

## Evaluation of different isolates of entomopathogenic fungus, *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes) against *Bemisia tabaci* (Hemiptera: Aleyrodidae)

Rahim Eslamizadeh<sup>1</sup>, Ahmad Said B Sajap<sup>1</sup>, Dzolkhifli B Omar<sup>2</sup> and Nur Azura Binti ADam<sup>2</sup>

1-Department of Forest Management, Forestry Faculty, UPM

2-Department of Plant Protection, Agriculture Faculty, UPM

**Corresponding author:** Rahim Eslamizadeh, email: r.eslamizadeh@areo.ir

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### Abstract

*Bemisia tabaci* (Gennadius) (Homoptera, Aleyrodidae) is one of the most important whitefly pests in different parts of the world including subtropical and tropical region as well as in the greenhouse production systems. In order to search for an effective biocontrol agent against this pest, we conducted this study to determine the effectiveness of the entomopathogenic fungus, *Paecilomyces fumosoroseus* against *B. tabaci*. The viability of *P.fumosoroseus* isolates conidia was determined by culturing them on potato dextrose agar (PDA) culture medium and assessing their germination after 24 h of incubation at  $24 \pm 2$  °C. Results of this experiment showed that percent of germination was 100, 100,  $98 \pm 1$ ,  $97 \pm 1$ ,  $96 \pm 2$ , 100,  $95 \pm 1$ , 100,  $98 \pm 2$ , and  $97 \pm 1$  for isolates P.F.UPM, P.F.49, P.F.39, P.F.24, P.F.40, P.F.29, P.F.16, P.F.1, P.F.12, and P.F.2, respectively. Mortality of egg and nymph of *B. tabaci* caused by different isolates of *P. fumosoroseus* was studied under laboratory conditions using fungal conidia suspension with a concentration of  $1 \times 10^6$  conidia mL<sup>-1</sup>. The mortality of *B. tabaci* eggs and second, third and fourth instars nymphs caused by the *P. fumosoroseus* isolates was from 42 to 91, 38 to 90, 37 to 89, and 41 to 86%, respectively. The P.F.UPM as the most lethal isolate caused 91, 90, 86 and 89% mortality on eggs and second, third and fourth instars respectively. Mortality caused by the other isolates ranged between 42 to 76%, 38 to 74%, 37 to 85%, and 41% to 84% for eggs and second, third and fourth instars respectively. There was a significant difference between various isolates for causing mortality on eggs and second, third and fourth instars respectively. According to the results, there was a strong correlation between mortality in all stages of *B. tabaci* life cycle and the fungal isolates. Conidia concentrations of *P. fumosoroseus* (UPM) isolate for concentration mortality response test were  $1 \times 10^8$ ,  $1 \times 10^7$ ,  $1 \times 10^6$ ,  $1 \times 10^5$  and  $1 \times 10^4$  conidia mL<sup>-1</sup>. In the concentration-mortality response tests with the second instar nymphs mortality reached up to 95% within 6 days after application of conidia suspension at the concentration of  $1 \times 10^8$  conidia mL<sup>-1</sup> and there were significant differences among various concentrations. The LC<sub>50</sub> of this fungus against the second instar nymph of *B. tabaci* six days after application was determined as  $2.77 \times 10^5$  conidia mL<sup>-1</sup>. The *P. fumosoroseus* isolate UPM obtained from *B. tabaci* was more effective against all stages of *B. tabaci* compared with the fungi isolated from *Pteroma pendula*. However, further studies have to be carried out to determine effectiveness of this isolate in the field conditions.

**Keywords:** Entomopathogenic fungi, *Bemisia tabaci*, *Paecilomyces fumosoroseus*, bioassay, biological control, whitefly

## Introduction

Whiteflies are very small sap-sucking insects that usually infest the leaf and foliage of the plants. The bodies and wings of most adult whiteflies are dusty with secreted white powdery material, thus leading to the common name of this group of insects. In recent years, they have become one of the most economically important groups of insects found on food crops and ornamental plants. Even though whiteflies are mainly subtropical and tropical in distribution, several injurious species occur in the temperate zones of the world, some of which are serious pests. Particularly damaging of the species that are vectors of viral diseases of vegetables (Suh & Hodges, 2008).

Infested plants may exhibit a range of symptoms due to direct feeding damage, contamination with honeydew and associated sooty molds, whitefly-transmitted viruses and phytotoxic responses. There may be one or a combination of the following symptoms: chlorotic spotting, vein yellowing, inter-vein yellowing, leaf yellowing, yellow blotching of leaves, yellow mosaic of leaves, leaf curling, leaf crumpling, leaf vein thickening, leaf enations, leaf cupping, stem twisting, plant stunting, wilting and leaf loss (Malumphy *et al.*, 2007). The importance of whiteflies as economic pests seems to develop constantly and various species of whiteflies (Aleyrodidae) are among the most severe of agricultural pests, causing millions of dollars in losses each year to various crops.

*Bemisia tabaci* was first described in 1889 as a tobacco pest in Greece and named *Aleyrodes tabaci* (Gennadius, 1889), and subsequently became known under the common names as tobacco whitefly, sweet potato whitefly, cotton whitefly, and cassava whitefly. *Bemisia tabaci* is a plant sap-sucking insect from Aleyrodidae family in the superfamily Aleyrodoidea (whiteflies). It is

readily compatible with new host plants and geographical regions and has now been reported from all continents, except Antarctica, and its polyphagous nature has been recorded in more than 600 plant species worldwide (Oliveira *et al.*, 2001). It causes direct injury by feeding on leaves and also indirect damage by promoting growth of black sooty mould on their honeydew secretions and by vectoring economically important plant viruses (Faria & Wraight, 2001). Control of *B. tabaci* has been accomplished through the use of common insecticides and unfortunately, the frequent use of insecticides has resulted in the development of resistant *B. tabaci* populations and whitefly resistance to insecticides is reported in many countries (Ferron, 1978).

The negative environmental effects and new ideas have encouraged the development of replacement pest management strategies, in which microbial control play a more important role. In terms of health, quality and quantity of food production. Microbial organisms (entomopathogenic fungi, bacteria and viruses) are new agents that can be used for control of several important greenhouse and field pests. Entomopathogenic fungi collected from nature have been isolated and propagation on artificial medium and specific hosts (Faria & Wraight, 2001).

In the present study, we tested the effectiveness of 10 isolates of *Paecilomyces fumosoroseus* (Hypocreales) entomopathogenic fungus against *B. tabaci* in laboratory conditions that were comparable to greenhouse conditions. The specific objectives of this study were: (1) to determine effectiveness of entomopathogenic fungi against *B. tabaci*, (2) to determine the effect of conidia concentration of the most promising isolates on the mortality of nymph, and (3) to compare infection rates between the eggs, second, third and fourth instar nymph stages of *B. tabaci*.

The result from this study is useful in the development of fungal biocontrol agents against *Bemisia tabaci*.

## Material and methods

### Collection of insects

The original whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae) (*B. tabaci* biotype Asia 1) on cucumber, *Cucumis sativus* (Cucurbitaceae) obtained from greenhouse of Faculty Agricultural, Universiti Putra Malaysia (UPM) were reared on cucumber in greenhouse at 65%±5 RH and 24°C±1. Every two weeks, new cucumber plant was planted and 20 day old plants were exposed to the adult whiteflies. Plants have infested by eggs were transferred to cages and divided to three groups. The first group was used for *P. fumosoroseus* bioassay against whitefly eggs kept until nymph emergence to evaluate the efficacy of the fungus against nymphal stages, second instar, (509 ±23.96  $\mu\text{m} \times 303 \pm 18.95 \mu\text{m}$ ), Third instar, (559 ± 22.41  $\mu\text{m} \times 352 \pm 17.75 \mu\text{m}$ ) and Fourth instar, (613 ± 14.67  $\mu\text{m} \times 358 \pm 11.02 \mu\text{m}$ ) of *B. tabaci*.

### Fungal isolates

Ten isolates of *Paecilomyces fumosoroseus* obtained from Forestry Faculty of UPM, were screened for assay against *Bemisia tabaci* (Aleyrodidae).

Fungal isolates screened against *Bemisia tabaci* and their source were P.F.UPM isolated from *B. tabaci* in greenhouse UPM-Malaysia in 2011 and P.F.49, P.F.39, P.F.24, P.F.40, P.F.29, P.F.16, P.F.1, P.F.12 and P.F.2 isolated from *Pteroma pendula* in Langkap-Malaysia in 2009.

The isolates were propagated from a single conidium to obtain pure cultures. So, to achieve this, 100  $\mu\text{L}$  of conidial suspensions ( $1 \times 10^6$  conidia  $\text{mL}^{-1}$ ) were inoculated on potato dextrose agar (PDA) medium with 1% yeast extract and incubated at 25 °C for 4 days. Following

incubation, a single colony was transferred to another petri dish onto PDAY medium and incubated at 25 °C for one week. Conidium was harvested by washing the dishes with an aqueous solution of 10 mL 0.1% Tween 80. Subsequently the conidium suspension was filtered through cloth filter to remove mycelium and then shaken for 5 min by using a vortex to homogenize the suspension. Conidia were counted in an improved Neubauer haemocytometer and aliquots were adjusted to the desired concentration (Sevimab *et al.*, 2009).

The viability of conidia of each isolate was determined by placing a drop of each diluted conidial suspension on PDAY. The drop was covered with sterile microscope cover slip and germination was assessed after 24 h of incubation at 24 ±2 °C. Conidia were considered to have germinated if the germ tube was longer than the diameters of the conidium. The numbers of germinated and ungerminated conidia were counted and viability percent was calculated. Isolates with viability more than 95% were used in the concentration-mortality response tests. Autoclaved broken white rice in polypropylene bags were used as solid substrate in the second stage. The bags were incubated up to 14 days at 25 ±1 °C for fungal development and sporulation. The plastic bags were then opened, the rice with conidia were spread out in clean plastic trays and placed inside a drying cabinet at 20 ±3 °C. After five days, the moisture content was around 20%. Conidia were then extracted from the rice by sieving through brass laboratory sieves (300  $\mu\text{m}$  mesh). After sieving, conidia were dried further in an auto-desiccator cabinet with a built-in hygrometer for four days to reduce the moisture content to around 5% (Moore & Prior, 1993).

### Screening tests

The isolates with viability of over 95% were used in the bioassays. In the screening tests,  $1 \times 10^6$

mL<sup>-1</sup> conidium suspensions were used for all isolates. Controls were treated only with sterile water containing 0.1% Tween 80 (Burgess & Thompson, 1971). Middle leaves of 2–3 week old cucumber plants, *Cucumis sativus* (Cucurbitaceae), that were the same age of the eggs, second, third and fourth instar nymphs of *B. tabaci*, were excised and the petioles held in place by absorbent cotton wool. The fresh eggs and nymphs were counted and placed on the leaves dipped in the respective conidium suspension ( $1 \times 10^6$  mL<sup>-1</sup>) for 2–3 s or in 0.1 Tween 80 aqueous solution (as control) in four replicates. After 2nd and 7th day, mortality was recorded. Only those *B. tabaci* which showed symptoms of fungal infection as manifested by sporulation of the fungus breaking through the cuticle were counted as a kill by the pathogen (Cuthbertson *et al.*, 2005).

#### Concentration-mortality response test

Conidial concentrations of *Paecilomyces fumosoroseus* UPM isolate was prepared from the initial concentration of  $5 \times 10^8$  conidia/ml, using a Neuberg haemocytometer, and serially diluting in test tubes with distilled water as the carrier to produce  $1 \times 10^8$ ,  $1 \times 10^7$ ,  $1 \times 10^6$ ,  $1 \times 10^5$  and  $1 \times 10^4$  conidia/ml. The initial concentrations were stored in the refrigerator at 4 °C prior to use (Burgess, 1998). In this pathogenicity test, middle leaves of 2–3 week old cucumber, *Cucumis sativus*, that were of the same age as the second instar nymph of *B. tabaci*, were excised and the petioles

were held in place by absorbent cotton wool. Fresh nymphs were counted and placed on treated leaves (dipped in the respective conidium suspension ( $1 \times 10^6$  mL<sup>-1</sup>) for 2–3 s or in 0.1 Tween 80 aqueous solution as controls) in three replicates. At the 2nd, 4<sup>th</sup>, 6<sup>th</sup>, 8<sup>th</sup>, and 10<sup>th</sup> day after treatment mortality was recorded. Only those *B. tabaci* that showed symptoms of fungal infection as manifested by sporulation of the fungus breaking through the cuticle were counted as a kill by the pathogen.

#### Statistical analysis

In the concentration-mortality response tests, the percentage mortality data were corrected using Abbott's formula and  $LC_{50}$  values were calculated by Probit Analysis (with statistical package Polo Plus version.1), based on Finney (Abbott, 1925). All experiments were repeated three times. In the screening tests, the percentage mortality data of diseased insects were corrected by Abbott's formula and analyzed as a completely randomized design using the analysis of variance procedure in the SAS statistical package. Treatment means were separated by Tukey's multiple range test at 0.05 level of significance.

## Results

#### Screening bioassay

Before conducting, the bioassay conidia from each isolates were tested for their viability. Result showed that the viability was 97 to 100 percent. (Table 1).

Table 1- Germination percentage of isolates 24 h after incubation at  $24 \pm 2$  °C.

<i>P. f.</i> isolates	UPM	49	39	24	40	29	16	1	12	2
Germination %	100±0	100±0	98±1	97±1	96±2	100	95±1	100	98±2	97±1

The *P. fumosoroseus* isolates produced different mortality rates among eggs, and second, third and fourth instar nymphs of *Bemisia tabaci*. In the case of *B. tabaci* eggs mortality caused by

the *P. fumosoroseus* isolates, the values ranged from 42 to 91%. The *P. fumosoroseus* UPM isolate caused 91% mortality and was the most lethal among tested isolate. Mortality caused by the other

fungi ranged from 42 to 76%, and were significantly different from each other. There was a significant relationship between mortality ( $r^2= 0.92$ ) and the fungal isolates (Fig. 1 and Table 2).

*B. tabaci* second instar nymph mortality caused by the *P. fumosoroseus* isolates ranged from 38 to 90% (Fig. 1 and Table 3). Mortality caused by the other fungi ranged from 38 to 74%, and were significantly different from each other. There was a strong relationship between mortality ( $r^2=0.94$ ) and fungal isolates (Fig. 1 and Table 2).

In this study the *B. tabaci* third instar nymph mortality caused by the *P. fumosoroseus* isolates ranged from 37 to 89%. The *P. fumosoroseus* UPM isolate caused 89% mortality and was again the most lethal of the 10 fungus isolates (Fig. 1 and Table 3). Mortality caused by the other fungi

ranged from 37 to 85%, and were significantly different from each other. There was a strong relationship between mortality ( $r^2= 0.98$ ) and fungal isolates (Fig. 1 and Table 2).

The *B. tabaci* fourth instar nymph mortality caused by the *P. fumosoroseus* isolates ranged from 41 to 86 %. *Paecilomyces fumosoroseus* UPM isolate caused 86% mortality and was the most lethal of the 10 fungus isolates tested (Fig. 1 and Table 3). Mortality caused by the other fungi ranged from 41 to 84 %, which were significantly different from each other. There was a strong relationship between mortality ( $r^2= 0.98$ ) and fungal isolates (Figure 1 and Table 3).

Table 2- Mean comparison of mortality of eggs and immature stages of *B. tabaci* treated with  $1 \times 10^6$  conidia/ml of *P. fumosoroseus* isolates.

<i>P. fumosoroseus</i>	Eggs	Second instar Nymph	Third instar Nymph	Fourth instar Nymph
Isolates	Means	Means	Means	Means
P. f- UPM	91.00±.71a	90.00±.50a	889.75±1.25a	86.25±.48a
P. f- 49	76.00±4.50ab	73.75±2.98ab	84.50±.95a	84.50±.95a
P. f- 2	67.00±2.54bc	60.50±2.90bc	59.00±1.16b	58.25±1.70b
P. f- 29	55.50±2.70cd	57.25±3.35cd	48.00±.70cd	43.25±1.25de
P. f- 40	50.50±.87cd	54.25±1.93cd	42.50±1.70de	42.75±1.25de
P. f- 39	48.75±8.27d	53.75±2.59cd	51.75±.47bc	51.50±.95bc
P. f- 1	46.50±6.22d	43.25±3.35de	41.50±3.79de	43.25±1.25de
P. f- 24	46.00±.41d	50.00±4.70cd	45.50±1.55cd	46.75±2.46de
P. f- 16	43.00±1.47d	48.50±5.80de	48.75±2.52cd	49.25±1.97cd
P. f- 12	42.00±.80d	38.25±.80e	36.75±1.10e	40.75±.48e
Control	0.97±.03e	2.95±.40f	4.13±.96f	3.90±.80f

Means followed by the same letter within each column are not significantly different (Turkey's, s multiple range test at 0.05 level of significance)

**Fungal conidia concentration-mortality response test**

The *P. fumosoroseus* UPM isolate was selected for the dose response test at different

concentrations based on its very high mortality effect against second instar nymphs of *B. tabaci*. Concentration-mortality response of *P. fumosoroseus* UPM isolate against the second instar

nymph of *B.tabaci* resulted in both time- and dose-dependent mortality (Figure 1). In the concentration-mortality response test on the second instar nymphs mortality reached 95% within 6 days after application of  $1 \times 10^8$  conidia  $\text{mL}^{-1}$  and there was significant differences among concentrations ( $P < 0.05$ ) (Figure 2).

#### LC<sub>50</sub>

According to goodness of fit test, the deviations observed were low enough to perform a

Probit analysis ( $X^2 = 12.27$ ,  $df = 12$ ,  $P < 0.05$ ). A linear relationship between dose and mortality was clear based on the t value for the slope ( $t = 8.690$ ). The Probit analysis was also used to calculate  $\text{Lc}_{50}$ . While mortality in the control unit was 1%, the  $\text{Lc}_{50}$  of this fungus against the second instar nymphs of *B .tabaci* six days after treatment was determined to be  $2.77 \times 10^5$  conidia  $\text{mL}^{-1}$  (Figures 1 and 3, Table 3).

Fig. 1- Cumulative mortality of *B. tabaci* second instar nymph at different doses of *P. fumosoroseus* UPM conidia.

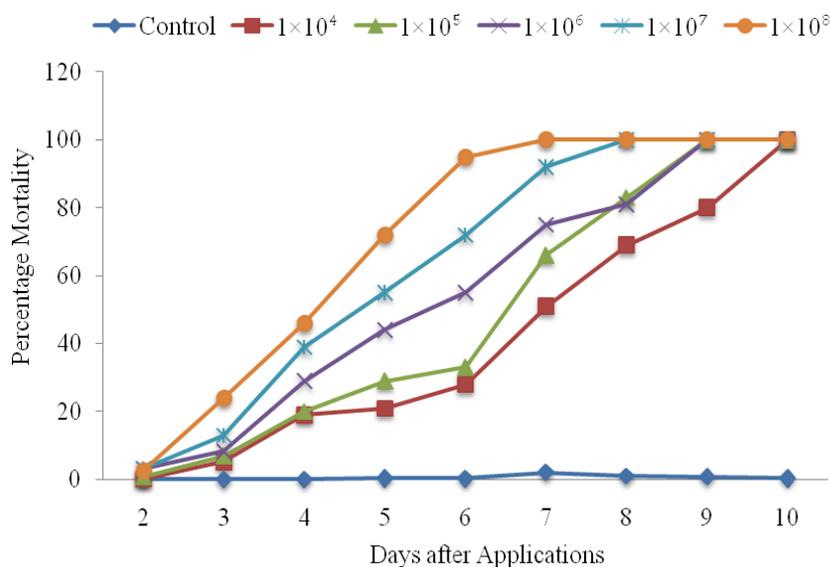


Table 3- Lethal Concentrations (LC) values of *P. fumosoroseus* UPM isolate against *B. tabaci* second instar nymph.

LC	Slope $\pm$ SE	(Con.)	95% Limits Fiducial	
			Lower	Upper
LC <sub>10</sub>	6.724 $\pm$ 0.774	$3.596 \times 10^4$	$2.659 \times 10^4$	$4.220 \times 10^4$
LC <sub>50</sub>	6.724 $\pm$ 0.774	$5.577 \times 10^5$	$4.964 \times 10^5$	$6.048 \times 10^5$
LC <sub>90</sub>	6.724 $\pm$ 0.774	$8.650 \times 10^6$	$7.820 \times 10^6$	$10.275 \times 10^6$
LC <sub>95</sub>	6.724 $\pm$ 0.774	$9.795 \times 10^7$	$8.650 \times 10^7$	$12.279 \times 10^7$
LC <sub>99</sub>	6.724 $\pm$ 0.774	$12.370 \times 10^8$	$10.382 \times 10^8$	$17.268 \times 10^8$

**Lt<sub>50</sub>**

Lt<sub>50</sub> values (days) were determined with 95% fiducial limits following immersion of *B. tabaci* second instar nymphs in aqueous suspensions of *P. fumosoroseus* at 1×10<sup>5</sup>, 1×10<sup>6</sup> and 1×10<sup>7</sup> conidia ml<sup>-1</sup>. Lt<sub>50</sub> values for 1×10<sup>5</sup> concentration of *P. fumosoroseus* UPM isolate varied from 5.58 to 6.3

days, with an average of 5.91 days, for the 1×10<sup>6</sup> concentration values varied from 4.97 to 5.52 days, with an average of 5.23 days, and for the 1×10<sup>7</sup> concentration values varied from 4.17 to 4.59 days, with an average of 4.38 days (Table 4).

Table 4- Median lethal time (Lt<sub>50</sub>) values of *P. fumosoroseus* UPM isolate at different conidia concentrations on *B. tabaci* second instar nymph.

UPM isolate	Lt <sub>50</sub>	95% Fiducial	Limits			
Conidia/ml	Slope ± SE	(Days)	Lower	Upper	χ <sup>2</sup>	df
1×10 <sup>4</sup>	3.79±0.5	6.28	5.85	6.83	6.89	19 0.0001
1×10 <sup>5</sup>	4.8±0.35	5.91	5.58	6.3	21.47	19 0.0001
1×10 <sup>6</sup>	4.5±0.32	5.23	4.97	5.52	6.97	19 0.0001
1×10 <sup>7</sup>	5.9±0.39	4.38	4.17	4.59	19.25	19 0.0001
1×10 <sup>8</sup>	7.12±0.6	3.94	3.7	4.1	38.48	19 0.0001

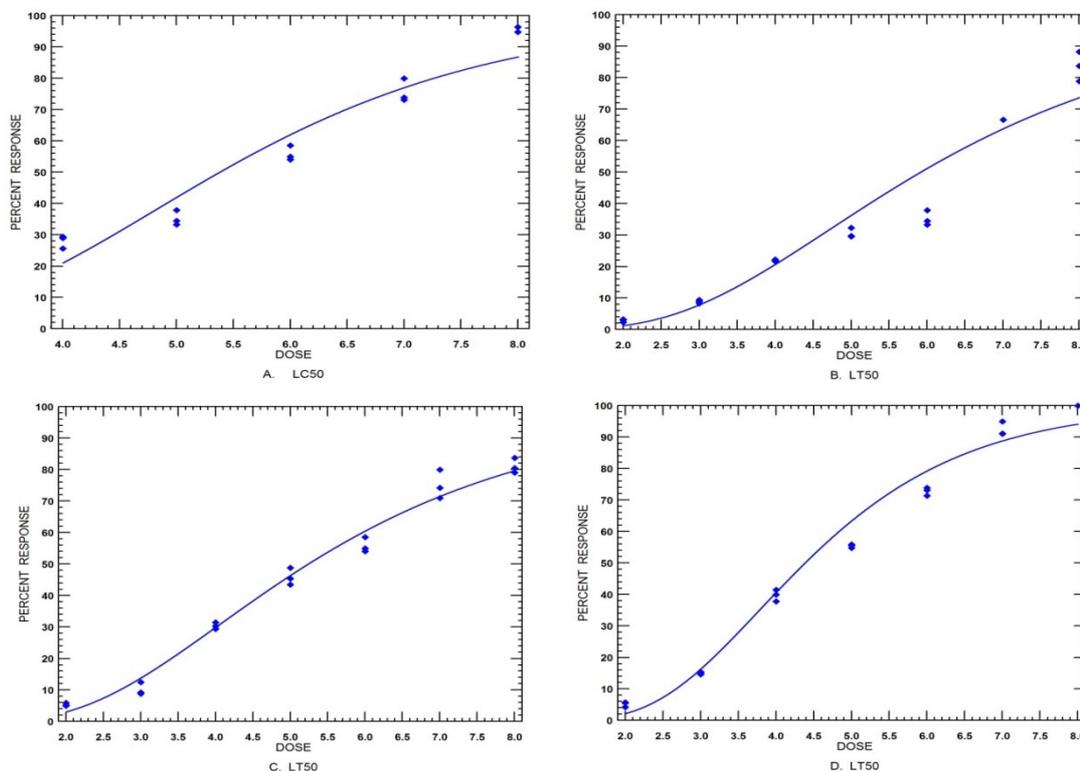


Fig. 2- Linear relation between dose and mortality of *B. tabaci* second instar nymph after application of fungal *P. fumosoroseus* UPM isolate at different doses of conidia

A: Lc<sub>50</sub> Six days after application

B: Lt<sub>50</sub> 1×10<sup>5</sup> conidia ml<sup>-1</sup>

C: Lt<sub>50</sub> 1×10<sup>6</sup> conidia ml<sup>-1</sup>

D: Lt<sub>50</sub> 1×10<sup>7</sup> conidia ml<sup>-1</sup>

## Discussion

The first criterion of our approach for selecting *P. fumosoroseus* isolates for whitefly control was the high mortality against the target organism. Entomopathogenic fungi are an important option in integrated pest management (IPM) programs. As fungi usually invade insect hosts by penetrating the cuticle, they are the only pathogens that have been developed for the control of insects with piercing and sucking mouthparts such as whiteflies and aphids (Latgé & Papierok, 1988; Lacey *et al.*, 1995). Screening examinations revealed that all P.f. isolates infected *B. tabaci* with 44 -95%.  $LC_{50}$  and  $Lt_{50}$  values determined to be  $5.577 \times 10^5$  conidia ml<sup>-1</sup> (6 days) and 5.23 days ( $1 \times 10^6$  concentration) respectively.

A novel and important finding was the effect of the UPM isolate on *B. tabaci*. Results showed that the *P. fumosoroseus* isolates produced significant mortality in eggs, and second, third, and fourth instar nymphs of *B. tabaci*. The *P. fumosoroseus* UPM isolate that was collected from *B. tabaci* was more active against all stages of *B. tabaci* than those fungi isolated from other insects. Also among P.f. isolates, the indigenous isolate 49 had 76±4.5, 73±2.98, 84±.95 and 84±.95 mortality percent in eggs, second instar, third instar and fourth instar nymph respectively. This means that the indigenous isolate 49 was also acceptable to isolate the effect of the pest control. Similarly,

various isolates of *L. lecanii* from aphids, whiteflies and other insects showed distinct differences in their ability to infect whiteflies. Strains collected from *T. vaporariorum* were usually more active against whiteflies than those isolated from other insects including aphids (Hall, 1982; Kitazawa, 1984; Masuda, 1992; Chandlaer *et al.*, 1993), and strains that originate from whiteflies have greater recycling potential in whitefly cadavers and populations (Hall, 1982; Chandlaer *et al.*, 1993).

These observations confirm that as a general rule fungi isolated from an insect will be more effective on those insects. In this study, each isolate had almost identical effects on different stages of *B. tabaci* and the mortality of each isolate against the different stages of *B. tabaci* was not significantly different.

## Conclusion

In conclusion, the results showed that the entomopathogenic *P. fumosoroseus* isolates are good biocontrol agents against all stages of *B. tabaci*, and the *P. fumosoroseus* UPM isolate was the most promising biological control agent against this pest and the UPM isolate *P. fumosoroseus* was selected for further experiments. However, further studies are needed to determine effectiveness of this isolate in the field.

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