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).

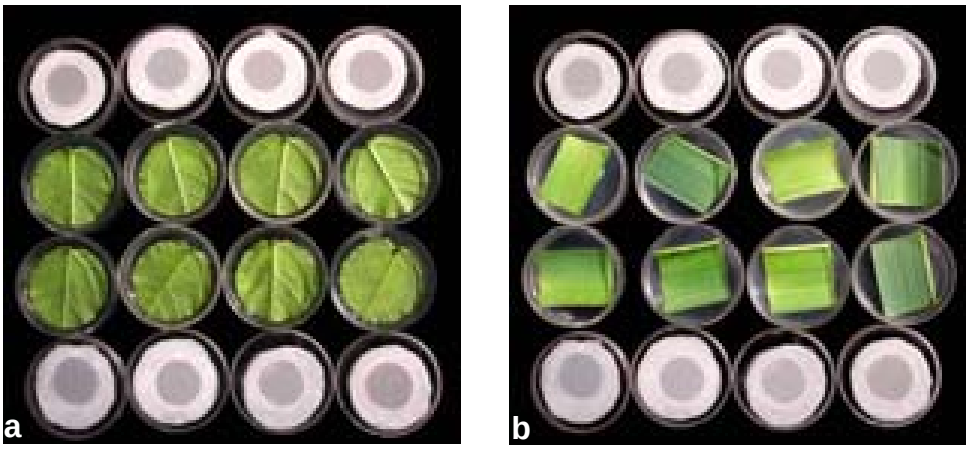

Fig. 1: Round Plexiglas for obtaining the uniformly aged and bioassay of *Frankliniella occidentalis* (a) and *Thrips tabaci* (b)

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).

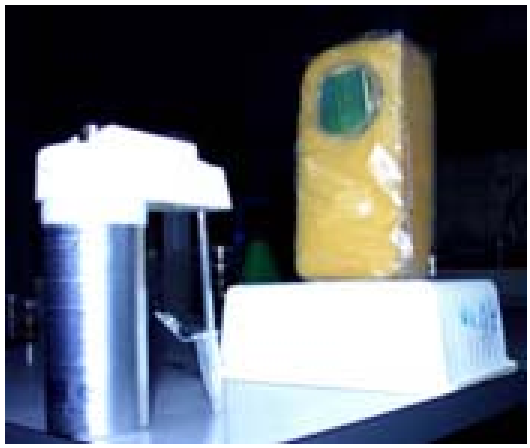

Fig. 2: Sprayer using for applied conidial suspensions

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.*