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Research Article

# Virulence of Iranian isolates of *Metarhizium anisopliae* on western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae)

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**Abstract:** The Western flower thrips *Frankliniella occidentalis*, an important greenhouse pest, has acquired rapid resistance to the chemical pesticides. Therefore, biological control is worth consideration as an alternative control method. Among the biological control agents, entomopathogenic fungi showed to be quite successful in some occasions. In this study, three Iranian isolates of *Metarhizium anisopliae* ('DEMI001', 'DEMI002' and 'DEMI003') were bioassayed for their lethal effects on the adults of the *F. occidentalis*, in vitro. The 'DEMI002' and 'DEMI003' had the lowest and highest $LC_{50}$ at concentrations of $3.06 \times 10^4$ and $1.90 \times 10^5$ conidia/ml, respectively. Also, the isolate 'DEMI002' had the lowest $LT_{50}$ of $4.39 \pm 2.13$ days at the concentration of $10^6$ conidia/ml. The mean comparison showed that there was a significant difference between DEMI002 and DEMI003 in terms of virulence at most of the concentrations. Consequently, the 'DEMI002' can be considered as a promising tool in biological control programs of the *F. occidentalis*.

**Keywords:** *Metarhizium anisopliae*, *Frankliniella occidentalis*, Western flower thrips, Bioassay

## Introduction

The Western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is an important pest, damaging a wide range of greenhouse crops (over 500 plant species in more than 50 plant families) (Strassen *et al*., 1986; Yudin *et al*., 1986; Broadbent *et al*., 1987; Steiner, 1990; Robb and Parrella, 1995;Lewis, 1997). It can directly damage its host plants through feeding on cell sap and indirectly through the transmission of harmful plant viruses such as TSWV and INSV (van Lenteren *et al*., 1992; Robb and Parrella, 1995; Kirk and Terry, 2003; Thungrabeab *et al*., 2006). It is difficult to control this pest with conventional insecticides because of its small size and cryptic habits (Robb and Parrella, 1995; Espinosa *et al*., 2002). The ability of *F. occidentalis* to develop resistance to chemical insecticides, has further complicated its control (Immaraju *et al*., 1992; Brodsgaard, 1994; Zhao *et al*., 1994), necessitating integrated management of this pest (Kirk, 2001), which includes application of enthomopathogenic fungi (Vestergaard *et al*., 1995; Ekesi and Maniania, 2000; Maniania *et al*., 2001, 2003; Meyer *et al*., 2001; Abe and

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Ikegami, 2005). Western flower thrips has acquired resistance to all major groups of insecticides. However, there is no evidence about developing resistance to entomopathogenic fungi regarding thrips or any other insects (Maniania *et al*., 2001).

Entomopathogenic fungi are currently being investigated for control of many important insect pests on various crops around the world, and are commercially available. Entomopathogenic fungus *Metarhizium anisopliae* (Metschnikoff) is an important biocontrol agent and has been formulated for application in insect pest management systems (Kpindou *et al*., 1997; Faria and Wraight, 2001; Feng *et al*., 2004). The objective of this study was to determine the virulence of local isolates of *M. anisopliae* to *F. occidentalis* and to find a suitable isolate for future studies on microbial control of *F. occidentalis*.

## Materials and Methods

### Insect

Thrips were collected in a greenhouse near Varamin (Tehran, Iran) and were reared in PVC containers (16 cm height and 4.5 cm diameter) with two peripheral ventilation holes, covered with mesh,. The containers (along with green beans, as food) were kept in a CT room at 25 ± 1 °C, 16L : 8D h, and 60 ± 10% RH. Only adult insects were used in tests. To ensure that the population is pure *F. occidentalis* 20 thrips were randomly selected from culture and microscopic slides were prepared.

### Fungal isolates

Three isolates of *Metarhizium anisopliae* ('DEMI001', 'DEMI002' and 'DEMI003'), kept at the fungal culture collection of the Department of Agricultural Entomology in Iranian Research Institute of Plant Protection, were used in this study. Isolates were cultured on Sabouraud's dextrose agar with yeast extract (SDA + Y medium) (Merck, Germany) in Petri dishes (7 cm in diameter). The cultures were kept for 2-3 weeks at 27 ± 1 °C in the darkness.

### Conidial suspensions

Conidia were harvested from the surface of 2- 3 week old cultures, by scraping and were suspended in 10 ml of Tween 80 (0.1% solution), in glass tubes. The liquid was stirred to produce a homogeneous conidial suspension. To separate the mycelium from suspension, it was passaged through cheese cloth. The viability of conidia was determined by spreading 100 ml of conidial suspention on water agar plates (5 cm in diameter). These plates were incubated at 27 ± 1 °C, in darkness for 16-20 hours and percentage germination was determined by counting 100 conidia on each plate. Conidia were considered as germinated when the germ tube was equal or greater than conidium lenghth (Schapovalof *et al*., 2014).

Only conidial suspensions with viability above 85% (Jenkins *et al*., 1998) were used for the bioassay tests.

Conidia were then quantified with an improved Neubauer hemocytometer under a light microscope at a magnification of X200. Preliminary tests were performed with seven concentrations ($10^2$, $10^3$, $10^4$, $10^5$, $10^6$, $10^7$ and $10^8$). After determining the range of lethal dose (25-75% mortality), five conidial suspensions at concentration of, $1 \times 10^4$, $1 \times 10^{4.5}$, $1 \times 10^5$, $1 \times 10^{5.5}$ and $1 \times 10^6$ conidia/ml were prepared for each isolate.

### Bioassay

Three isolates of *M. anisopliae* were bioassayed by the immersion method, against *F. occidentalis* in vitro. Five concentrations for each isolate, 3 replicates for each concentration and 15 (three-day-old) adult thrips were used for each replicate. The insects were collected by aspirator, that its tube was attached to the bottom of a microtube (1.5 ml volume) (the bottom of the microtube was cut and covered with mesh). A plastic pipet tip was placed to the top of the microtube. So, thrips were directly transferred into the microtube. After detaching the plastic pipette tip and the tube, 10 ml of conidial suspension was then poured over thrips into the microtubes. After 5 seconds the suspension was drained out through plastic mesh and microtubes transferred into plastic Petri dishes (6 cm in diameter). A sterile tissue paper along with a slice of green bean were placed at the bottom of Petri dishes. Thereafter, the lids were sealed with Parafilm and the petri dishes were transferred into an incubator (with the same CT room conditions mentioned above). After 24 hours, when the suspension dried and thrips initiated to move actively, microtubes were removed from Petri dishes and the exact number of insecs in each dish was recorded and then the lids of Petri dishes were replaced with lids that were covered with mesh. High relative humidity is necessary for conidial germintion of entomopathogenic fungi (Ramoska, 1984). Therefore, each treatment was transferred into dishes with a wet tissue at the bottom (to maintain high humidity). Mortality was recorded on a daily basis for 10 days. Cadavers were surface sterilized and transferred into sterile Petri dishes with wet cotton and were placed in an incubator. Sporulation was checked. The experiment was repeated twice to ensure that results confirmed each other. Control insects were treated only with Tween 80, 0.01% solution.

**Data analysis**

Data were analyzed in a completely randomized design. To calculate $LT_{50}$ and $LC_{50}$ Curve Expert and Priprobit softwares were used, respectively. Mean comparisons were performed using Duncan's multiple range test in SAS 9.1 software. To draw the regression line, Excel software was used. The data was manipulated with Abbot formula to remove the effect of control.

## Results

Mortality of thrips increased in a conidial-dose-dependent manner and the range of mortality for all isolates on day 10 was 9.3-94.3%. The lowest and highest mortality rates were observed in 'DEMI003' ($10^4$ conidia/ml) and DEMI002 ($10^6$ conidia/ml) plots, respectively (Table 1).

From day 10 onward, the mortality in all fungal treatments did not increase. Thus, the mortalities recorded on day 10 were used to estimate the mortalities of adults due to different isolates (Table 1). The mortalities differed significantly among the conidial concentrations of all isolates. All isolates at high concentration ($10^6$ conidia/ml) caused more than 50% mortality. At low concentration (up to $10^5$ conidia/ml), however, only 'DEMI002' caused more than 50% mortality and 'DEMI003' didn't cause more than 50% mortality up to $10^{5.5}$ conidia/ml concentration. At all concentrations, 'DEMI002' caused higher mortality than 'DEMI003' (Table 1). Thus, different isolates had different abilities to infect *F. occidentalis* adults and their impact largely depended on the conidial concentrations. Among three isolates, the 'DEMI002' and 'DEMI003' had the lowest and highest $LC_{50}$ value with $3.06 \times 10^4$ and $1.90 \times 10^5$ conidia/ml, respectively. Furthermore, the isolate 'DEMI002' had the lowest $LT_{50}$ of $4.39 \pm 2.13$ days at the concentration of $10^6$ conidia/ml (Table 2). The mean comparison test showed that there is a significant difference among three isolates (Table 1).

The linear relationship between the logarithm of conidial concentrations of each isolate and the adult mortalities was determined by probit analysis (Table 3). Based on the estimate of the $LC_{50}$, the three tested isolates, although having different virulence, were infectious to *F. occidentalis*. Compared to the most virulent isolate ('DEMI002'), a relative potency was estimated for the other isolates (Table 3). By dividing $LC_{50}$ of each isolate to the lowest $LC_{50}$. The relative potency of 'DEMI003' was 6.2 times more than the value of 'DEMI002' indicating that 'DEMI002'was 6.2 times more virulent than DEMI003 on *F. occidentalis*.

Results show that isolate **'**DEMI002**'** could be a useful agent for controlling the *F. occidentali*.





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**Table 1** Comparison of adult mortality rates of *Frankliniella occidentalis*,10 days after exposure to different concentrations of *Metarhizium anisopliae* isolates under laboratory condition.

| Isolates | Concentration (con/ml) | No. of treated adults (total)[1] | Adult mortality (± SE) (%) after 10 days[2] | P-value | df | F |
|---|---|---|---|---|---|---|
| DEMI001 | $10^4$ | 94 | 28.06 ± 1.53a | 0.0038 | 8, 2 | 16.30 |
| DEMI002 | $10^4$ | 97 | 27.27 ± 4.28a | $P < 0.01$ | | |
| DEMI003 | $10^4$ | 85 | 9.30 ± 2.08b | | | |
| DEMI001 | $10^{4.5}$ | 99 | 36.13 ± 7.21ab | 0.0231 | 8, 2 | 7.54 |
| DEMI002 | $10^{4.5}$ | 87 | 61.03 ± 13.25a | $P < 0.05$ | | |
| DEMI003 | $10^{4.5}$ | 88 | 19.57 ± 1.16b | | | |
| DEMI001 | $10^5$ | 92 | 47.70 ± 2.35b | 0.0017 | 8, 2 | 22.31 |
| DEMI002 | $10^5$ | 87 | 65.37 ± 2.60a | $P < 0.01$ | | |
| DEMI003 | $10^5$ | 83 | 45.90 ± 1.66b | | | |
| DEMI001 | $10^{5.5}$ | 87 | 53.67 ± 1.01ab | 0.05 | 8, 2 | 5.11 |
| DEMI002 | $10^{5.5}$ | 90 | 72.90 ± 11.28a | $P = 0.05$ | | |
| DEMI003 | $10^{5.5}$ | 85 | 46.16 ± 2.60b | | | |
| DEMI001 | $10^6$ | 89 | 86.47 ± 2.59ab | 0.03 | 8, 2 | 6.48 |
| DEMI002 | $10^6$ | 84 | 94.30 ± 3.96a | $P < 0.05$ | | |
| DEMI003 | $10^6$ | 91 | 77.03 ± 3.47b | | | |

[1] The number of adults at a given concentration pooled from all replicates.
[2] Means with different letters within same concetration are significantly different based on Duncan's test.

**Table 2** Lethal time of mortality ($LT_{50}$) of *F. occidentalis* after treatment by *M. anisopliae* isolates under laboratory condition.

| Isolates | Concentration | Regression model | Coefficient of correlation (r) | $LT_{50}$ (days) | ± SD |
|---|---|---|---|---|---|
| DEMI002 | $10^{5.5}$ | Logistic | 0.99 | 5.66 | 1.83 |
| DEMI002 | $10^6$ | Logistic | 0.99 | 4.39 | 2.13 |
| DEMI001 | $10^{5.5}$ | Logistic | 0.99 | 9.2 | 1.83 |
| DEMI001 | $10^6$ | Logistic | 0.99 | 5.1 | 3.82 |
| DEMI003 | $10^{5.5}$ | Logistic | 0.99 | 10.9 | 2.34 |
| DEMI003 | $10^6$ | Logistic | 0.99 | 4.79 | 1.80 |

**Table 3** The $LC_{50}$ for different isolates of *Metarhizium anisopliae* against *Frankliniella occidentalis* adults, 10 days after exposure under laboratory condition.

| Isolates | Intercept | Slope | df | Chi Square | $LC_{50}$ with 95% CL (con/ml) | Relative potency |
|---|---|---|---|---|---|---|
| DEMI002 | -3.831 | 0.854 | 3 | 11.27 | $3.06 \times 10^4$ ($1.21 \times 10^4$ - $6.54 \times 10^4$) | 1.0 |
| DEMI001 | -3.651 | 0.747 | 3 | 7.06 | $7.75 \times 10^4$ ($3.23 \times 10^4$ - $2.14 \times 10^5$) | 2.5 |
| DEMI003 | -4.962 | 0.940 | 3 | 3.90 | $1.90 \times 10^5$ ($1.42 \times 10^5$ - $2.65 \times 10^5$) | 6.2 |

CL: confidence limits.

## Discussion

Application of entomopathogenic fungi for biocontrol of thrips has been studied for many years. The success in the use of enthomopathogenic fungi largely depends on the selection of highly virulent isolate (Thungrabeab *et al.*, 2006). Based on our results, all isolates were found to be pathogenic to *F. occidentalis* but flower thrips showed different levels of susceptibility to *M. anisopliae* isolates. 'DEMI002' was the most virulent to *F. occidentalis* (94.30 ± 3.96% mortality at $10^6$ con./ml concentration). Such a





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difference among the isolates of fungal species against an insect species has been reported by Ekesi *et al*. (1998). They screened 22 strains of entomopathogenic fungi against *Megalurothrips sjostedti* and found that four isolates of *M. anisopliae* were highly pathogenic to this insect. Vestergaard *et al*. (1995) identified strains of *M. anisopliae*, which compared to other pathogenic fungi, were more pathogenic to *F. occidentalis*. They found that the lowest $LC_{50}$ was $3\times10^5$ con./ml. According to the lowest amount of $LC_{50}$ of our study ($3.06\times10^4$ con./ml), the 'DEMI002' can be comparable with the isolate used by Vestergaard *et al*. (1995), although the conditions of the two experiments were different. Azaizeh *et al*. (2002) found that *M. anisopliae* was able to reduce the population growth of *F. occidentalis* on cucumber.

Nikpour (2008), used two Iranian isolates of *M. anisopliae* ('DEMI001' and 'DEMI002') for controlling *Thrips tabaci*, and found that 'DEMI001' ($LC_{50} = 0.3\times10^3$ con./ml) was more virulent than 'DEMI002', and the lowest $LT_{50}$ (5.2 day) was observed with 'DEMI001'. This contrasts our results on *F. occidentalis*, where larger numbers of insects were infected by 'DEMI002' and the lowest $LT_{50}$ ($4.39 \pm 2.13$ day) was observed.

Differences in virulence between fungal species and isolates have also been reported in case studies for other insect species (Moorhouse *et al*., 1993; Thungrabeab *et al*., 2006). Another study showed that *M. anisopliae*, compared to *Beauveria bassiana* and *Lecanicillium muscarium*, was more virulent to *F. occidentalis* (Gouli *et al*., 2009).

It is reported that high range of mortality rates is more probable to be observed in *M. anisopliae* bioassays (Thungrabeab *et al*., 2006) and this may confirm our results (9.3-94.3% Mortality). Ansari *et al*. (2008) reported that pre-pupae and/or pupae of thrips are highly susceptible to *M. anisopliae* infection and two isolates caused >85% mortality. Whereas, Vestergaard *et al*. (1995) reported that the larval and pupal stages of *F. occidentalis* are more resistant to infection by *M. anisopliae* than adults. The differential susceptibility may occur due to the interaction between the insect integument being penetrated by the fungus and ecdysis of larval and pupal stages (Maniania *et al*., 2001).

Several reports revealed that entomopathogenic fungi successfully control *F. occidentalis*, *Thrips palmi* and *T. tabaci* in the field (Satio, 1991; Maniania *et al*. 2001, 2003). However, since susceptibility to fungal isolates differes among the thrips species, selection of a fungal isolate as a biological control agent for thrips should be undertaken with care (Abe and Ikegami, 2005).

Although in some cases, no significant differences were observed among virulence of isolates used in this study, the relative potency criteria showed that DEMI002 is more potent than other isolates and additional studies on environmental and ecological aspects of this isolate may help us to procure a biological control agent to use in IPM programs.

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# زهرآگینی جدایه‌های ایرانی *Metarhizium anisopliae* (Hypocreales: Clavicipitaceae) روی تریپس غربی گل *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae)

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**چکیده:** تریپس غربی گل *Frankliniella occidentalis* یکی از آفات مهم گلخانه می‌باشد که به‌سرعت به حشره‌کش‌های شیمیایی مقاومت پیدا کرده است. بنابراین کنترل بیولوژیک می‌تواند به‌عنوان یک روش جایگزین برای کنترل آن مطرح باشد. از میان عوامل کنترل بیولوژیک قارچ‌های بیمارگر حشرات در مواردی کاملاً موفق عمل نموده‌اند. در تحقیق حاضر اثرات کشندگی سه جدایه ایرانی از قارچ *Metarhizium anisopliae* (DEMI001، DEMI002 و DEMI003) در شرایط آزمایشگاهی روی حشرات کامل تریپس غربی گل بررسی شد. جدایه‌های DEMI002 و DEMI003 به‌ترتیب با $LC_{50}$ های $۳/۰۶ \times ۱۰^۴$ و $۲/۹۰ \times ۱۰^۵$ بیش‌ترین و کم‌ترین میزان کشندگی را نشان دادند. هم‌چنین جدایه DEMI002 با $LT_{50}$ ۲/۱۳ ± ۴/۳۹ روز در غلظت $۱۰^۶$ کنیدی بر میلی‌لیتر کم‌ترین زمان کشندگی ۵۰٪ را داشت. مقایسه میانگین‌ها نشان داد که در اکثر غلظت‌ها اختلاف معنی‌داری بین DEMI002 و DEMI003 ازنظر زهرآگینی وجود دارد. بنابراین جدایه DEMI002 را می‌توان جدایه‌ای امیدبخش در برنامه‌های کنترل بیولوژیک تریپس غربی گل دانست.

**واژگان کلیدی:** *Metarhizium anisopliae*، *Frankliniella occidentalis*، تریپس غربی گل، زیست‌سنجی



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