	$(9.90\pm1.20)\times10^3$ bcA	0.567±0.022***
Leek	$(2.79\pm1.63)\times10^4$ bA	0.465±0.020***	$(2.09\pm0.57)\times10^4$ bA	0.621±0.145**
Cucumber	$(7.84\pm4.04)\times10^3$ cA	0.662±0.100**	$(5.69\pm3.78)\times10^3$ cA	0.549±0.033***
Cotton	$(7.15\pm1.18)\times10^4$ aA	0.397±0.065**	$(3.70\pm0.79)\times10^4$ aA	0.642±0.110*
Swan river daisy	$(8.95\pm7.34)\times10^3$ cA	0.455±0.063**	$(6.68\pm1.67)\times10^3$ cA	0.483±0.109*
Saintpaulia	$(7.29\pm1.77)\times10^4$ aA	0.465±0.007***	$(4.18\pm1.82)\times10^4$ aA	0.500±0.044***
Thrips tabaci				
Bean	$(1.65\pm0.49)\times10^4$ bA	0.560±0.195*	$(1.19\pm0.18)\times10^4$ bA	0.615±0.023***
Leek	$(1.86\pm0.44)\times10^4$ bA	0.461±0.096**	$(1.42\pm0.26)\times10^4$ bA	0.524±0.068**
Cucumber	$(1.76\pm0.55)\times10^4$ bA	0.554±0.155*	$(6.08\pm1.96)\times10^3$ bA	0.552±0.171*
Cotton	$(3.89\pm1.52)\times10^5$ aA	0.573±0.094**	$(1.68\pm0.36)\times10^5$ aA	0.411±0.105*
Swan river daisy	$(1.06\pm0.74)\times10^4$ bA	0.468±0.159*	$(1.46\pm0.66)\times10^3$ bA	0.421±0.053**
Saintpaulia	$(5.71\pm2.58)\times10^5$ aA	0.680±0.092**	$(3.88\pm1.85)\times10^5$ aA	0.443±0.073**

Means in columns with different small letters indicate significant differences among different host plants within the same isolate (two factors ANOVA, P≤0.05; Duncan's multiple rang test). Means in rows with the same capital letters are not significantly different between the two fungi isolates within the same host plant (T-test, P≤0.05).

[a] T-test on slope of regression equation

*** Significantly different from zero (P <0.001)

** Significantly different from zero (P <0.01)

* Significantly different from zero (P <0.05)

3.1.5 Side effects on non-target arthropods

The results of side effects of *B. bassiana* and *M. anisopliae* on five non-target beneficial arthropods are given in figure 37 and 38. Both fungi were found to be non-pathogenic to *H. nitidus* and *C. septempunctata*. However, *M. anisopliae* had pathogenicity to *D. tamaninii* and *C. carnea*, in which *D. tamaninii* was more susceptible than *C. carnea* with corrected mortalities of 10 and 4%, respectively. Mortality in control was 9.3% on *D. tamaninii* and 5.7% on *C. carnea*. On the other hand, *B. bassiana* and *M. anisopliae* were pathogenic to *P. persimilis* with mortalities of 76.6% and 56.2%, respectively, while the mortality in control was 5.7%.

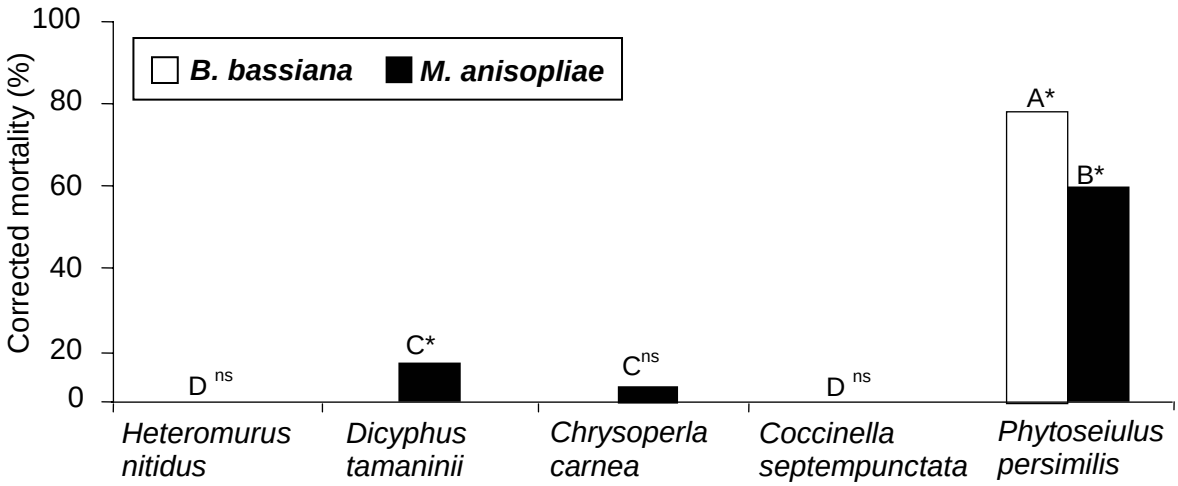


Fig. 37: Corrected mortality of non-target arthropods treated with *Beauveria bassiana* and *Metarhizium anisopliae* at concentration of 10×10^8 conidia/ml (Bar with different letters indicate significant differences among different non-target arthropods within the same isolate
* significant difference between fungal isolates at $P\leq0.05$; ns = not significant between fungal isolates)

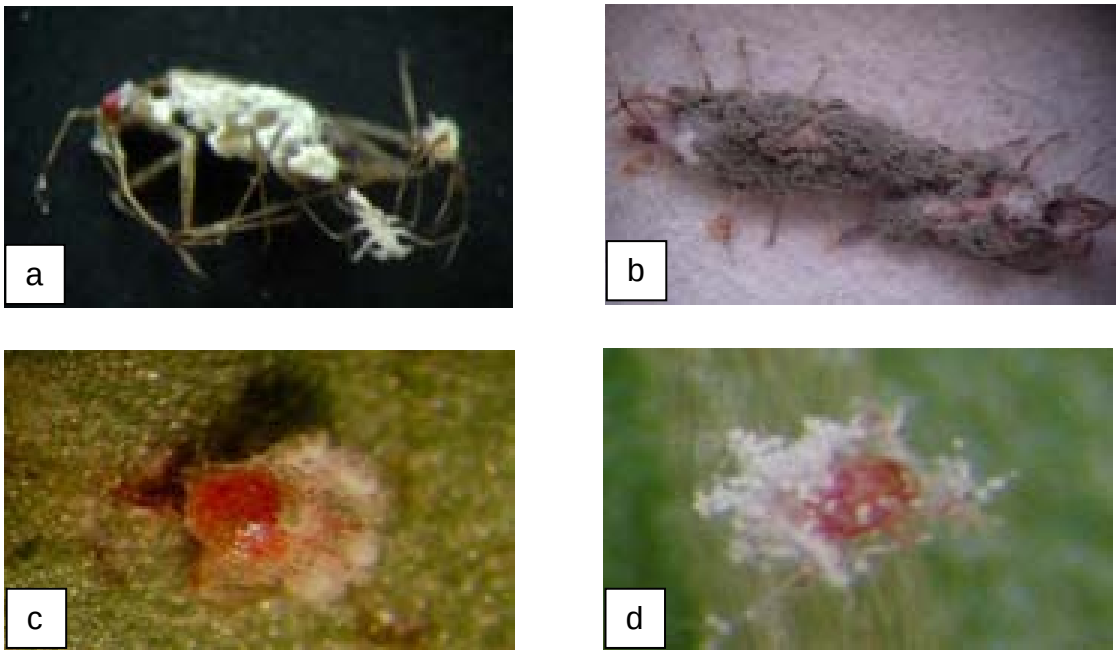


Fig. 38: *Metarhizium anisopliae* infected *Dicyphus tamaninii* adult (a), *Chrysoperla carnea* larva (b), *Phytoseiulus persimilis* adult (c) and *Beauveria bassiana* infected *Phytoseiulus persimilis* adult (d)

3.2 Experiments under greenhouse conditions

This study's part deals with the results of the experiment, which had been conducted to investigate persistence including conidia viability and efficiency of *B. bassiana* and *M. anisopliae* conidia after prolonged exposure conidia on the leaf surfaces of different host plants in greenhouse and also potential to use these fungi for controlling of *F. occidentalis* in greenhouse.

3.2.1 Persistence of *Beauveria bassiana* Bb.5335 and *Metarhizium anisopliae* Ma.7965 conidia on different host plants

3.2.1.1 Conidia viability after prolonged exposure

3.2.1.1.1 Crop plants

Figure 39 represent conidial viability of *B. bassiana* and *M. anisopliae* on bean, leek, cucumber and cotton. Crop host plants influenced the conidial viability of *B. bassiana* and *M. anisopliae* on leaf surface. The conidia of both fungi took from the leaf surface of all crop plants immediately after application were not different. After 3 days post applications, differences in conidia viability among crop host plants were found. Percentage conidia viability of *B. bassiana* were 86.33% on bean, 88.33% on leek, 89.67% on cucumber and 68.33% on cotton, while those of *M. anisopliae* were 88.00, 79.6, 87.00 and 66.67% on bean, leek cucumber and cotton, respectively. Conidia viability declined over time. Overall, average conidia viability was highest on cucumber, followed by bean and leek, while it was lowest on cotton. Conidia of *B. bassiana* and *M. anisopliae* were still viable for 30 days after application on the leaf surface of cucumber and bean with percentages viability of 21 and 17%, respectively for *B. bassiana*, while those of *M. anisopliae* were 17%. No conidia viability was evident on leaf surface of leek and cotton at 24 and 21 days after application, respectively.

3.2.1.1.2 Ornamental plants

The results of present study found that conidia of *B. bassiana* and *M. anisopliae* were viable on leaf/flower surfaces of swan river daisy higher than saintpaulia. For instance, at 15 days after application percentage conidia viability of *B. bassiana* conidia was 51.67 and 29.92% on swan river daisy and saintpaulia, respectively, while those of *M. anisopliae* were 36.67 and 26.33% on the above mentioned ornamental plants (Fig. 40).

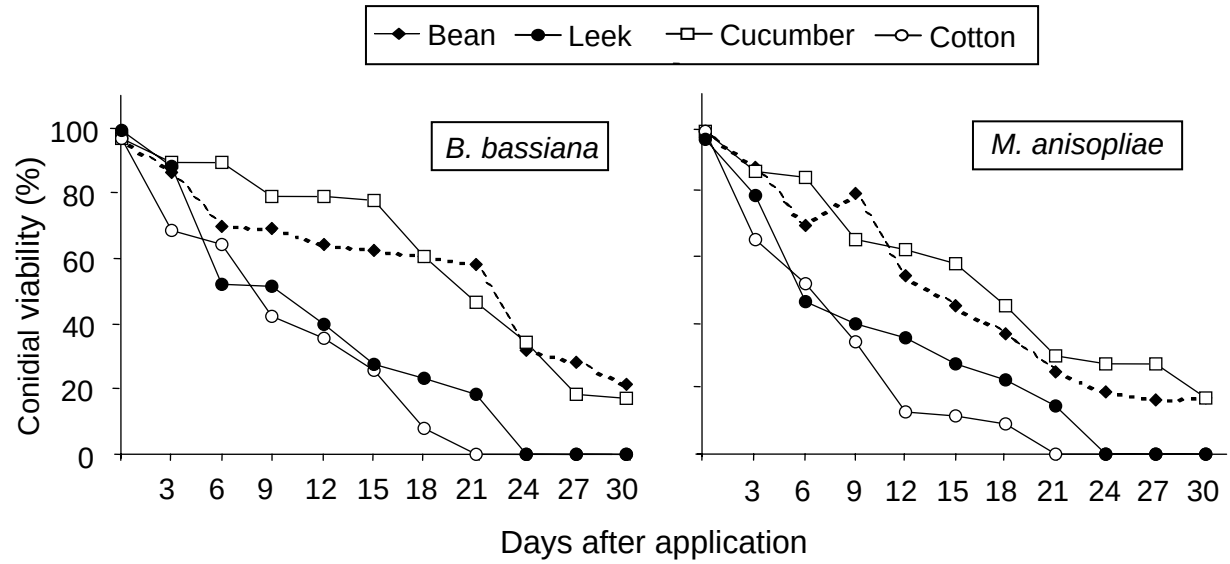


Fig. 39: Percentage conidia viability of *Beauveria bassiana* and *Metarhizium anisopliae* conidia on leaf surfaces of different crop host plants at different days after application

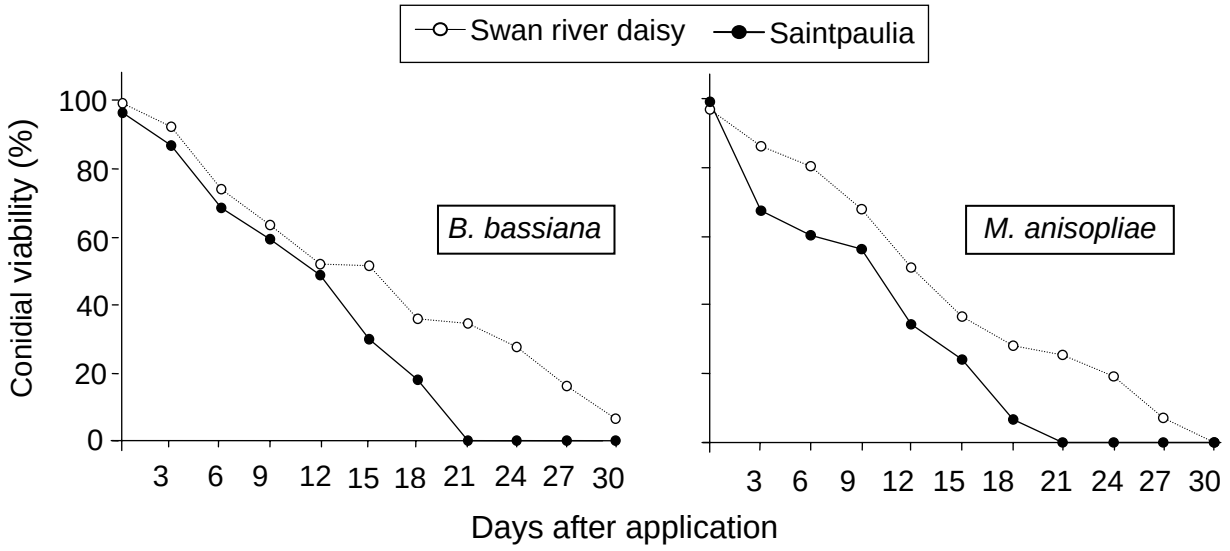


Fig. 40: Percentage conidial viability of *Beauveria bassiana* and *Metarhizium anisopliae* conidia on flower surfaces of swan river daisy and saintpaulia at different days after application

3.2.1.2 Efficiency of conidia after prolonged exposure

3.2.1.2.1 Crop plants

Figure 41 shows the mortalities of *F. occidentalis* larvae caused by *B. bassiana* and *M. anisopliae* conidia on leaf/flower surface after prolonged exposure. Mortality of *F. occidentalis* regarded to conidia viability, and differed greatly among crop plants. Mortality of *F. occidentalis* on cucumber was high throughout experiment, while low mortality was observed on cotton.

Percentage mortality decreased with time after application. For example, the mortality caused by *B. bassiana* on cucumber decreased from 98% at the day application to 29% one-month after application, while on cotton it reduced from 98 % (at the day application) to 0% 18 days after application. As the results of *M. anisopliae*, the mortality at the day application was 96.68, 100 and 98.89% on bean, cucumber and cotton, respectively, while it was 47.28% on bean, 51.23% on cucumber and 0% on cotton at 18 day after inoculation.

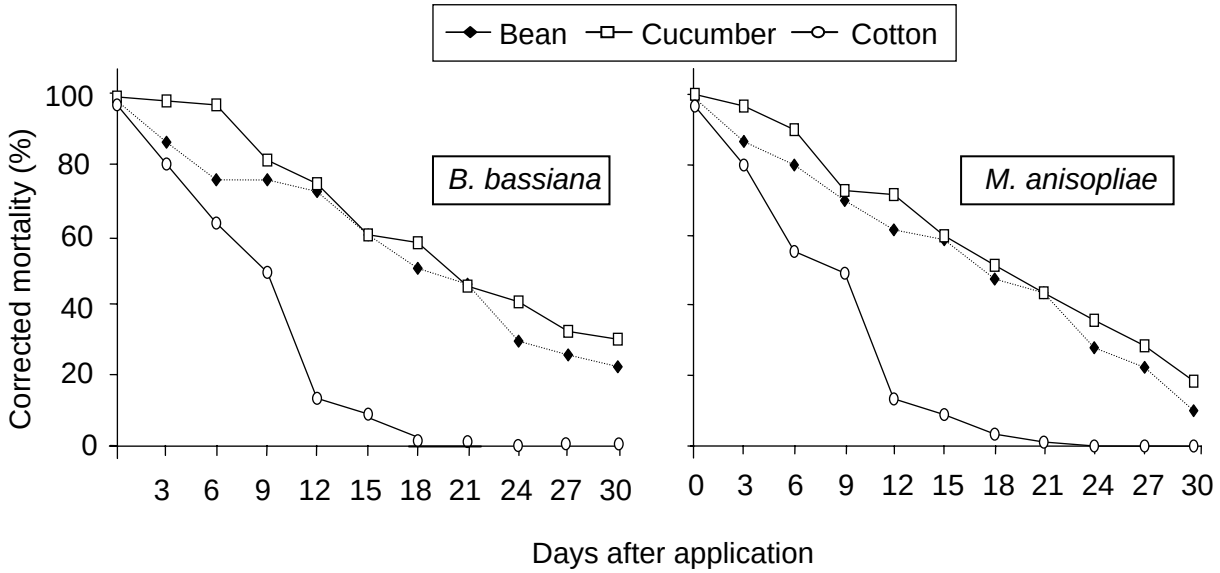


Fig. 41: Percentage corrected mortality of *Frankliniella occidentalis* caused by *Beauveria bassiana* and *Metarhizium anisopliae* conidia after pronged exposure on crop plants at different days after application

The results of *T. tabaci* showed the same tendency as *F. occidentalis*, that the mortalities caused by *B. bassiana* and *M. anisopliae* conidia on leaf/flower surface after prolonged exposure were different among different crop host plants and times after application. The percentage of mortality at the day of conidia application was high and showed a decrease later, which was host plant related. At the end of 30 days, the remaining conidia of both fungi isolates on cucumber leaves were still infectious to *T. tabaci* with mortality of 22.22% by *B. bassiana* and 10% by *M. anisopliae*, while there was no mortality of *T. tabaci* on leek and cotton (Fig. 42).

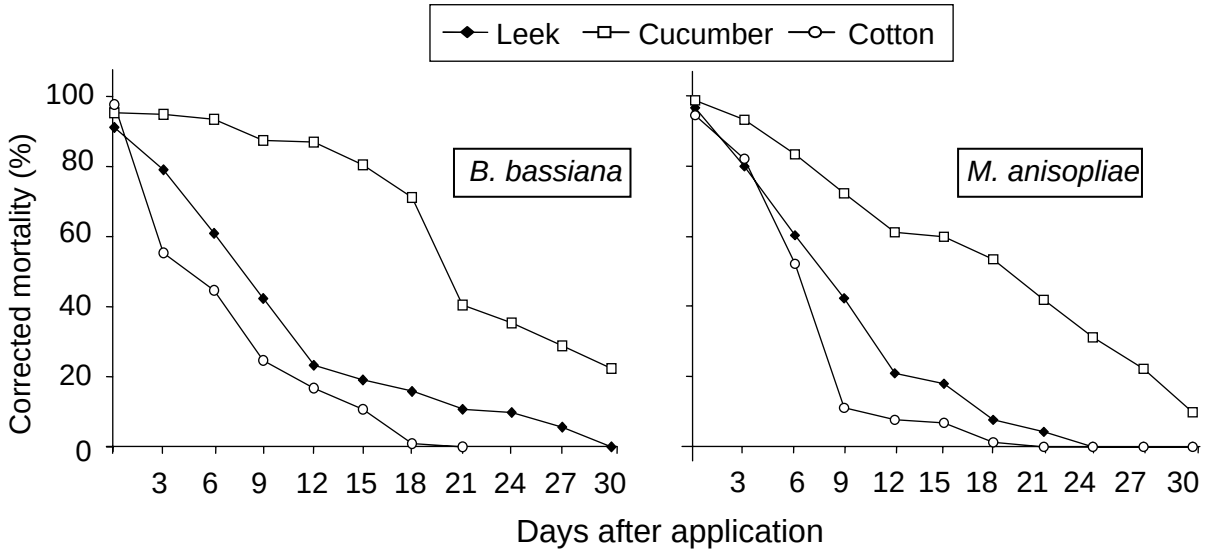


Fig. 42: Percentage corrected mortality of *Thrips tabaci* caused by *Beauveria bassiana* and *Metarhizium anisopliae* conidia after prolonged exposure on crop plants at different days after application

3.2.1.2.2 Ornamental plants

The mortality of *F. occidentalis* larvae on swan river daisy caused by *B. bassiana* decreased from 100% at the day application to 18% on day 30 and 96% to 24% caused by *M. anisopliae*, while those of both fungi on saintpaulia were 98% on the day of application to no deaths within 18 days (Fig. 43).

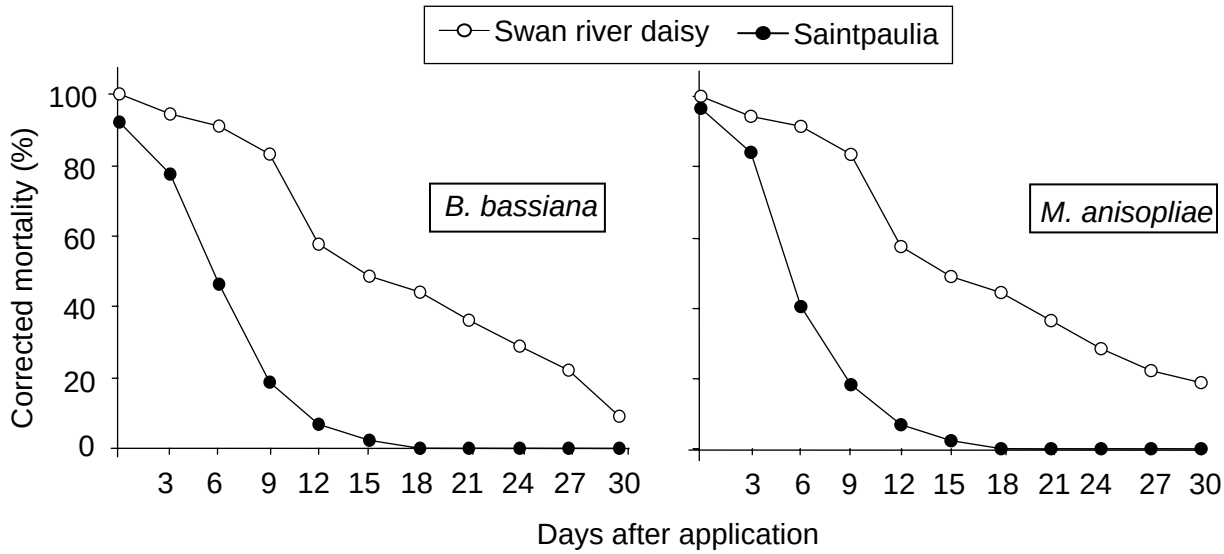


Fig. 43: Percentage corrected mortality of *Frankliniella occidentalis* caused by *Beauveria bassiana* and *Metarhizium anisopliae* conidia after pronged exposure on ornamental plants at different days after application

B. bassiana and *M. anisopliae* showed the same efficiency that the mortality of *T. tabaci* decreased in time, gradually decline on swan river daisy and steeper decline on saintpaulia (Fig. 44).

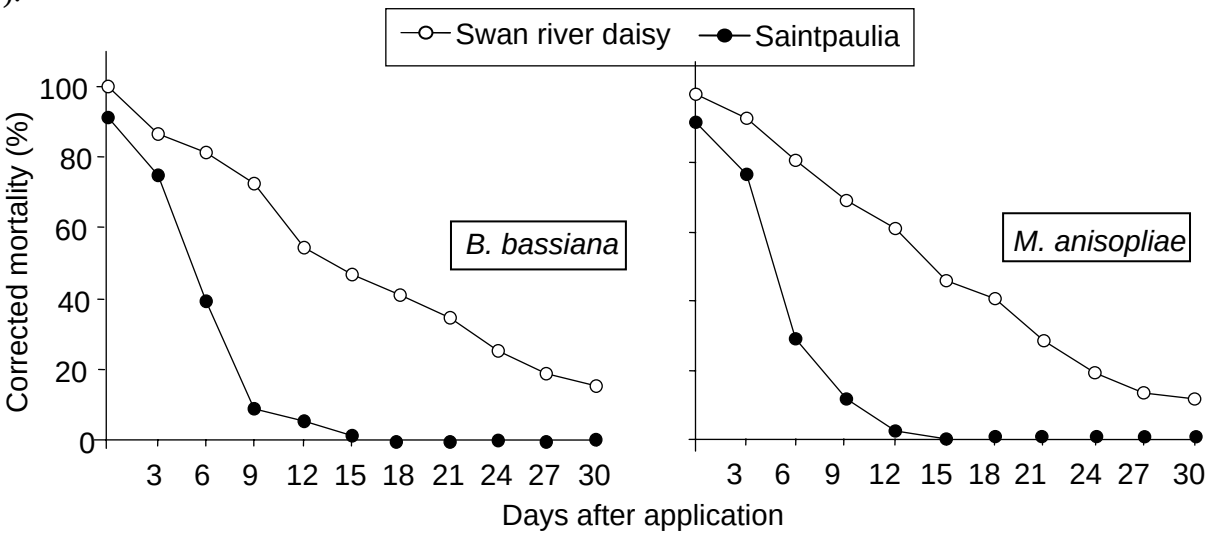


Fig. 44: Percentage corrected mortality of *Thrips tabaci* caused by *Beauveria bassiana* and *Metarhizium anisopliae* conidia after pronged exposure on ornamental plants at different days after application

3.2.1.2.3 Lethal time (LT_{50})

LT_{50} values of *F. occidentalis* larvae fed on different host plants treatment with *B. bassiana* and *M. anisopliae* conidia after prolonged exposure were inversely proportion to time after application. Those increased with increasing time after application, for instance LT_{50} values caused by *B. bassiana* at the day of application were 3.6, 3.3, 4.2, 4.0 and 4.4 days on bean, cucumber, cotton, swan river daisy and saintpaulia, while 7.3, 7.3, 11.2, 7.4 and 17 days at 15 days after application on above mentioned host plants (Fig. 45).

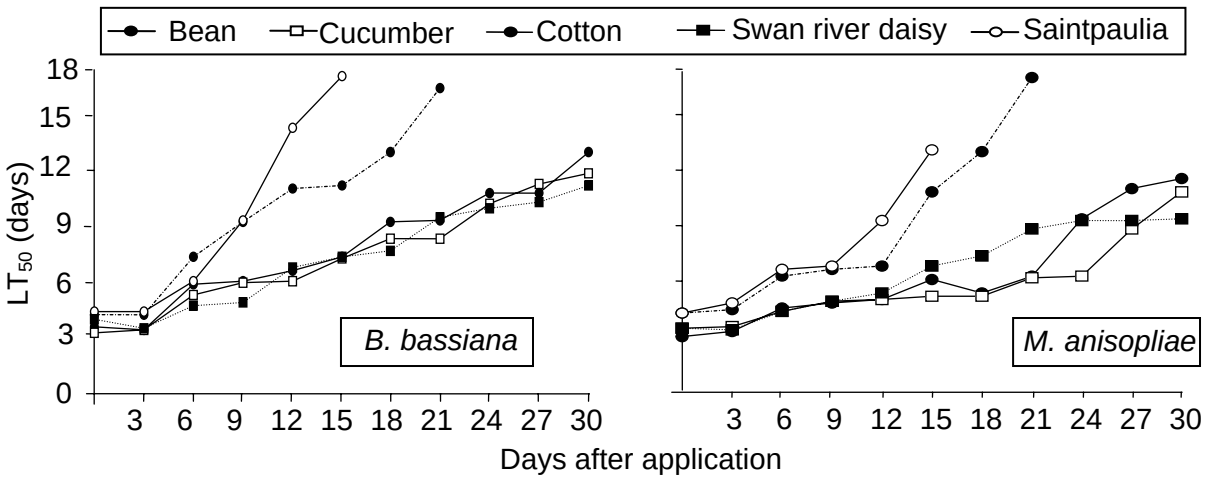


Fig. 45: LT_{50} values of *Frankliniella occidentalis* caused by *Beauveria bassiana* and *Metarhizium anisopliae* on different host plants at different days after application

The results observed on *T. tabaci* had the same trend, as on *F. occidentalis* that LT_{50} values were different among host plants. The shortest LT_{50} were found on cucumber, while saintpaulia had the longest LT_{50} throughout the monitoring period. For example, LT_{50} values on cucumber caused by *B. bassiana* were 3.63, 3.74, 5.64, 5.94, 6.12, 7.00, 8.19, 8.63, 8.63, 11.12, 13.37 and 12.83 days, while those were 5.98, 6.43, 6.02, 10.34, 17.34 and 17.06 days on saintpaulia (Fig. 46).

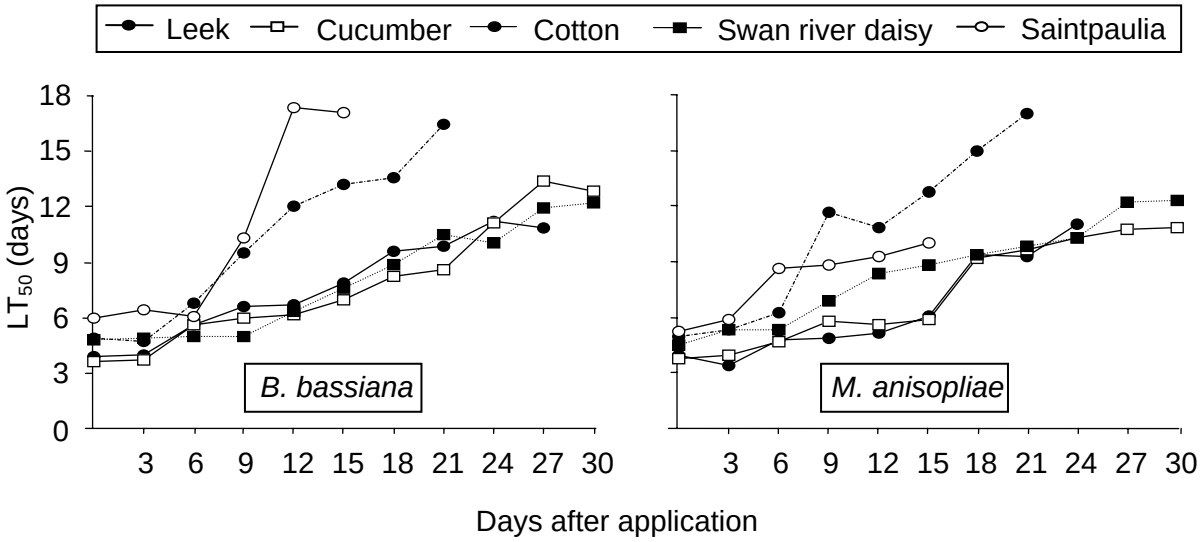


Fig. 46: LT_{50} values of *Thrips tabaci* caused by *Beauveria bassiana* and *Metarhizium anisopliae* on different host plants at different days after application

3.2.2 Efficiency of *Beauveria bassiana* Bb.5335 and *Metarhizium anisopliae* Ma.7965 for controlling *Frankliniella occidentalis* in greenhouse

The results showed that the mean initial populations of *F. occidentalis* (larva and adult) were 20.5, 22.7 and 21.1 thrips/plant in the experiments of *B. bassiana*, *M. anisopliae* and control, respectively (Fig. 47). After application, the mean numbers of thrips populations per plant reduced significantly different ($P \leq 0.05$) in both fungi treatments as compared to untreated control in the 7 and 14 days. The mean numbers in the 7 days after application were 10.6, 7.5 and 26.7 thrips/plant in the experiments of *B. bassiana*, *M. anisopliae* and control, respectively, while the means were 9.9, 12.3 and 26.9 thrips/plant after 14 days on the experiment as mentioned above. No significant difference was observed at 21 days and 28 days after application.

The results indicated that within 7 days after application *M. anisopliae* had higher efficiency to reduce *F. occidentalis* population than *B. bassiana* thereafter, *B. bassiana* was more efficient.

However, there was no significant difference in efficacy between the two fungi treatments throughout the experiment.

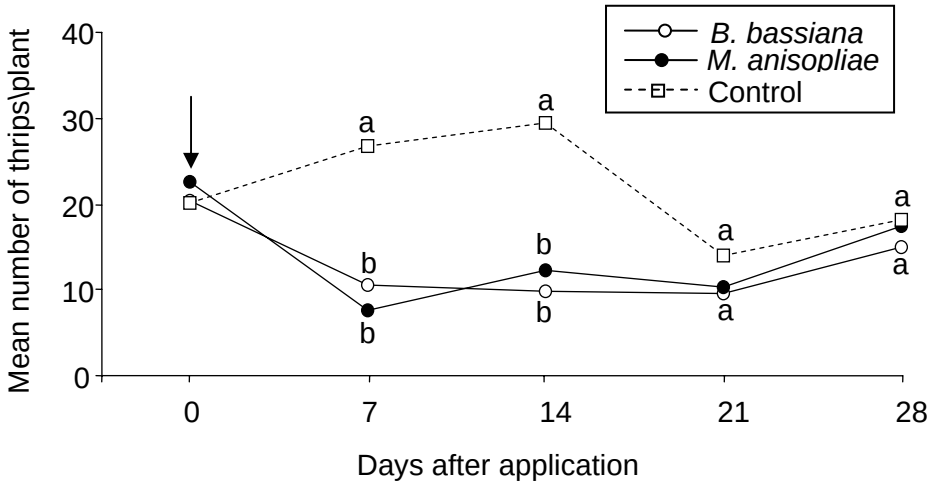


Fig. 47: Mean number of *Frankliniella occidentalis* population after application *Beauveria bassiana* and *Metarhizium anisopliae* on cucumber in greenhouse

The thrips population after 7 days application out-performed than later time in bringing down population density where it reduced the population 60.55% (*B. bassiana*) and 75.80% (*M. anisopliae*) compared to the control (Tab. 12).

The density of thrips in controls decreased substantially at 21 days and increased at 28 days. This might have been caused by food constrain because the leaves were heavily infested that reduced the thrips population after cucumber flowering thrips populations had build up.

Tab. 12: Percentage reduction in *Frankliniella occidentalis* population on cucumber after application *Beauveria bassiana* and *Metarhizium anisopliae* compared to the control

Name of fungi	Reduction population (%±SE) of *Frankliniella occidentalis* after (days)			
	7	14	21	28
Beauveria bassiana	60.55±6.37 ab	67.27±2.07 a	32.70±10.61 bc	18.01±4.22 c
Metarhizium anisopliae	75.80±5.13 a	47.16±2.07 b	26.07±11.27 bc	4.04±4.13 c

Means in rows with different small letters indicate significant differences among different percentages of population reduction within the same isolate (one way ANOVA P≤0.05; Duncan's multiple rang test).

4 DISCUSSION

In order to enrich knowledge of entomopathogenic fungi from Thailand as biological control agents and to use them successfully to control pest thrips, it was necessary to assess their efficiency through key criteria for selection of biological control agents. Therefore, the experiments were conducted to screen highly efficient isolates. The biological and ecological characteristics, mode of infections and influences of abiotic and biotic factors affecting the efficiency as well as side effects on non-target arthropods of selected fungi isolates were also determined. Finally, the experiments under greenhouse conditions were set up to evaluate their persistence and efficiencies for controlling thrips in greenhouse.

Screening high efficiency isolates

The use of entomopathogenic fungi for control of pest thrips has long been recognized. However, the successful in the use of entomopathogenic fungi largely depends on the selection of high efficiency isolates. The results of the preliminary bioassays indicated that 37 and 36 out of 41 isolates of different entomopathogenic fungi from Thailand were pathogenic to *F. occidentalis* and *T. tabaci*, respectively. This is the first study to state that *Akanthomyces*, *Aschersonia*, *Cordyceps* and *Torrubiella* are pathogenic to thrips.

Beauveria, *Metarhizium* and *Paecilomyces* were more pathogenic than isolates from other genera. This was in agreement with HALL and PAPIEROK (1982) and MOORHOUSE et al. (1993) who stated that *Metarhizium*, *Beauveria* and *Peacilomyces* were the best-known fungi with respect to their wide geographical distributions, broad host ranges and their great potentials as biological control agents. Additionally, VESTERGAARD et al. (1995) identified strains of *M. anisopliae*, which were more pathogenic than any of the *V. lecanii* strains assayed on *F. occidentalis*. Furthermore, *M. anisopliae* could reduce both the adult and larval populations of *F. occidentalis* on chrysanthemum (MANIANIA et al. 2001). EKESI et al. (1998) screened 22 strains of entomopathogenic fungi against *Megalurothrips sjostedti* (TRYBOM) (Thys., Thripidae), and found that 2 strains of *B. bassiana* and 4 strains of *M. anisopliae* were highly pathogenic to *M. sjostedti*. GILLESPIE (1986) and FRANSEN (1990) reported that in laboratory studies, *T. tabaci* was susceptible to *M. anisopliae*, *B. bassiana*, *P. fumosoroseus* and *V. lecanii*.

The results presented here demonstrate that there was variation in pathogenicity within fungal genera, species and isolates. Seven out of 8 isolates of *Beauveria* were highly pathogenic, while only 4 among 8 isolates of *Metarhizium* had high pathogenicities. From 7 isolates of

Peacilomyces, only 3 were highly pathogenic. Within isolates, the differences in pathogenicity were more pronounced for *Metarhizium* than for *Beauveria*. This contrasts with EKESI et al. (1998), who found that the differences in virulence were more obvious for *B. bassiana* strains than for *M. anisopliae* ones. Differences in pathogenicity between fungal species and isolates have also been reported in *T. tabaci* (THUNGRABEAB et al. 2005) and for other insect species (POPRAWSKI et al. 1985, MOORHOUSE et al. 1993).

The entomopathogenic fungi used in this study were isolated from different hosts. Many investigators reported that pathogenicity was not always related to the original host or geographic origin (PRIOR 1990, MOORHOUSE et al. 1993). VESTERGAARD et al. (1995) found that *V. lecanii* isolated from thrips was weakly pathogenic to *F. occidentalis* while EKESI et al. (1998) reported that *B. bassiana* isolated from *M. sjostedti* was only moderately pathogenic to its original host. FENG and JOHNSON (1990) noted that the original host has no significant influence on the virulence, and entomopathogenic fungi isolated from soil showed high pathogenicity to insects. EKESI et al. (1998) stated that 1 isolate of *B. bassiana* and 2 isolates of *M. anisopliae* from soil were highly virulent to *M. sjostedti*.

One of the first steps of testing the efficacy of an entomopathogenic fungus for biological control was choosing an appropriate isolate. Virulence against the target pest was a key criterion; because the fungus has been passage through a natural host, its virulence should be maintained (FARGUES and ROBERT 1983). The results of the secondary bioassay indicated that *M. anisopliae* Ma.7965 and Ma.6079, *B. bassiana* Bb.5335 and Bb.4591 as well as *P. fumosoroseus* Pfu.5338 were superior among 41 isolates tested. Differences in virulence among isolates were reported in all the entomopathogenic fungi assayed. SENGONCA et al. (2006) revealed that among 41 isolates, 14 isolates (6 isolates of *Beauveria*, 4 isolates of *Metarhizium*, 3 isolates of *Paecilomyces* and 1 isolate of *Cordyceps*) were highly virulent to *F. occidentalis*. Moreover, CHANDLER et al. (2005) reported that 3 out of 40 isolates caused greater mortalities to *Tetranychus urticae* KOCH (Acari: Tetranychidae) than control.

The susceptibility of insects to entomopathogenic fungi varied with the life stage (FERRON 1985). Understanding which stage was most susceptible to the infection was thus important for development of management tactics. The different life stages of *F. occidentalis* showed various susceptibilities to entomopathogenic fungi. The results obtained in this study showed that the larval stages were more susceptible to infection by entomopathogenic fungi than pupal and adult

stages. This finding was in contrast with the findings of VESTERGAARD et al. (1995), who reported that the larval and pupal stages of *F. occidentalis* were more resistant to infection by entomopathogenic fungi than the adult stage. As well the adult stage of *M. sjostedti* was found to be more susceptible to infections than larval and pupal stages (EKESI and MANIANIA 2000). Different susceptibilities of various life stages to entomopathogenic fungi have been reported for other insect species. GINDIN et al. (2000) found that pathogenicity of *V. lecanii* to silverleaf whitefly, *Bemisia argentifolii* BELLOWS and PERRING (Hom., Aleyrodidae) decreased with the proceeding developmentat. The susceptibility of *T. vaporariorum*.to *A. aleyrodis* reduced with age. The older instars were less susceptible while the adults were seldom infected by the fungi (FRANSEN et al. 1987), whereas the early stages of *Helicoverpa* spp. have been reported to be less susceptible to *N. rileyi* than the later stages (MOHAMED et al. 1977).

These differences in susceptibility at various developmental stages could be assumed to be due to interaction between the insect integuments being penetrated by fungus and the ecdysis of host. Cuticle thickness or metamorphosis might also influence the susceptibility as well. Penetration might be difficult because the composition of the cuticle was changing. The inability of the fungus to penetrate the cuticle of the older instar stages could be due to the failure of the fungus to produce enzymes or because increasing amounts of antifungal substances present in the cuticle inhibited the germination and penetration processes. The latter case might be explained by differences between the cuticle of the adults and larvae. Furthermore, the adult insect might remove spores from its body by grooming, leading to the protection itself in some ways.

Biological and ecological characteristics

The results of biological characteristics could serve as a basis for selection of isolates as biological control agents against *F. occidentalis* and *T. tabaci*. Morphological characteristics of *B. bassiana* Bb.5335 and *M. anisopliae* Ma.7965 have shown similar characteristics to those of the original description (SAMSON et al. 1988, HUMBER 1997).

In the present study, *M. anisopliae* conidia survived longer period than those of *B. bassiana*, and they gradually declined in viability, which corresponded to the observations by DAOUST and ROBERT (1983). They noted that *B. bassiana* and *P. farinosus* conidia lost viability most rapidly, while *M. anisopliae* conidia survived longer at humidity extreme but increased temperatures.
Temperature was an abiotic limiting factor that affected rates of germination, conidiation, growth, and survival of entomopathogenic fungi (BENZ 1987). Optimal temperatures for

entomopathogenic fungal germination, conidiation and growth often ranged between 20-30°C, but variation in temperature tolerance within isolates could also be significant. Many researchers reported that germination of entomopathogenic fungi was dependent on temperature. DIMBI et al. (2004) reported that there were significant differences among the isolates because of effect of temperature, where germination at 15 and 35°C were low. Moreover, EKESI et al. (1999) indicated that among 2 strains of *B. bassiana* and 4 strains of *M. anisopliae,* there were no significant differences in germination at 15, 20, 25 and 30°C, while significant differences occurred among the fungal strains at 35°C. In these experiments, it was found that temperature did not greatly affect the germination capacity of *B. bassiana* and *M. anisopliae* and that there were no significant differences in germination at temperature levels of 18, 25, 30 and 35 °C. These could be explained by the fact that they had a broad temperature range of germination. The potential of isolates to tolerate high or low temperatures was normally related to the climatic data of their geographic origin. A relationship between thermal tolerance and climate of origin has been shown for other isolates of entomopathogenic fungi. The two fungi isolates used in the present study originated from Thailand, where is located in the tropical region, therefore high temperature had no effect on germination. However, the germination at the high temperature (35°C) was similar to the moderate temperatures, while the elongation of germ tube was shorter. This point was supported by SHIMAZU (2004), who found that *B. bassiana* F-263 germinated at 34°C, but the growth of germ tubes stopped after 7 days of inoculation. It was interesting to note that the highest temperature of 35°C did not affect the germination of these two selected fungi isolates. Moreover, the current results indicated that temperature clearly influenced the speed of germination. Temperatures below or above the optimal temperature resulted in on increase in time taken for germination.

A temperature ranges from 18-30°C affected slightly on the conidial production. The most rapid ones occurred at 25 and 30°C. At 35°C, the temperature retarded the conidiation process. The corroborative results for this study were confirmed that *B. bassiana* and *M. anisopliae* were unable to sporulate in vitro at temperatures below 10°C and above 35°C (WALSTAD et al. 1970).

On the other hand, this study confirmed that the colony growth of *B. bassiana* and *M. anisopliae* were highly affected by temperature. Extreme temperature of 35°C inhibited the colony growth. Many researchers stated that the optimal temperature for colony growth of entomopathogenic fungi was generally around 20-25°C, although this depended on fungus species and strain (GLARE and MILNER 1991, GOETTEL and INGLIS 1997). However, this could vary depending on

the geographic origin (SHIMAZU 2004). Previous studies have shown that a considerable number of *B. bassiana* and *M. anisopliae* isolates exhibited a highest growth rate at 28°C with a few isolates growing better at 30 than at 25°C (FARGUES et al. 1997, EKESI et al. 1999). In the present experiments, growth of *B. bassiana* and *M. anisopliae* were better at 30 than at 25°C, implying that these isolates could be considered to have a higher optimal temperature than the other isolates in the previous study. These results, therefore, were agreed with the maximum growth rates of the fungal isolates of tropical origin that have been reported to be between 25-30°C (DIMBI et al. 2004).

Relative humidity exerted a direct influence on germination, conidiation and colony growth. Both fungi isolates performed poorly at low relative humidity. Interaction between the physiological characteristics and the moisture could play an important role in limiting the survival of fungi (DAOUST and ROBERT 1983). The results herein indicated that the physiological processes, which governed germination, conidiation and colony growth, were affected by relative humidity in different ways.

Light was an important factor in the biology of most fungi, particularly as it related to conidiation, spore discharge and survival (PLATT and MORRALL 1980). Germinations of *B. bassiana* and *M. anisopliae* in this study reduced under darkness condition, comparing to continuous light and 16:8 h (L:D) photoperiod. The conidiation and colony growth provided the same trends as the conidia germination under continuous light, be more pronounced than under darkness and 16:8 h (L:D) photoperiod.

UV radiation was well documented to influence germination, conidiation and colony growth. Different isolates of entomopathogenic fungi could not have the same response and susceptibility to UV light. In the present study, *B. bassiana* and *M. anisopliae* conidia still had high germinations after long UV exposure. Similarly, MÜLLER-KÖGLER (1965) discovered that conidia of fungus were not damaged after long UV exposure. However, FARGUES et al. (1996) indicated that the conidia from 65 isolates of *B. bassiana*, 23 isolates of *M. anisopliae*, 14 isolates of *M. flavoviride* and 33 isolates of *P. fumosoroseus* were detrimentally affected after being irradiation by artificial sunlight (295 to 1,100 nm at an ultraviolet-B irradiance of 0.3 Wm^2) for 2 h or more. Moreover, after 14 isolates of *B. bassiana*, *M. anisopliae* and *M. flavoviride* were exposed to 4, 8, 16 and 24 h of UV light from a sunlight simulator at 40°C, conidial viability decreased markedly in all isolates with increasing UV exposure. MOORE et al.

(1996) noted that direct exposure to simulated sunlight for only a few hours affected half-lives of *M. anisopliae* conidia. Both fungi isolates could produce conidia under UV light but conidiation of *M. anisopliae* was less sensitive to UV light than *B. bassiana*. Colony growths of both fungi tested had little responses to UV light. This agreed with FARGUES et al. (1996), who observed that isolates of *P. fumosoroseus* from tropical regions were more resistant to solar simulator radiation than isolates from temperate regions.

Mode of infection

Infection process of entomopathogenic fungi to insect occurred via a series of integrated, systematic interactions between the fungus and the insect. These interactions were complex and dependent upon specific host-pathogen interactions (CHARNLEY 1989, KHACHATOURIANS 1991, 1996, HAJEK and ST. LEGER 1994). Understanding these processes will provide a rational basis for selection of efficiency isolates, improve and explain fungal virulence or host resistance as well as helping in the production of more efficient biological control agents. BOUCAIS and PENDLAND (1991) stated that the infection process of *B. bassiana* and *M. anisopliae* were as follows: attachment of conidia on the insect's cuticle, their germination, germ tube formation, penetration and internal colonization culminating in host death.

Attachment of conidia on the surface of host cuticle was the first step in the establishment of pathogenesis. Entomopathogenic fungi displayed different strategies in their attachments to insects. The surface of *B. bassiana* and *M. anisopliae* conidia consisted of a bare protein-rich hydrophobic rodlet layer that interacted with insect epicuticle for attachment. Conidia of many entomopathogenic fungi would bind in a non-specific manner to the epicuticle surfaces of both susceptible and resistant host insects (BOUCIAS and PENDLAND 1991). However, production and penetration of germ tubes did not normally occur on non-host insects. In addition to chemical requisites, the surface topography of the cuticle has also been shown to play a role in appressorial formation (ST. LEGER et al. 1991). These results demonstrate that conidia of *B. bassiana* and *M. anisopliae* randomly attached at equivalent rates over the head, thorax, abdomen and leg of the larva and pupa, while the conidia attached mostly to the wings on the adult. Corresponding with these results, VESTERGAARD et al. (1999) stated that the conidia of *M. anisopliae* attached mostly to the wings of adult *F. occidentalis*, but the number declined from 73% to 40% within 72 h after incubation at 23°C. Likewise, ALTRE et al. (1999) reported that the number of conidia attaching was often related to the fungal virulence and the susceptibility of the

host, similar to current observations that the number of conidia attachment on the cuticle was from high to low on larva, pupa and adult, respectively. The topography and general chemical properties of the epicuticle might enhance conidial attachment. Conidia were frequently found to be trapped in areas containing large numbers of setae, intersegmental membranes, legs and the antennal segmental. Comparative experiments have also revealed that substantially greater numbers of these conidia bound to larval cuticle than to the adult cuticle.

Germination of *B. bassiana* and *M. anisopliae* conidia on the cuticle of larval, pupal and adult *F. occidentalis* varied with temperature and life stage. At 25, 30 and 35°C, the conidia germinated within 12 h after the inoculation at all life stages, while the germination was delayed at 18°C. These results were similar to finding of VESTERGAARD et al. (1999), who reported that the conidia of *M. anisopliae* germinated on larval, pupal and adult stages within 12 h after inoculation at 23°C. Characteristics of an insect, a potential fungal pathogen and their environment could contribute to the failure of a fungus to infect the insect (CHARNLEY 1989, HAJEK 1997). Moreover, the germination process required both an appropriate humidity regime and available nutrients for germ tube production (WOODS and GRULA 1984). The present results strongly supported the theory that the germination periods for *B. bassiana* and *M. anisopliae* varied with various life stages and temperatures. Germination of conidia on the integument of *F. occidentalis* differing with time, successive on larvae than on pupa and adult, could be attributed to the variation in the composition of integument of life stages. We hypothesized that the nutrient on the cuticle played a role of germination.

The current results indicated that germling of *M. anisopliae* produced appressorium at the ends of germ tubes on the hard, sclerotized parts of the body, intersegmental membranes and on the thin membranous wings. This showed clearly that the topography, chemistry, and the physical properties of the cuticle influenced germ tube formation. Probably, the different textures of larval, pupa and adult integuments might provide varying stimulants for germ tube formation, which resulted in the wide variation in the initiation of conidia germination on different developmental stages. BUTT et al. (1995) reported similar observations for *M. anisopliae* on aphid and flea beetle.

Entomopathogenic fungi invaded their hosts by direct penetration of the host exoskeleton or cuticle. The epicuticle was the first barrier encountered. Penetration of this layer was either by infection pegs produced from underside of appressoria or by direct entry through germ tubes.

Penetration of fungi on intact insect cuticle appeared to be a combination of mechanical force and enzymatic degradation, the relative contribution of the two components depending on the structure and composition of the cuticle encountered (CHARNLEY 1989). In addition to nutrient, surface topography influenced appressorial formation in *M. anisopliae*. This study and previous study, it was assumed that the fungi were different in virulence on different life stages, because they were unable to degrade cuticle or overcome cuticle based on host defences.

From the results obtained, *M. anisopliae* conidia germinated on the thrips surface and often differentiated to form an appressorium, while *B. bassiana* penetrated the thrips through direct hyphal penetration. VESTERGAARD et al. (1999) reported that *M. anisopliae* conidia germinated and produced appressoria on larval, pupal and adult stages; but no data was available on *B. bassiana*. However, ALTRE and VANDENBERG (2001) reported that *P. fumosoroseus* did not appear to produce appressoria on the cuticle of diamondback moth larvae. Fungi apparently penetrated cuticles directly with undifferentiated germ tubes. In addition, *V. lecanii* did not produce appressoria on the cuticle of *F. occidentalis* (SCHREITER et al. 1994).

Entomopathogenic fungi penetrated the host cuticle shortly after germination or after limited hyphal growth (BUTT et al. 1989, WRAIGHT et al. 1990, ST. LEGER et al. 1991). The present study revealed that the penetration of *B. bassiana* and *M. anisopliae* on the thrips cuticle took place between 36 to 48 h after infection, depending on the life stage and temperature. The dramatically changing environmental conditions could reduce the efficacy of the pathogen. Delaying or preventing infection was also observed in this experiment, where penetration pegs on the larval stages were observed at 36 h after inoculation under 25°C consequently 48 h after inoculation at 18°C. Additionally, WRAIGHT et al. (1990) stated that the fungi penetrated the host cuticle between 24-48 h under ideal conditions. Previous studies with *V. lecanii* on potato aphid, *Macrosiphum euphobiae* (THOMAS) (Hom., Aphididae) and *M. anisopliae* on *F. occidentalis*, showed that the penetration occurred within 30 h (ASKARY et al. 1999, VESTERGAARD et al. 1999). The current study it was found that the majority of successful penetration occurred was observed at the abdominal region of all life stages, particularly at arthrodial membranes, at joints and between segments.

Penetration on adults took more time than that of other life stages. This could be explained by the fact that conidia were mostly attached on the wings, which had heavy sclerotization. If the cuticle of insect was heavily sclerotized, the penetration to the hemocoel might take several days

and in such cases the hyphae tended to grow between the cuticular lamellae (KUMAR et al. 2004). The degree of cuticular sclerotization also had a strong influence on the penetratibility.

B. bassiana and *M. anisopliae* killed the thrips by enzymes and mechanical force to penetrate and/or colonize the host cuticle, so that it could release nutrients that in turn encouraged mycelial growth rather than penetration. During conidium germinate, it utilized the nutrients from the insect and thus could stress the insect and make it susceptible to the infection. In addition, the toxin that they secreted might play an important role in thrips mortality.

In conclusion, the development period of the fungi in the larva thrips at 25°C revealed that most conidia of both fungi germinated within 12 h. They took place 36 h to penetrate into cuticle. The fungi began to develop on the thrips at 48 h and the whole thrips was covered with mycelia at 60 h. At 72 h after inoculation, the fungi grew cover the cadavers. However, different development period times were observed at different temperatures and life stages.

Efficiency of *Beauveria bassiana* Bb.5335 and *Metarhizium anisopliae* Ma.7965

Influence of abiotic factors

It was clear that abiotic factors influence the efficacy of biological control agents as on entomopathogenic fungi. Understanding the influence of abiotic factors is necessary to maximize their efficiency.

Temperature was one of the factors that affected fungal growth and pathogenicity against insects, the same fungal isolate might show difference in their efficacy. The results obtained in the current study showed that *B. bassiana* and *M. anisopliae* caused mortalities in both *F. occidentalis* and *T. tabaci*, but the onset of the efficacy differed with temperature. Fungal isolates were more effective to both thrips species at 25 and 30°C. This corresponded with the optimum temperature for germination and mycelial growth, when being cultured on media (THUNGRABEAB, unpublished observations). However, when temperature decreased below (18°C) or increased above (35°C) the optimal temperature, these two fungi isolates still displayed infectively, but caused significant reductions of the efficiency. FERRON (1978) reported that the temperature lower than optimal one retarded the development of mycosis without necessarily affecting the total mortality. The efficacy of the two fungi tested against *F. occidentalis* and *T. tabaci* depended clearly on temperature. Therefore, the results corroborated the previous reports that efficacy increased as temperature until an optimum level was reached.

THUNGRABEAB et al. (2006) reported that 5 isolates of entomopathogenic fungi, e.g. 2 isolates of *B. bassiana*, 2 isolates of *M. anisopliae* and 1 isolates of *P. fumosoroseus* showed significantly higher efficacies to *F. occidentalis* and *T. tabaci* at 25 and 30°C than at 16°C. VESTERGAARD et al. (1995) mentioned that *F. occidentalis* was susceptible to *M. anisopliae* at a temperature range of 18-26°C, but the mortality was higher at higher temperatures of 23 and 26°C comparing with 18 and 20°C. EKESI et al. (1999) noted that there was a significant decrease in fungal infection to *M. sjostedti* by some tropical *M. anisopliae* strains at 20°C in comparison to 25 and 30°C. Furthermore, in the present study it was found that *B. bassiana* showed higher efficiency at initiating disease in bioassay at low temperature than *M. anisopliae*, whereas *M. anisopliae* was far more effective at high temperature than *B. bassiana*. Such a difference has also been illustrated in some other studies for different fungi (CAMPBELL et al. 1996, THOMAS and JENKINS 1997). It was possible that the host cuticle component might changes the thermal tolerance of the fungi at different steps of their developments on the insect host, i.e. conidia germination, vegetative growth on the cuticle surface, through the integument and in the hemolymph (FERRON et al. 1991). Additionally, HEGEDUS and KHACHATOURIANS (1996) revealed that the insects increased their body temperatures slightly at ambient temperatures of less than 15°C. At 17-20°C the insect and the ambient temperatures were equivalent and at temperatures higher than 20°C the body temperature was slightly lower than ambient air and insect body temperature to stabilize upon shifting from either 20-32°C or 32-20°C with insect's body temperature lagging behind. According to the results of conidia germination, conidia germinated at 35°C were similar to those at optimum temperature, but mortality was lower. Thus, it was probable that the fungi incorporated into body of thrips could not proliferate. Several researchers have reported correlations between optimum temperature for fungal growth and fungal infection (MANIANIA and FARGUES 1992, TEFERA and PRINGLE 2003, DIMBI et al. 2004).

In this study, it was found that *B. bassiana* Bb.5335 and *M. anisopliae* Ma.7965 were far more effective to both thrips species within a broad range of temperature. Therefore, they could be used as biological control agents for controlling *F. occidentalis* and *T. tabaci* in the agricultural ecosystem.

Relative humidity has long been recognized to strongly influence on the efficacy of entomopathogenic fungi to control insect pests. High humidity condition was an absolute requirement for establishment of fungi–induced infection of insects. However, some fungi

isolates might show a broad range of well-tolerated humidity (OSBORNE and LANDA 1992). WRAIGHT et al. (2000) reported that no significant difference was found in efficacies of *B. bassiana* and *P. fumosoroseus* against *B. argentifolii* at 25-54% RH. VIDAL et al. (2003) noted that mycoinsecticide Mycotal based on *V. lecanii* and Naturlis-L. base on *B. bassisana* showed high control potentials for whiteflies in spite of ambient dry conditions, prevailing in the laboratory (56-75% mortality at 49-54% RH) and in the field (76% at 39-85% RH). The current results confirmed that, there was no significant difference between the efficiencies of *B. bassiana* and *M. anisopliae* against *F. occidentalis* and *T. tabaci* at 60 and 85% RH, while the efficiencies of both fungi reduced at 40% RH. Several authors indicated that at least 12 h of high humidity (85-95%) were required for elevating levels of infection and spread of *V. lecanii*. Nevertheless, some hyphomycetous infections were reported to occur under low ambient humidity condition, for example, the infection of *B. argentifolii* took place under laboratory and field conditions at a low humidity (40%) (LACEY et al. 1996). This was in agreement with the present study that *B. bassiana* and *M. anisopliae* were efficacious against *F. occidentalis* and *T. tabaci* at 40% RH. In these cases, FARGUES et al. (1997) suggested that the infection was due to the conditions prevailing within the microhabitat of the insect host, which would have been expected to be more humid than the ambient conditions. In addition, it has been well known now that humidity conditions at leaf surface might differ substantially from ambient conditions (WRAIGHT et al 2000). Some authors have suggested that the RH in the boundary layer near the leaf surface would be increased because of plant transpiration (FRANSEN 1990, LACEY et al. 1996). These conditions would support fungi development and infection to insects. Besides, previous studies had suggested that ambient humidity condition was not likely to affect the efficacy of fungi to infect and kill insects, but could affect the ability of the fungi to sporulate. This study confirmed that ambient humidity conditions only had a weak influence on efficacy of *B. bassiana* and *M. anisopliae* against two thrips species. However, the microhabitat of thrips and microclimate of host plant is still unknown.

In conclusion, temperature and relative humidity affected the efficiencies of the two fungi isolates and on the host thrips susceptibility, in which host-pathogen interaction came into play.

Influence of host plant

The interactions of entomopathogens, insect hosts and host plants have been recognized. VIDAL et al. (1998) found that the larvae of *Bemisia tabaci* (GENNADIUS) (Hom., Aleyrodidae) were

similarly susceptible to *P. fumosoroseus*, when the larvae were reared on cabbage (*Brassica sativa* L.), cucumber or tomato (*Lycopersicon esculentum* MILLER). The results could be compared with the present study, where *F. occidentalis* reared on bean, leek, cucumber and swan river daisy were similarly susceptible to *B. bassiana* and *M. anisopliae*. It could be hypothesized that these host plants had no effect on the susceptibility of *F. occidentalis* and *T. tabaci* to *B. bassiana* and *M. anisopliae*. On the other hand, both thrips species reared on cotton and saintpaulia were consistently significantly less susceptible to the infection by either fungus than those reared on bean, leek, cucumber and swan river daisy. The mechanism behind the host plant effect was not known, but it could be presumed that the cotton plant and saintpaulia produced fungicidal or fungistatic compounds that might offer some protection to thrips feeding on it or inhibit the fungal infection. The inhibition could occur in at least two different ways: the conidia became inactivated when they encountered the layer of cotton or saintpaulia, or the thrips might acquire a secondary plant metabolite that interacted with the infection process in vivo. Previously, it has been reported that the terpernoid gossypol, phenolic acids, flavonols, flavones, anthocyanins, volatile terpenes, sesquiterpenoid naphtols and ketones, terpenoid aldehydes, fatty acids and lipids alone or in combination, produced by many cultivars of cotton, might have been involved in the inhibition of fungi at the third trophic level (POPRAWSKI and WALKER 2000). To our knowledge, no information regarding saintpaulia has been published.

The results reported here reveal that larvae of *F. occidentalis* and *T. tabaci* reared on different host plants differed in their susceptibilities to *B. bassiana* and *M. anisopliae* infections. Thus, the results from this study indicated that the host plant inhibited the efficacy of entomopathogenic fungi on or in the host insects. This was in agreement with a previous study from TODD et al. (2005), who found that there was a significant effect of host plant on *F. occidentalis* susceptibility to *B. bassiana*, in which thrips exposed to treated kidney beans foliage were six times more susceptible to *B. bassiana* infection than thrips exposed to treated impatiens (*Impatiens wallerana* L.). There was no study on the efficacy of entomopathogenic fungi against thrips on different host plant species, i.e. cotton, swan river daisy and saintpaulia. However, host plant effects on efficacy of entomopathogenic fungi to other insects have been reported. POPRAWSKI et al. (2000) reported that third-instar nymphs of *T. vaporariorum* were highly susceptible to the infection by *B. bassiana* and *P. fumosoroseus* when reared on cucumber, whereas the insects reared on tomato plants were significantly less susceptible to the infection. Furthermore, RAMOSKA and TODD (1985) indicated that chinch bug, *Blissus leucopterus*

leucopterus (SAY) (Het., Lygaeidae) fed on sorghum and corn were more resistant to *B. bassiana* than those on barley, and *B. argentifolii* reared on cotton was consistently significantly less susceptible to infections by either fungus than those reared on melon (POPRAWSKI and WALKER 2000).

From the practical point of view, the results herein indicate that the utilization of *B. bassiana* Bb.5335 and *M. anisopliae* Ma.7965 for controlling *F. occidentalis* and *T. tabaci* are adaptable to bean, leek, cucumber and swan river daisy.

Side effects on non-target arthropods

B. bassiana and M. anisopliae were known to have broad host ranges (ZIMMERMAN 1993), and the use of such biological control agents to control insect pests might have effect on beneficial arthropods, such as natural enemies that were also important biological control factors. However, these fungi have been recorded from different numerous insect orders, and appeared to have limited host range and were specific to single order of arthropod hosts. Furthermore, isolates or strains within a species of fungi were frequently only virulent to a few arthropod species (FENG et al. 1994). The risk of using entomopathogenic fungi also depended on the innate susceptibility of the insect, the degree of its exposure and the environmental conditions.

The current study, it was indicated that *B. bassiana* and *M. anisopliae* were found to be non-pathogenic to collembolan, *H. nitidus*. These results were supported by BROZA et al. (2001), DROMPH and VESTERGAARD (2002), who noted that *B. bassiana*, *B. brongniartii*, *Hirsutella* spp. *M. anisopliae* and *V. lecanii* did not affect the mortalities of three collembolan species, *Folsomia fimetaria* L. *Proisotoma minuta* (TULLBERG) (Collembola: Isotomidae) and *Hypogastrura assimilis* (KRAUSBAUER) (Collembola: Hypogastruidae).

Entomopathogenic fungi infections on natural enemies were not particularly common and many natural enemy species appeared to be resistant (GOETTEL et al. 1990). The present results support this theory that *C. septempuntata* showed resistance to *B. bassiana* and *M. anisopliae*. However, previously, JAMES and LIGHTHART (1994) reported that *M. anisopliae*, *B. bassiana* and *P. fumosoroseus* have the potential to infect *Hippodamia convergens* GUÉRIN MÉNEVILLE (Col., Coccinellidae), whereas *N. rileyi* did not. *Serangium parcesetosum* SICARD (Col., Coccinellidae) had significantly lower survivorship when sprayed with *B. bassiana* than with *P. fumosoroseus* (POPRAWSKI et al. 1998). MAGALHÃES et al. (1988) noted that *B. bassiana* caused mycosis in

60% of adult *Coleomegilla maculata lengi* TIMBERLAKE (Col., Coccinellidae) and in 35% of adult *Eriopis connexa* (Col., Coccinellidae), when conidia were applied directly to the insects. However, both coccinellid species were not infected following exposure to spore showers of *Zoophthora radicans* (BREFELD) BATKO (Zygomycetes: Entomophthorales). TODOROVA et al. (1994) remarked that different stains of *B. bassiana* showed different efficacies on larvae of *C. maculata lengi*. The different ecological host ranges of different entomopathogenic fungus isolates, e.g. co-evolution between hosts and pathogens could partially explain the different susceptibilities found in this study and in previously reported studies.

Entomopathogenic fungi could infect most genera of insects and mites, but different species or isolates were different pathogenicities and virulence. It could be quite specific and might only infect one type of host. The present results found that *M. anisopliae* were pathogenic to *D. tamaninii* and *C. carnae*, while *B. bassiana* had no negative effect on both insects. Few cases have been published on the effects of entomopathogenic fungi on *C. carnea*. SEWIFY and EL ARNAOUTY (1998) reported that *V. lecanii* impaired the feeding and searching capacity of *C. carnea*. No data on *D. tamaninii* was reported in the review for either of these two fungi or other fungi species. The present study examined for the first time that *B. bassiana* and *M. anisopliae* were highly pathogenic to *P. persimilis*. No comparable data was available on such effects of entomopathogenic fungi on this predatory mite. However, JACOBSON et al. (2001) revealed that Naturalis-L based on *B. bassiana* had no detrimental effect on *Amblyseius cucmeris* OUDEMANS (Acari: Phytoseiidae).

It could be concluded that *B. bassiana* and *M. anisopliae* were non-pathogenic to some beneficial soil insects and have low pathogenicity to insect natural enemies, but were moderate pathogenicity to predatory mite *P. persimilis*.

Experiments under greenhouse conditions

Persistence of *Beauveria bassiana* Bb.5335 and *Metarhizium anisopliae* Ma.7965 on different host plants

The ability of entomopathogenic fungi to persist in the host plant was important for the effectiveness of fungi to control insects. The persistence of *B. bassiana* and *M. anisopliae* conidia, determined in this study by conidial viability and 1st larval instar's mortality, was largely influenced by the host plant, whether on crop or on ornamental host plant. The conidial viability recorded in cucumber was the highest while the lowest conidial viability was found on

cotton. The previous study also reported that conidia of the same fungal isolate differed in their viabilities on different host plants. INGLIS et al. (1993) stated that conidia of *B. bassiana* on leaves of alfalfa survived much longer than those in wheatgrass. Similarly, *B. bassiana* could survive longer on lettuce than on celery (KOUASSI et al. 2003). *A. aleyrodis* conidia survived longest on cucumber, followed by poinsettia and lowest on gerbera (MEEKES et al. 2000).

The efficacies of *B. bassiana* and *M. anisopliae* conidia after prolonged exposure against *F. occidentalis* and *T. tabaci* were better on bean, cucumber and swan river daisy than on leek, cotton and saintpaulia under greenhouse conditions, whereas the mortality in the controls of these plants were similar. This could be explained that the conidia were more persistent on leaves of bean, cucumber and swan river daisy than on leek, cotton and saintpaulia leaves. Host plants influencing the persistence of entomopathogenic fungi to control insects e.g. *T. vaporariorum*, *Lygus lineolaris* PALISOT DE BEAUVOIS (Het., Miridae) and *P. xylostella* have been reported (INGLIS et al. 1993, FURLONG and PELL 1997, MEEKES et al. 2000, WRAIGHT et al. 2000, KOUASSI et al. 2003). However, there was no literature so far about influence of host plant on persistence of fungi to thrips. This was the first study, which stated that the same fungal isolate, *B. bassiana* or *M. anisopliae* differed in its persistence on the six host plants to control thrips. *B. bassiana* and *M. anisopliae* were highly efficacious against 1st larvae of *F. occidentalis* and *T. tabaci* on cucumber and less efficacious against the same insect species on saintpaulia. Therefore, the results presented herein and with other published information could be due to a number of reasons. Firstly, the composition of the phyllosphere microbiota differed among and within host plant species. Some microorganisms were exclusively associated with one plant; others were more opportunistic. Several interactions existed between microbes on the leaf surface, such as competition for nutrients, antibiosis and mycoparasitism, and the leaf microbiota might compete with the entomopathogens (ELAD et al. 1996). Secondly, the chemical composition and cuticular waxes of the host plant could differ substantially. Chemical substrates leached from the leaf might act as nutrient sources for fungi, while leaf cuticles contained components that could also be exploited. On the other hand, leaves of certain plants might exude fungistatic substances such as catechol, salicyclic acid, tannic acid or terpernoid gossypol, which could inhibit germination or restrict germ-tube growth (VEGA et al. 1997, POPRAWSKI and WALKER 2000). Conidial viability and percentage mortality on cotton was low, presumably for the same reason. Difference in chemical plant substances might also have an effect on the host insect, thus influence fungal infection indirectly. Fungal inhibitors produced by the plant might

protect the insect as well (RAMOSKA and TODD 1985). Thirdly, the morphology of the host plant and its canopy characteristics might play a role. Leaf surface features, such as size and shape, surface topography and canopy characteristics varied among plant species. Cucumber had large, hairy leaves, whereas cotton had smaller, rather smooth leaves. These features affected the leaf boundary layer, in which insect-fungus interactions took place. Broad and/or hairy leaves had a thicker boundary layer than narrow leaves and leaves with a smooth surface. Similarly, temperature, placement of leaves on a plant (sunny or shady) and turbulence would also influence the thickness of this layer. The microenvironment of the fungus, and especially relative humidity, was of great importance to its longevity.

The current data indicated that conidia persistence declined over time. This point was supported by many researchers, which stated that direct sunlight, temperature and humidity have played an important role in reducing conidial viability on the foliage (FARGUES et al. 1996, MOORE et al. 1996). JAMES et al. (1995) reported that *B. bassiana* conidial persistence and infectivity up to 28 days on alfalfa leaves. *A. aleyrodis* conidia stayed viable and were able to infect 90% of whitefly nymphs 31 d after application (MEEKES et al. 2000). Similarly, present results indicated that the remaining conidia on leaves after 30 days were still infective under greenhouse conditions of high UV, fluctuating temperature and relative humidity. It could be explained that these fungi isolates might adapt well to survive, and have high enough pathogenicity to maintain insect control even after substantial decline in conidial viability. In addition, it has been well known that temperature and humidity conditions at the leaf surface might differ substantially from ambient conditions (WRAIGHT et al. 2000), because fungi did require moisture for development. Sufficient moisture existed within the microhabitat of many insect hosts or within the microenvironment of the host's body surface to support infection was essentially independent of ambient moisture conditions (FARGUES et al. 1997). Conidial rate decline observed on cucumber was not significant, even under the same abiotic conditions as were present for cotton. This could be explained by differences of crop types.

In conclusion, the type of host plant must be considered to guarantee success of foliar applications for *B. bassiana* and *M. anisopliae* in the greenhouse control of both thrips species.

Efficiency of *Beauveria bassiana* Bb.5335 and *Metarhizium anisopliae* Ma.7965 for controlling *Frankliniella occidentalis* in greenhouse

Many isolates of entomopathogenic fungi have been found to be effective to control insects in laboratory conditions, but be failed to suppress insects population in greenhouse conditions. Thus, it would be necessary to evaluate efficacy of *B. bassiana* Bb.5335 and *M. anisopliae* Ma.7965 in greenhouse before their adoption as biological control agents.

The results of these experiments demonstrated that *B. bassiana* and *M. anisopliae* had efficiency to control *F. occidentalis* in greenhouse. The mean number of *F. occidentalis* individuals/plant was significantly higher in the control experiment than in the experiments with *B. bassiana* and *M. anisopliae*. Various researchers have shown that different entomopathogenic fungi could effectively control *F. occidentalis* in greenhouse. HEYLER (1993) and MANIANIA et al. (2001) reported that *V. lecanii* and *M. anisopliae* had the potential to reduce both larval and adult populations of *F. occidentalis* on chrysanthemum. In addition, BROWNBRIDGE (1995) stated that *M. anisopliae* reduced *F. occidentalis* population on chrysanthemum by about 72% and about 47% by *B. bassiana*. In addition, *M. anisopliae* reduced population of *F. occidentalis* on cucumber by about 60% (VAN DER SCHAAF et al. 1990).

The present study showed that *B. bassiana* and *M. anisopliae* had the same efficacies to control *F. occidentalis*. Although, at the 1st and 2nd week after application *M. anisopliae* showed higher efficacy to control *F. occidentalis* than *B. bassiana*, while at 3rd weeks after application *B. bassiana* performed to control *F. occidentalis,* however, there was no significant difference between their efficiencies. This points was supported by early stages of this studies on biological characteristic, which indicated that *B. bassiana* had resistance to UV light than *M. anisopliae*; the results of persistence also found that *B. bassiana* was more persistent on plants under greenhouse conditions than *M. anisopliae*. In present study, no significant difference was observed at 3 and 4 weeks after application. It could be explained firstly by the fact that cucumbers were flowering and thus the thrips populations had built up. Secondly, the survival of conidia decreased over time. This finding confirmed the results previously presented by AZAIZEH et al. (2002), who had suggested that biweekly application of *M. anisopliae* was found to be effective in reduction of the population growth of *F. occidentalis* in cucumber under greenhouse conditions, particularly when the initial thrips density was low (with 2 or 3 thrips per leaf) to moderate (with 3 or 4 thrips per leaf). But, when the cucumber was initially heavily infested with

F. occidentalis (10-15 thrips/leaf), it caused only a modest reduction in *F. occidentalis* population and only 4 weeks after application was the reduction significant. In these studies, it found that cucumber was heavily infested with *F. occidentalis*, *B. bassiana* and *M. anisopliae* still caused highly reduction *F. occidentalis* population. MANIANIA et al. (2003) revealed that weekly and biweekly applications of *M. anisopliae* on onion crops in the field significantly lowered the thrips density and damage in comparison to the untreated control.

The present results suggested that *B. bassiana* Bb. 5335 and *M. anisopliae* Ma.7965 were efficient to be biological control agents against *F. occidentalis* on cucumber crops. Although for maximum benefit, the uses of *B. bassiana* and *M. anisopliae* to control *F. occidentalis* in greenhouse conditions would require application at two-week interval. However, further experiments should be required development of the appropriate produced formulations of the effective entomopathogenic isolates under field conditions.

Conclusion

From 41 isolates of different entomopathogenic fungi from Thailand, 2 isolates, e.g. *B. bassiana* Bb.5335 and *M. anisopliae* Ma.7965 displayed high efficiencies against various developmental stages of *F. occidentalis* and *T. tabaci* under both in laboratory and greenhouse conditions. They were relatively safe on non-target beneficial insects, but were moderately pathogenic to predatory mite *P. persimilis*. Additionally, these two selected fungal isolates showed superior abilities in a broad range of good tolerance to high or low temperatures, resistance to low relative humidity as well as long persistence on the leaf surface under high UV, fluctuating temperature and relative humidity. Consequently, these fungi were very promising to be used as biological control agents within the framework of IPM programs. However, there are still some points to be further investigated; the efficiency and persistence of the fungi under field conditions, side effects on other non-target arthropods, their combined use with natural enemies, safety to environment, production and formulation of fungi as well as strategies of application to successfully suppress these two thrips.

SUMMARY

In the present study, efficiency of 41 different entomopathogenic fungal isolates, belonging to 25 species from 11 genera isolated from different hosts in Thailand as biological control agents were investigated against *Frankliniella occidentalis* (PERGANDE) and *Thrips tabaci* LINDEMAN (Thys., Thripidae) in the laboratory and under greenhouse conditions.

In the laboratory, the result of preliminary screening showed that 37 out of 41 isolates, belonging to *Akanthomyces* sp., *Aschersonia* spp., *Beauveria* spp., *Cordyceps* sp., *Hirsutella* spp., *Metarhizium* spp., *Paecilomyces* spp., *Torrubiella petchii* (HYWEL-JONES) and *Verticillium* spp. had pathogenicities against *F. occidentalis*. The same isolates, except *T. petchii* were found to be pathogenic to *T. tabaci*. *Hymenostilbe* sp., *Hypocrella discoidea* (BERK and BROOME) and *Cordyceps pseudomilitaris* (HYWEL-JONES) were found to be non-pathogenic against both thrips species. Fungi isolates varied significantly in term of pathogenicity, and could be classified into three pathogenicity groups. Secondary screening, in the high pathogenicity group, there were 16 isolates and these isolates were screened for their degree of virulence. It was found that 5 isolates, i.e. 2 isolates of *B. bassiana* (Bb.4591, Bb.5335), 2 isolates of *M. anisopliae* (Ma.6079, Ma.7965) and 1 isolate of *P. fumosoroseus* (Pfu.5338) displayed the highest degree of virulence. Tertiary screening, the efficiency of 5 virulent isolates was investigated against various life stages of both thrips species. The efficiency of the 5 fungi isolates differed at various life stages. The susceptibility of both thrips to fungi decreased from larvae over pupae to adults. *B. bassiana* Bb. 5335 and *M. anisopliae* Ma.7965 had a significantly highest efficacy throughout the following series of screenings and were used in further experiments.

In further experiments, biological and ecological characteristics of *B. bassiana* Bb. 5335 and *M. anisopliae* Ma.7965 such as morphological and physiological characteristics were studied. Morphological characteristics showed that colonies of *B. bassiana* appeared pale-yellow, conidia were hyaline with globose to ellipsoidal in shape and ranged from 2.16-3.26 µm in diameter. The colonies of *M. anisopliae* were green, conidia were cylindrical with rounded ends, ranging from 6-6.8 µm long and 2-2.7 µm wide.

Physiological characteristics, conidia viability decreased as time increased, where *M. anisopliae* conidia were viable for at least one year longer than those of *B. bassiana*. The experiments on germination at different environmental factors showed that temperature at 18, 25, 30 and 35°C did not greatly affect the germination rate, while clearly influenced the speed of germination.

Relative humidity (RH) exerted a direct influence on germination. Percentage germination of both fungal isolates at 62, 85 and 100% RH had a significantly higher germination than at 32% RH. Similarly, low RH caused a decrease of germination speed. Light-dark conditions had no effect on germination but a speed of germination of both fungal isolates was delayed under dark condition. Ultraviolet light had negative effect on germination and speed of germination. The investigations into conidiation at different environmental factors found that the timing and abundance of conidia production varied among fungi and temperatures. The optimal conidia production of *B. bassiana* took place mostly at 25°C followed by 18 and 30°C, while at 30°C followed by 25 and 18°C were optimal for *M. anisopliae.* At 35°C, *M. anisopliae* could produce conidia, while *B. bassiana* could not produce. Relative humidity influenced conidia production of both fungi isolates. The conidiation increased with increasing RH. The peak of conidia production were found at 100% RH followed by 85, 62 and 32% RH, respectively. As results of light-dark conditions, both fungi isolates produced more conidia under continuous light than darkness and 16:8 (L:D) conditions. The conidiation of *M. anisopliae* more affected than that of *B. bassiana*. Ultraviolet light affected the conidiation of both fungi in which *M. anisopliae* had greater response to UV light than *B. bassiana*. The colony growth at different environmental factors showed that temperature significantly affected colony growth of *B. bassiana* and *M. anisopliae*. They were slower at 18 and 35°C compared to 25 and 30°C. The relative humidity had significantly affected colony growth, it was slower at 32% RH compared to 62, 85 and 100% RH. As colony growth under light-dark conditions, the colony growth was faster under continuous light than others. Ultraviolet light affected the colony growth of both fungi. *B. bassiana* had more response to the UV light than *M. anisopliae*.

Mode of infection of *B. bassiana* Bb.5335 and *M. anisopliae* Ma.7965 to *F. occidentalis* showed that conidia of *B. bassiana* and *M. anisopliae* had randomly attached over the cuticle of *F. occidentalis*. Both fungi isolates germinated on the cuticle of all life stages started at 12 h post inoculation (pi). Stages of thrips and temperatures significantly affected germination and elongation. Penetration of fungi into the cuticle started from 36 h pi, but percentage and speed of penetration were dependent on life stage and temperature. *B. bassiana* did not form an appressoria. The fungi infected by direct hyphal penetration, while *M. anisopliae* formed an appressoria before penetration. The colonization behaviour observed on *B. bassiana* and *M. anisopliae* were similar. The fungus developed mycelia inside the haemocoel within 48 h pi. At 60 h pi, the mycelium appeared to cover the whole thrips cuticle. The hyphal bodies developed

through the thrips cuticle within 72 h pi. However, stage of thrips and temperature was found to have effects on development of pathogenicity process. Pathogenicity process was longest on adult, followed by pupal and larval instars and they were delayed at 18 and 35°C compared to 25 and 30°C.

Efficiency of *B. bassiana* Bb.5335 and *M. anisopliae* Ma.7965 against *F. occidentalis* and *T. tabaci* under different abiotic factors found that temperature was influenced the efficacy of both fungi. Those had a significantly higher ($P \leq 0.05$) efficacy against both thrips species at 25 and 30°C than at 18 and 35°C. Different relative humidity levels also illustrated that two fungi isolates were more effective against both thrips species at 60 and 85% RH than at 40% RH. The LC_{50} values decreased with increasing RH. Host plant influenced the efficacy of the two fungi against *F. occidentalis* and *T. tabaci* in which LC_{50} values varied with the host plant, whether on crop or ornamental plant. Both thrips species reared on bean, leek, cucumber and swan river daisy were highly susceptible to fungal infection, but were significantly less susceptible when reared on cotton and saintpaulia.

Side effects on non-target arthropods showed that *B. bassiana* and *M. anisopliae* were non-pathogenic to *Heteromurus nitidus* TEMPLETON (Collembola: Entomobryidae) and *Coccinella septempunctata* LINNAEUS (Col., Coccinellidae). On the other hand, those were moderately pathogenic to *Phytoseiulus persimilis* ATHIAS-HENRIOT (Acari: Phytoseiidae), but only *M. anisopliae* was found to be pathogenic to *Dicyphus tamaninii* WAGNER (Het., Miridae) and *Chrysoperla carnea* (STEPHENS) (Neur., Chrysopidae).

Under greenhouse conditions, the persistence of *B. bassiana* Bb.5335 and *M. anisopliae* Ma.7965 conidia was influenced by host plant, whether on crop or ornamental plant. Among all the crop plants used, cucumber had the highest significantly conidia viability, while it was the lowest on cotton. Conidia of both fungi isolates were viable on the leaf surfaces of cucumber and bean for one month. Ornamental plants had also effect on conidia viability. Conidia of both fungi isolates were more viable on flower surfaces of swan river daisy than saintpaulia. Efficiency of conidia after prolonged exposure based on percentage mortality found that the mortality of both *F. occidentalis* and *T. tabaci* regarded to conidia viability and crop plants. The mortality of both thrips on cucumber was high throughout experiment, while low mortality was observed on cotton. The mortality on ornamental plants showed that mortality of both thrips species on swan river daisy was significantly higher than that on saintpaulia. LT_{50} values of *F. occidentalis* and *T.*

tabaci larvae treatment with *B. bassiana* and *M. anisopliae* differed greatly among host plants and they increased with increasing time after application.

Efficiency of *B. bassiana* Bb.5335 and *M. anisopliae* Ma.7965 for controlling *F. occidentalis* in greenhouse showed that *B. bassiana* and *M. anisopliae* displayed higher effectiveness in controlling *F. occidentalis* in greenhouse. Significant reductions in *F. occidentalis* population were recorded compared to control within 14 days after application.

From a practical point of view, the results of current study indicated those entomopathogenic fungi from Thailand, *B. bassiana* Bb.5335 and *M. anisopliae* Ma.7965 have the potential to be used as biological control agents against *F. occidentalis* and *T. tabaci* in both laboratory and greenhouse.

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