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Insecticidal effect of *Fusarium subglutinans* on *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae)

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Summary *Fusarium subglutinans* (Ascomycota: Nectriaceae) is known to have lethal effects on aphid species, while there are limited studies associated with other arthropods. In this study, the effect of different spore concentrations (1x10^4, 1x10^6 and 1x10^8 spores/ml) of *F. subglutinans* 12A, isolated from *Aphis gossypii* in Adana-Karataş (Turkey), was investigated on *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) females and on 2nd instar nymphs (only 1x10^6 spores/ml). The application method was by dipping and observations on mortality of females were conducted 24, 48, 72, 96 hours and 7 and 9 days after application. Mycosis was also observed on dead individulas. Mortality of nymphs was recorded during 8 days after application. Higher average dead females were found in the treatments compared to the control, but there was not significant difference between the tested concentrations (Mycosis rate recorded in 1x10^6 spores/ml was higher than those in 1x10^4 and 1x10^8 spores/ml). The highest and lowest mycosis rates were observed on the 7th and 3rd day, respectively. Average number of dead 2nd instar nymphs recorded in 1x10^6 spores/ml did not differ from control.

Additional keywords: Biological control, entomopathogenic fungi, pest, thrips

Introduction

The Western Flower Thrips (WFT) *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) is a serious pest feeding on leaves, fruits and flowers and causing direct and indirect damages on agricultural crops and ornamental plants (Bryan and Smith, 1956; Miliczky and Horton, 2011; Demirözer *et al*., 2012). Due to significant number of thrips vectors for viral pathogens, they are known as destructive pests worldwide.

The WFT spread to the World from North-West of the United States (Kirk and Terry, 2002). The first presence of WFT in Turkey was recorded in vegetable fields of Antalya (Western Mediterranean region) in 1993 (Tunç and Göçmen, 1994) and in a very short time it suppressed the *Frankliniella intonsa* (Trybom) which was the main thrips species in cotton fields in Çukurova region (East Mediterranean) (Atakan *et al*., 1998; Atakan and Özgür, 1998; 2000; Atakan, 2003; Doğanlar and Aydin, 2009).

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Short generation period, high reproductive capacity and thigmotactic behaviour of *F. occidentalis* are reasons that make it difficult to control. In addition, rapid resistance development ability against insecticides also contributes to the difficulty in the control of *F. occidentalis*. The WFT is known to be resistant to carbamates (bendiocarb, formetanate, methiocarb), organophosphates (diazinon), spinosyn (spinosad) and pyrethroids (acrinathrin, deltamethrin, fenvalerate, permethrin) (Jensen, 2000; Bielza, 2008; Cloyd, 2009).

Besides the difficulties in the suppression of thrips populations, chemical insecticides are known to have side effects on the natural enemies of the WFT (Goettel and Hajek, 2000; Pell *et al*., 2001; Jones *et al*., 2005; Demirözer *et al*., 2012). Since entomopathogens are specific to their hosts and they reproduce they are a desirable alternative in pest control (Charnley and Collins, 2007). Additionally, low risk on the non-target organisims supports safeuse of entomopathogens in control practices (Eilenberg *et al*., 2001; Augustyniuk-Kram & Kram, 2012; Shadid *et al*., 2012).

There are 750 known species of entomopathogenic fungi, which belong to 85 ge-

nus of the Plyla Ascomycota and Zygomycota (Samson *et al*., 1988; McCoy *et al*., 1988; Gillespie and Moorhouse, 1989). A considerable number of these species belong to the genera *Beauveria, Entomophthora, Metarhizium, Neozygites, Nomuraea* and *Lecanicillium* (Desphande, 1999; Shadid *et al*., 2012). Although *Fusarium* spp. (Ascomycota: Nectriaceae) cause diseases in a number of economically important plants, the species *Fusarium subglutinans* isolated from aphids has entomopathogenic action on arthropods (Gerin, 1998; Erkiliç *et al*., 1999; Satar *et al*., 2000).

The aim of this study was to determine the insecticidal effect of three different spore concentrations ($1x10^4$, $1x10^6$ and $1x10^8$ spores/ml) of *F. subglutinans* 12A on adult females of *F. occidentalis.* The mycosis rate was recorded on dead individuals of the thrips. Additionally, the effect of $1x10^6$ spores/ml concentration was investigated on the 2nd instar nymphs of *F. occidentalis*.

Materials and Methods

The study plant was pepper (*Capsicum annuum*). Adult females and 2nd instar nymphs of *F. occidentalis* used in the experiments came from laboratory colonies kept at 25±1°C, 60-70% RH and 16:8 h L:D. The isolate 12A of *F. subglutinans* from *Aphis gossypii* in Adana-Karataş, Turkey was used for making suspensions of the fungus.

In the study, spore concentrations of $1x10^4$, $1x10^6$ and $1x10^8$ spores/ml were prepared by using suspension of *F. subglutinans* 12A; they were cultured on potato dextrose agar (PDA) and incubated at 25°C for 10 days. Spore concentrations were determined by using Thoma counting chamber. In the control, distilled water and Tween 20 (0.1 %) was used.

The experiment was conducted in glass Petri dishes (9 cm diameter) containing pepper leaf discs (5 cm diameter) on filter papers, to feed the thrips. In each treatment 10 individuals, newly emerged adult females or 2nd instar nymphs, were used. Before the treatment, thrips were deprived food for 1 hour. The application method was by dipping for 5 seconds. After treatment the thrips were transferred by using a moisturized fine paint brush to the Petri dishes, which were then covered with parafilm to prevent their possible escape. The experimental design was a complete randomized block with five replications. The Petri dishes were kept in climate-controlled rooms at 25±1°C, 60-70% RH, and 16:8 h D:L.

Observations on mortality were made at 24, 48, 72 and 96 hours, and 7 and 9 days after the dipping. Mycosis observations were performed between the third and ninth day of the study. Counting on the 2nd instar nymphs was initiated 24 hours after the dipping and repeated every 24 hours until the 8th day of the experiment. Re-isolation was made at the end of the counting process on dead individuals.

Statistical Analysis

Square root transformation was applied to the data of dead individuals. Inverse angle transformation was applied to the mycosis data obtained from dead flesh (body) of adults. Data were analysed using repeated measurement analysis of variance in a factorial design (treatment x time). Linear relation between dead individuals and mycosis rates was investigated by calculating the Pearson correlation coefficient. The Mann-Whitney 'U' test was applied to the data obtained from 2nd instar nymphs of *F. occidentalis*, since the data were non-parametric. Significance level was $P< 0.05$.

Results

The mean numbers of dead females of *F. occidentallis* after dipping in solutions of three different spore concentrations of *F. subglutinans* 12A are presented in Table 1. The control had the lowest mean number of dead females, which was significantly different from the other treatments ($P<0.05$). The mean

Table 1. Mean mortality of adult females of *Frankliniella occidentalis* and mycosis rate on dead individuals after treatment with three different spore concentrations of *Fusarium subglutinans* 12A.

Treatments (spores/ml)	Mortality Mean ± s.e.	Mycosis rate Mean ± s.e.
10^4	3.67 ± 0.756 a	0.989 ± 0.230 b
10^6	3.47 ± 0.724 a	1.794 ± 0.351 a
10^8	4.20 ± 0.732 a	1.455 ± 0.262 ab
Control	0.70 ± 0.180 b	

Means with different letter in the same column differ significantly (*P <0.05*); s.e.: standard error

mortality obtained in three spore concentrations did not differ significantly between them (P>0.05).

In the concentrations 10^6 and 10^8 spores/ml, first deaths of adults were observed 24 hours after dipping, whereas this was 48 hours in 10^4 spores/ml (Figure 1). In the control, deaths were observed three days after dipping into the water and the mortality percentage was 16% on the 8th day of the experiment. Mortality percentage was 100% in the three spore concentrations of *F. subglutinans* 12A on the 9th day of the experiment (Figure 1).

The highest mycosis rate was recorded in 10^6 spores/ml (1.794 ± 0.351) and was higher than the average mycosis rates of 10^8 and 10^4 spores/ml concentrations (P<0.05; Table 1). The Pearson correlation coefficient between the death and mycosis rates was 0.68 and it was found significant (P<0.01). In addition, a linear relationship was determined between the death and mycosis rates and mycosis became visible three days after the application (Table 1). The highest mycosis rates were observed on the 7th and 9th day after application and the lowest rate was recorded three days after application in all spore concentrations. The Pearson correlation coefficient between the time and mycosis rate was 0.93 and it was significant (P<0.01).

Deaths of 2nd instar nymphs at 10^6 spores/ml concentration of the fungus were observed starting on the second day of spore applications. The results showed that percentage of mortality was more than 50% on the 8th day of the study (Figure 2). The 'R' value of average dead individuals was 0.412 and it was different from the control (P<0.01).

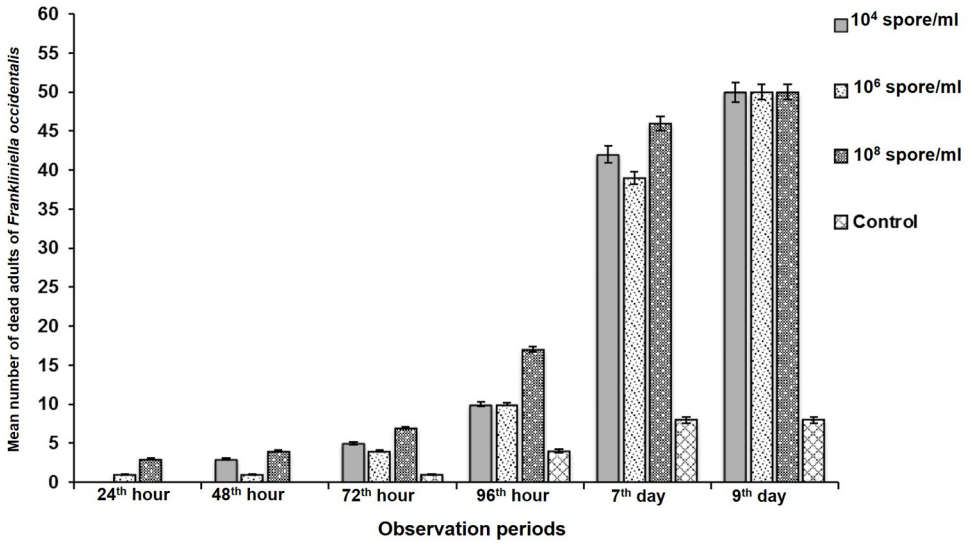

Figure 1. Mean number of dead adult females of *Frankliniella occidentalis* after treatment (dipping) with three different spore concentrations of *Fusarium subglutinans*.

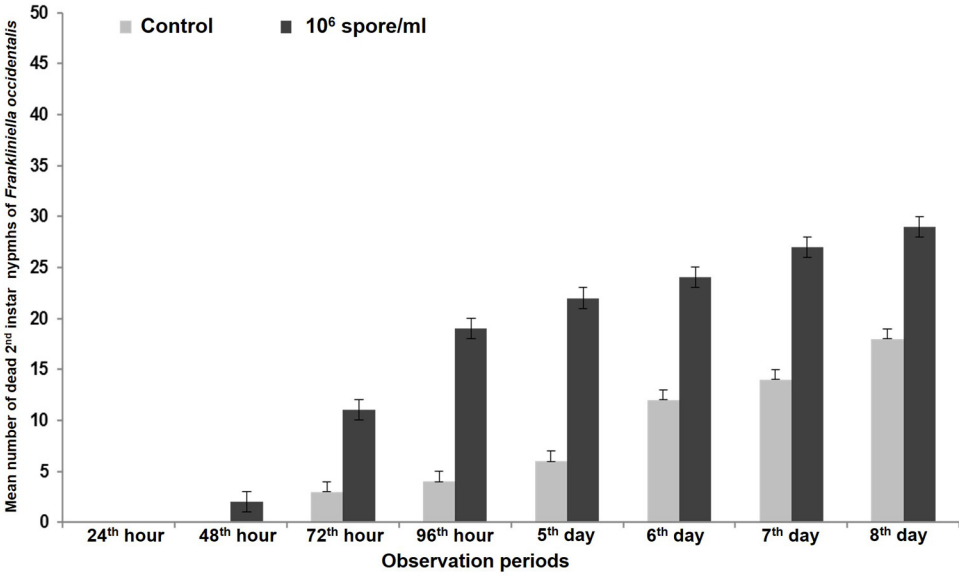

Figure 2. Mean number of 2nd instar nymphs of *Frankliniella occidentalis* after treatment (dipping) with 1x10^6 spores/ml concentration of *Fusarium subglutinans* 12A.

Discussion

Previous studies focused on efficacy of *F. subglutinans* on several aphid species (Erkiliç *et al*., 1999; Satar *et al*., 2000; Satar and Koç, 2004; Arici *et al*., 2012). Satar *et al*. (2000) reported that 1x10^7 spores/ml concentration of *F. subglutinans* caused 16% and 45.5% deaths on *A. gossypii* (Hemiptera: Aphididae) which fed on cotton and eggplant, respectively, and 12.9% on *Myzus persicae* (Hemiptera: Aphididae) which fed on eggplant.

In the present study, 1x10^4-1x10^8 spores/ml concentrations of *F. subglutinans* 12A had similar efficacy (in terms of mortality) against the thrips *F. occidentallis* but the 1x10^6 spores/ml concentration was found appropriate for mycosis. Lethal effect of *F. subglutinans* could be expected to vary on different host plants, pest species and different life stages of pests. The mortality rate of the 10^6 spores/ml concentration of *F. subglutinans* 12A was 58% on the 2nd instar nymphs of the thrips. The concentration 10^6 spores/ml of *F. subglutinans* was the most effective on *A. gossypii* and *A. fabae* in studies by Satar and Koç (2004) and; Arici *et al*. (2012).

Other fungi recorded to have biocidal effect on *F. occidentalis* include *Beauveria bassiana*, *Lecanicillium* (*Verticilium*) *lecanii* (Ascomycota: Cordycipitaceae) and *Metarhizium anisopliae* (Clavicipitaceae) (Jacobson *et al*., 2001; Ludwig and Oetting, 2002; Maniania *et al*., 2002). Whereas recorded mortality rates were 20-70 % for *L. lecanii*, these were 93.5-100% for *M. anisopliae* on different life stages of *F. occidentalis* (Vestergaard *et al*., 1995; Gouli *et al*., 2009). Several efficacy studies of different spore concentrations of *B. bassiana* showed that this fungus caused 67-96% deaths on pre adult stages of *F. occidentalis*. In addition 1x10^7 conidia/ml was the most effective spore concentration in several other studies Gouli *et al*., 2009; Gao *et al*., 2012; Wu *et al*., 2014), However these studies have no data on mycosis rates of the pest.

In conclusion, *F. subglutinans* 12A had a lethal effect on *F. occidentalis* and the fungus was found more effective on adults than the 2nd instar nymphs. Developing hyphae and spores of entomopathogenic fungi provide

infection to other individulas in the pest population (Moutia, 1936; Shahid *et al*., 2012). Present study results suggest that three days are required for mycosis to become visible from the application and seven days later mycosis rate reaches the highest level. Based on the results obtained from this study it is recommended future studies to focus on *F. subglutinans* 12A mode of action on arthropods, infection features and side effects of this fungus on natural enemies.

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Εντομοκτόνος δράση του μύκητα *Fusarium subglutinans* στο θρίπα της Καλιφόρνιας, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae)

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Περίληψη Ο μύκητας *Fusarium subglutinans* (Ascomycota: Nectriaceae) είναι γνωστό ότι έχει θανατηφόρο δράση σε είδη αφίδων, ενώ υπάρχουν περιορισμένες μελέτες που σχετίζονται με άλλα αρθρόποδα. Σε αυτή τη μελέτη, εξετάστηκε η επίδραση διαφορετικών συγκεντρώσεων σπορίων ($1x10^4$, $1x10^6$, και $1x10^8$ σπόρια/ml) του 12A *Fusarium subglutinans*, το οποίο είχε απομονωθεί από την αφίδα *Aphis gossypii* στα Adana-Karataş (Τουρκία), στο θρίπα *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), σε ενήλικα θηλυκά άτομα και σε νύμφες δεύτερης ηλικίας (μόνο $x10^6$ σπόρια/ml). Η μέθοδος εφαρμογής ήταν με εμβάπτιση και παρατηρήσεις σχετικά με τη θνησιμότητα των θηλυκών ατόμων διεξήχθησαν 24, 48, 72, 96 ώρες και 7 και 9 ημέρες μετά την εφαρμογή. Επίσης παρατηρήθηκε το φαινόμενο της μύκωσης σε νεκρά άτομα. Η θνησιμότητα των νυμφών καταγράφηκε κατά τη διάρκεια 8 ημερών από την εφαρμογή. Κατά μέσο όρο, περισσότερα θηλυκά άτομα βρέθηκαν νεκρά στις επεμβάσεις με το μύκητα σε σχέση με το μάρτυρα, αλλά δεν υπήρχε σημαντική διαφορά μεταξύ των συγκεντρώσεων που δοκιμάστηκαν (καταγράφηκε υψηλότερος ρυθμός μύκωσης στη συγκέντρωση $1x10^6$ σπόρια/ml από εκείνους στις συγκεντρώσεις $1x10^4$ και $1x10^8$ σπόρια/ml). Τα υψηλότερα και τα χαμηλότερα ποσοστά μύκωσης παρατηρήθηκαν την $7^η$ και $3^η$ ημέρα, αντίστοιχα. Ο μέσος αριθμός νεκρών νυμφών δεύτερης ηλικίας που καταγράφηκε στη συγκέντρωση $1x10^6$ σπόρια/ml δε διέφερε από το μάρτυρα.

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