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Target-oriented dissemination of the entomopathogenic fungus *Fusarium subglutinans* 12A by the Western Flower Thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae)

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Abstract Target-oriented dissemination of entomopathogenic fungi may have benefits for spore dispersal to pest populations. In this laboratory study, target-oriented dissemination of *Fusarium subglutinans* 12A was investigated using female *Frankliniella occidentalis* Pergande for the control of thrips. The aim was to determine changes on mortality with infected / un-infected thrips number ranging from one to five or while the moulded cadavers were in the environment and lastly, with spore concentration applied to the food or habitat. Investigations were conducted using Petri dishes and single-cell cage bioassays and spore concentration (1×10^6 spore/ml) of fungus was applied using the dipping method. When the infected thrips were released to uncontaminated individuals mean mortality rate reached 48.2% and an increase in the number of inoculated individuals had no significant effect on mortality rates. On the contrary, an increase in the number of uncontaminated individuals had a significant effect on mortality rates, in which four uncontaminated thrips were released with infected thrips and the mortality rate reached 60.4%. Results revealed that the presence of 1×10^6 spore/ml of fungus in the habitat raised the mortality rate to $90.9 \pm 2.4\%$ on the 7th day. In the experiment where cadavers were present in the environment, the mortality rate reached $45.7 \pm 3.7\%$ on the 8th day. Overall, the results suggest that *F. subglutinans* 12A can be effectively disseminated to uninfected individuals by releasing conspecifics of *F. occidentalis* inoculated with 1×10^6 spore/ml and the most effective factor was found habitat applications to the determining dissemination of fungus.

Keywords Biological control · Thrips · Transmission · *Fusarium subglutinans* · Entomopathogenic fungi

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Introduction

There are more than 5000 known species of thrips (Thysanoptera) of which 87 are considered important pests in agricultural production (zur Strassen 2003; Mound 2009). Thrips cause injury to plants through the feeding activity of adults and immatures and also through the oviposition of eggs into plant tissue (Cloyd 2009; Demirözer et al. 2012). Many species of thrips also vector viral pathogens, in addition to the mechanical damage they inflict on the plants (Moritz et al. 2004). The western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) is a cosmopolitan species and it plays an active role in the transmission of Tomato spotted wilt virus which is one of the most destructive plant viruses and some other important tospoviruses (Pappu et al. 2009; Webster et al. 2011; Cluever et al. 2015). The species exhibit hiding behavior under the calyx on the fruit or in places of contact between fruits and stems or leaves and for this

reason the required contact with insecticides may be limited (Kirk 1997; Jensen 2000; Hansen et al. 2003; Herron and James 2005). In addition, the polyphagous nature of the western flower thrips provides an extraordinary ability for resistance to pesticides (Reitz and Funderburk 2012). It is known that *F. occidentalis* has developed resistance to numerous insecticides with different modes of action including carbamate, organochlorine, organophosphate, pyrethroids, neonicotinoids, spinosyns, phenylpyrazoles, avermectins and pyriproxyfen (Immaraju et al. 1992; Zhao et al. 1995; Broadbent and Pree 1997; Demirözer et al. 2012; Gao et al. 2012). The present situation demonstrates that relying solely on insecticides for the management of thrips is not economical or sustainable and an integrated pest management strategy is needed in order to minimize thrips populations (Avery et al. 2009; Funderburk et al. 2011a, b; Demirözer et al. 2012).

Entomopathogenic fungi are thought to be a sustainable alternative to manage thrips due to a lack of resistance and little to no impacts on non-target organisms (Goettel and Hajek 2000; Eilenberg et al. 2001; Pell et al. 2001; Jones et al. 2005; Thungrabeab and Tongma 2007; Down et al. 2009; Augustyniuk-Kram and Kram 2012; Shahid et al. 2012; Demirözer et al. 2016). There are more than 750 species of entomopathogenic fungi that belong to Ascomycota, Hypocreales and Zygomycota. Many species belonging to *Beauveria*, *Entomophthora*, *Fusarium*, *Lecanicillium*, *Metarhizium*, *Neozygites* and *Nomuraea* have effects on *F. occidentalis* and other pest thrips (Montserrata et al. 1998; Ludwig and Oetting 2002; Maniania et al. 2002; Gouli et al. 2009; Gao et al. 2012; Niassy et al. 2012; Wang et al. 2013; Kivett 2015; Zhang et al. 2015; Lee et al. 2017; Mousavi et al. 2017). Although the *Fusarium* genus is known to be plant pathogens (Dean et al. 2012), there is no disease symptoms have been reported related with *F. subglutinans* on plants. An entomopathogenic fungus species *F. subglutinans* has many strains which can destroy various arthropods including *F. occidentalis* (Gerin 1998; Logicco et al. 1998; Erkılıç et al. 1999; Satar et al. 2000; Satar and Koç 2004; Demirözer et al. 2010, 2016; Uzun et al. 2016).

The principal method of inoculation of the target thrips with the entomopathogenic fungus is through an application of conidia on plants. Due to the thigmotactic behavior and pollen needs of western flower thrips, the pest spend most of its time under the calyx and inside the flowers, decreasing the chance of inoculation by the fungus (Ugine et al. 2005). Additionally, application by hydraulic high-pressure sprayer or an exhaust nozzle sprayer could decrease conidia germination or viability by approximately 50% (Griffiths and Bateman 1997; Nilsson and Gripwall 1999). Spores of entomopathogenic fungi can disseminate through the movement of target or non-target arthropods (Baverstock et al. 2009, 2010). Therefore, target-oriented spore dispersal has been proposed as a solution to disseminate the pathogen via host and/or non-host arthropods (Zhu and Kim 2012). Infected hosts is thought to be an effective method for transmission and dispersing of entomopathogens (Fuxa and Tanada 1987; Roy et al. 2001). Numerous studies are available using host or non-hosts to deliver pathogens to the target pest. (Butt et al. 1998; Roy et al. 2001; Bruck and Lewis 2002; Dowd and Vega 2003; Bird et al. 2004; Carreck et al. 2007; Avery et al. 2009; Down et al. 2009; Moran et al. 2011; Tinzaara et al. 2015). The well known pollinators *Apis mellifera* L. (Hymenoptera: Apidae) and *Bombus impatiens* Cresson (Hymenoptera: Apidae) have been used for dissemination of the spores. These species effectively delivered the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* to the targeted plant parts where the pests are typically located (Carreck et al. 2007; Kapongo et al. 2008). In some studies it has been found that coccinellids are capable of disseminating entomopathogenic fungi for the control of aphids (Roy et al. 2001; Pell and Vandenberg 2002; Roy et al. 2008).

Individuals, infected with the entomopathogenic fungi, constitute new inoculum sources and increase the infection possibilities in the population (Lerche et al. 2008). Dissemination within the population depends on host-specific behavior and movements. Frequently, *F. occidentalis* occurs as a swarm on host plants exhibiting aggressive interactions such as fighting, thereby providing a definite contact within populations (Terry and Dyreson 1996). Therefore, we think that Western Flower Thrips could individually collect spores from a moulding cadaver or from infected individuals in the population. Although *F. occidentalis* exhibits similar behavior to social insects, there is no report to show host-oriented spore dispersal. The present study was aimed to determine if host-oriented spore dispersal of

F. subglutinans 12A is an effective method of dissemination to *F. occidentalis* in different scenarios.

Material and methods

Insect and fungal material

The main materials of this study were *F. subglutinans* 12A (isolated from *Aphis gossypii* Glover Hemiptera: Aphididae, Adana-Karataş in Turkey) and four-year-old laboratory colony of *F. occidentalis* (thrips were collected from wild plants in the campus of Isparta Applied Sciences University in 2014 and maintained over generations in a chamber room at 25 ± 1 °C, 60–70% RH, and 8:16 h D:L, and identified according to Hoddle et al. 2012) and chili pepper plants (*Capsicum annuum* L.) (Balıkesir seed company, Turkey).

In this study, *F. subglutinans* spore concentration 1×10^6 spore/ml (Demirözer et al. 2016) was prepared according to method of Cheraghi et al. (2012) with minor modifications. The isolate was cultured on PDA at 25 °C for 10 days. Sterile distilled water was poured onto the mycelium of *F. subglutinans* and the mycelium was collected with a scalpel and the liquid was collected to extract spores. Suspensions were then filtered through sterilized cotton filters to obtain pure conidial suspensions and the spores were counted using a hemocytometer. The spore suspension was adjusted to 1×10^6 spore/ml concentration with sterile distilled water. Sterile distilled water containing Tween 20 (0.1%) was used in all control trials.

Methods

The studies were carried out in glass Petri dishes (9 cm diameter) in experiments 1–4 and experiment 5 was conducted in single cell cages. A complete randomized block design was used in all experiments. In order to provide for the feeding and sheltering needs of *F. occidentalis*, in the experiments 1–4, leaf discs of peppers (3 cm diameter) were used as food sources and filter papers were placed in each Petri dish.

In all experiments, the females of *F. occidentalis* newly hatched from pupae were captured by aspirator from the rearing box and transferred by wet fine brush into the mesh and spore inoculum (1×10^6 spore/ml) was applied using the dipping method (5 s). Observations began 72 h after inoculation and continued until all individuals were deceased in experiments 1–3. In experiment 4, observations commenced 24 h after the release of uncontaminated individuals into Petri dishes and continued until 8 days after release (24-h intervals). In experiment 5, observations were carried out on the 5th and 7th days.

In experiments 1 and 2, spore inoculum exposed individuals were not included in the analysis (The number of infected individuals that were released in each experiment). Observations were carried out under the stereo-microscope (40X zoom) and the number of dead thrips and mycosis occurred individuals were recorded separately. The mycosis observations carried out only in experiments 1 and 2. In all experiments, re-isolation was made at the end of the counting process on dead individuals. Throughout the study, the petri dishes and cell cages were maintained at 25 ± 1 °C, 60–70% RH, and 8:16 h D:L.

Dissemination experiments of Fusarium subglutinans 12A

Experiment 1 In the first experiment a range of one to five inoculated females were released into Petri dishes containing 10 uncontaminated females. The Petri dishes were then covered with parafilm to prevent escape. All trials were conducted with three replications for each number of inoculated subjects.

Experiment 2 This experiment was also conducted in glass Petri dishes, and the contents of the Petri dishes and the number of replications was identical to experiment 1. In experiment 2, uncontaminated females of *F. occidentalis* in numbers ranging from one to five were released into Petri dishes containing 10 inoculated females.

Experiment 3 In this experiment, pepper leaf discs (3 cm diameter) were also dipped (5 s) into spore inoculum. Next the leaf discs were placed into the Petri dishes and 10 uncontaminated females were transferred by fine brush onto the leaf discs. All trials were conducted with five replications.

Experiment 4 In this trial, two females of *F. occidentalis* were dipped (5 s) into spore inoculum and released into the Petri dishes. Five days later (when the mycosis had

occured), ten uncontaminated females of *F. occidentalis* were released into the petri dishes now containing two dead and moulded cadavers. The experiment was conducted with five replications.

Experiment 5 In this experiment, pepper plants were germinated in seedling trays containing a commercial growing mixture (The Galaxy® Growing Mix, The Galaxy Company, Antalya, Turkey) at 25 ± 1 °C, 60 ± 10% relative humidity and 16: 8 [L: D]. Two-week-old pepper seedlings were transplanted into 11.5-cm-high by 11.5-cm-diameter pots containing 430 g growing medium consisting of *Pinus* spp. bark, peat moss and sand (2:1:1). Trials were carried out on 60 days old pepper plants with a minimum of 1–3 flowers. Ten uncontaminated females were released on flowers and the plant parts containing the flowers were covered with a 100 ml cylindrical container (ventilated on two sides) to prevent the escape of thrips. The females were dipped into the spore inoculum in numbers ranging from one to five and were released into single cell cages. Each pepper plant was entirely covered with a plastic bag (5 L) for incubation of the entomopathogen fungus during the 48 h after release. The experiment was conducted with three replications for each number of thrips.

Statistical analysis

In this study mortality rate was calculated according to Abbott (Abbott 1925) and data were transformed (SIN (Sqrt (x*0.01)) prior to analysis. One-way Anova test was applied to the transformed data followed by Tukey's HSD (Honestly Significant Difference) test was performed ($p < 0.05$). Correlation and regression analyses were performed as well ($p < 0.01$). The Statistical software package SPSS 20.0 was used for all statistical analyses.

Results

The first experiment investigated whether the number of inoculated thrips (ranging from one to five) would have an effect on mortality rate and the data indicated that increasing the number of infected individuals had no significant effect on mortality rates ($p > 0.05$). The mortality rate was 39.1% when one inoculated thrips was released, with mortality rates reaching 51.8, 51.6, 48.2 and 50.1% in the trials in which 2, 3, 4 and 5 inoculated thrips were released; respectively (Fig. 1). The mean number of individuals displaying mycosis was significant when five inoculated thrips were released (5.53 ± 0.66; $p < 0.05$). In the other trials, there was no significant difference ($p > 0.05$) and the mean numbers were between 1.03–2.27.

In experiment 2, change in the number of uncontaminated individuals significantly affected mortality rates. The mortality rate in the trial where four uncontaminated thrips were released was significant (60.4%, $p < 0.05$), and the mortality rates reached 53.9, 51.4, 47.4 and 44.1 in the trials where 2, 3, 1 and 5 uncontaminated thrips were released; respectively (Fig. 2). The mean number of individuals exhibiting mycosis was significant where three uncontaminated thrips were released into the Petri dishes (2.43 ± 0.23; $p < 0.05$). The other mean numbers were between 0.20–2.23 and was not significant.

The experiment 3 was carried out to determine effects of inoculum applied to the food or habitat. The mortality rate was found to be 53.1 ± 4.4% on the fifth day and the mortality rate reached 90.9 ± 2.4% on the 7th day.

The experiment 4 was implemented to investigate the effects of infected cadavers (where mycosis had already occurred) on the uncontaminated thrips population. In this trial, while the mortality rate was 15.5 ± 2.4% on the 5th day, it reached 45.7 ± 3.7% on the 8th day (Fig. 3).

The experiment 5, carried out on the pepper plants, displayed similar results to experiment 1. The increase in the number of inoculated individuals had no significant effect ($P > 0.05$) on mortality rates of uncontaminated individuals that were released into the single cell cages. Five days after inoculation, mortality rate was 19.2% where four inoculated females were present (Fig. 4). However, on the seventh day after inoculation, the mortality rate reached 34.2% where five inoculated females were used (Fig. 5). Five and seven days were considered the maximum tolerable time interval by farmers to see the effect of the applied pesticide, therefore these days were chosen to conduct the experiment 5.

Discussion

The different experiments of our study indicate that *F. subglutinans* 12A (applied at a rate of 10^6 spore/ml) can be successfully diffused by conspecifics of *F. occidentalis* into the thrips population. When the

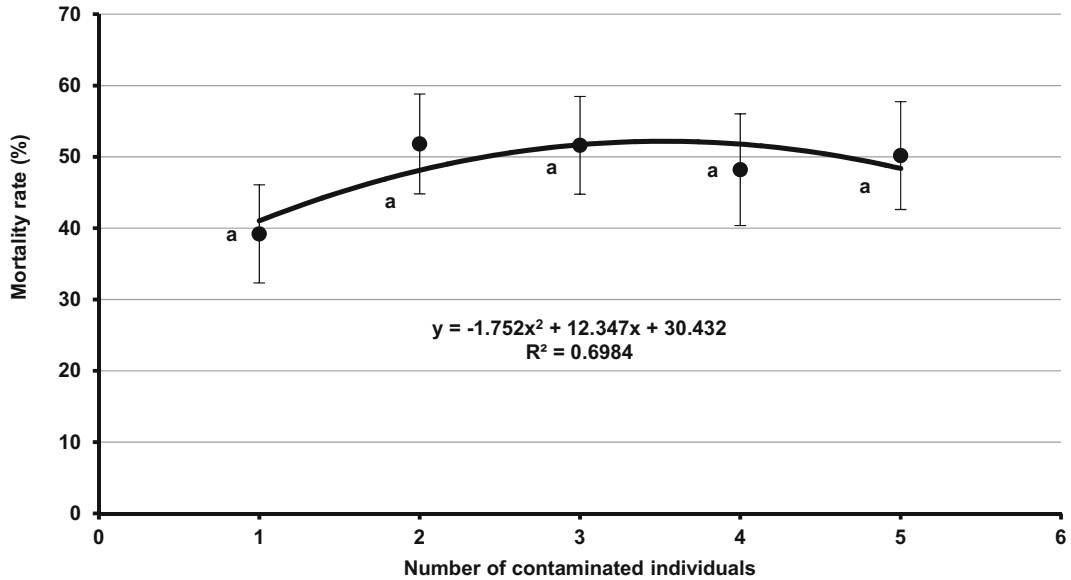

Fig. 1 Mortality rates of *Frankliniella occidentalis* Pergande in the Petri dish trial of the release of inoculated to uncontaminated thrips (Experiment 1) (Transformed data [SIN(x*1/2)] were used for statistical analysis)

inoculated thrips were released into the population, the highest mortality rate was 51.8% and when uncontaminated individuals were released into a Petri dish with inoculated individuals, the mortality rate was 60.4%. Though our investigation focused on dissemination of entomopathogenic fungi in a pest population by conspecifics, we noticed that other researchers generally utilized non-host arthropod species to disseminate fungi to the target pest. The thrips predator *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae) infected with *Lecanicillium longisporum* (Petch) (Zare & Gams) were released to *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) and *F. occidentalis* individuals and populations of both pests were reduced by 66% and 95%;

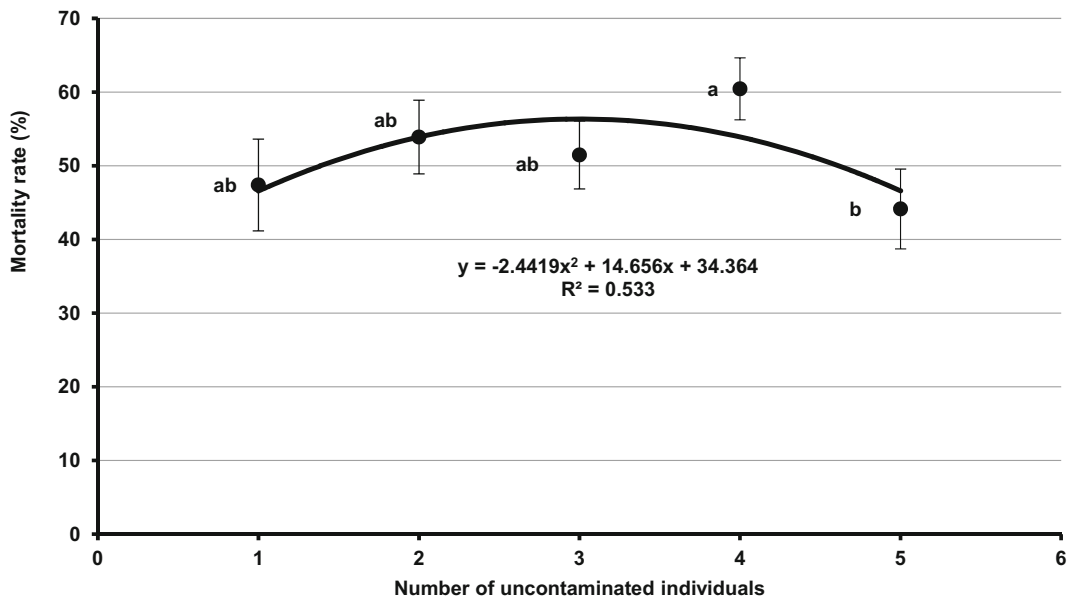

Fig. 2 Mortality rates of *Frankliniella occidentalis* Pergande individuals in the Petri dish trial of the release of uncontaminated to inoculated thrips (Experiment 2) (Transformed data [SIN(x*1/2)] were used for statistical analysis)

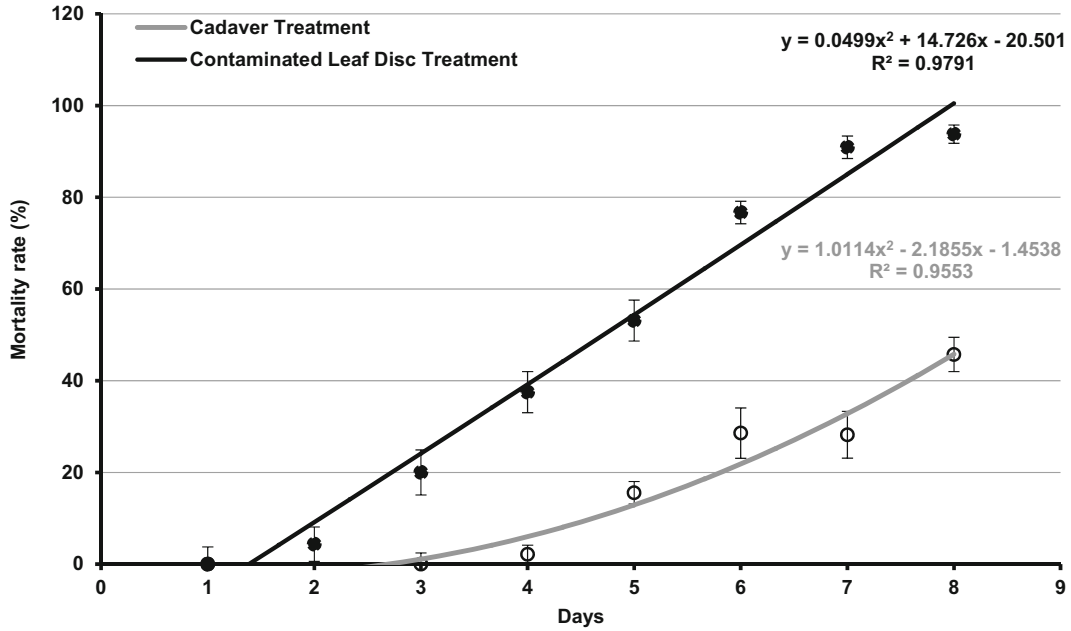

Fig. 3 Mortality rates of *Frankliniella occidentalis* Pergande individuals across time in the leaf disc and cadaver treatments (Experiment 3 and 4) (Transformed data [SIN(x*1/2)] were used for statistical analysis)

respectively (Down et al. 2009). The black garden ant (*Lasius niger* L.) (Hymenoptera: Formicidae) infected with *L. longisporum* likewise caused a significant decline in the population of *Aphis fabae* Scopoli (Hemiptera: Aphididae) and *Dysaphis plantaginea* Passerini (Hemiptera: Aphididae) (Flower 2002; Bird et al. 2004). However this method is not always succesful, as was found in the case of *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) infected with *Erynia neoaphidis* Remaud & Hennebert, which

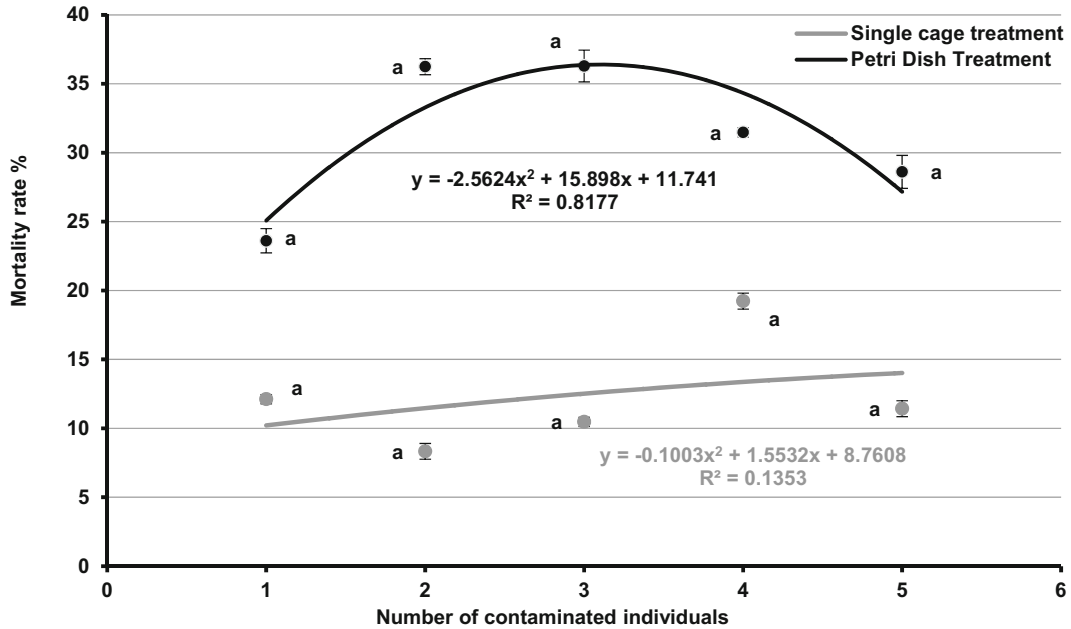

Fig. 4 Mortality rates of *Frankliniella occidentalis* Pergande individuals in the single cell cages and Petri dishes on the 5th day after fungus application (Experiment 1 and 5) (Transformed data [SIN(x*1/2)] were used for statistical analysis)

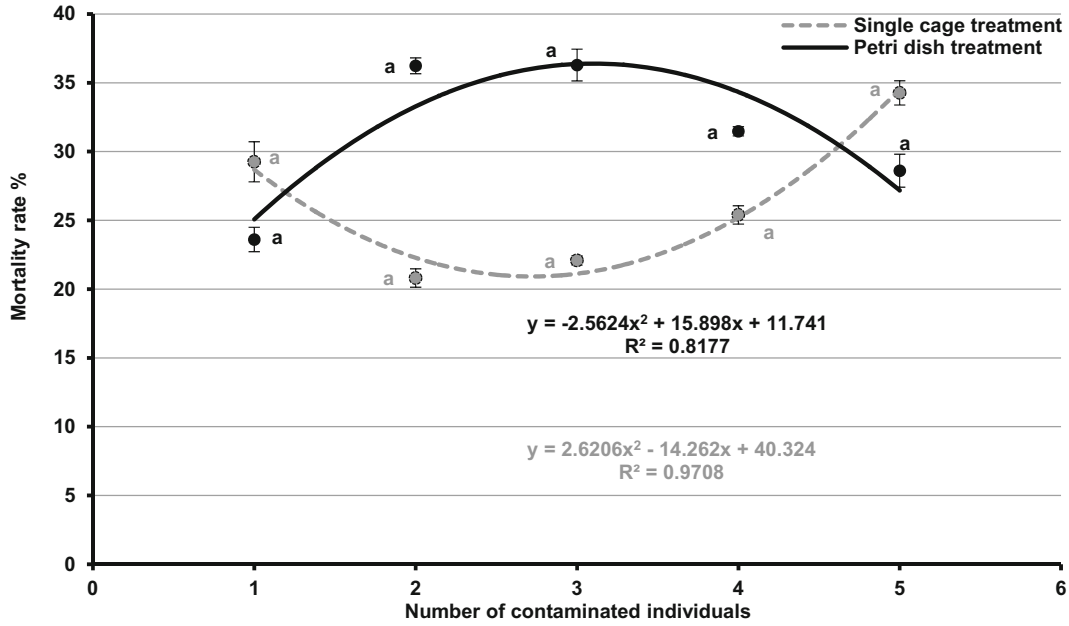

Fig. 5 Mortality rates of *Frankliniella occidentalis* Pergande individuals in the single cell cages and Petri dishes on the 7th day after fungus application (Experiment 1 and 5) (Transformed data [SIN(x*1/2)] were used for statistical analysis)

did not cause succesful inoculation of *Sitobion avenae* (Fabricius, 1775) whereas *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae) was infected with the fungus (Roy et al. 2001).

The most hopeful result was obtained in experiment 3 where the mortality rate reached approximately 94% on the 8th day where the inoculum was applied to the leaf surface. In autodissemination of entomopathogenic fungi succesful transmission depends on the attaching of the fungus conidia to the arthropod body, and staying alive is very important to the effectiveness of a fungi-based pest management strategy (Roy et al. 2001; Dowd and Vega 2003; Tsutsumi et al. 2003; Scholte et al. 2004; Maniania et al. 2006). Moreover, the concentration of entomopathogenic fungi on the leaf surface can also increase the host infection rate (Bailey et al. 2007). Avery et al. (2009) reported that the mortality rate of *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) was >95% on the 8th day in the leaf disc trial where *Isaria fumosorosea* Wize was applied. It was reported that the transmission of entomopathogenic fungi could be affected by the number of individuals in which hyphae development has already occurred on cadavers (Furlong and Pell 2001; Avery 2002; Klinger et al. 2006; Avery et al. 2009). In experiment 1, we found that when the number of inoculated thrips increased, the mean number of thrips exhibiting mycosis was as high as the number of uninfected individuals. Additionally, the number of cadavers in which mycosis has occurred could be the determining factor in the spread of the fungal spores to the pest populations. Likewise, the highest infection rate was recorded in a trial in which 30 cadavers of *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae) were infected with *Erynia neoaphidis* Remaud & Hennebert in comparison with the trials containing 1, 5, 15 cadavers (Roy et al. 2001). In experiment 4, we determined that the introduction of just two cadavers caused 45.7% mortality among uncontaminated thrips individuals.

In this study, target-oriented dissemination of the entomopathogenic fungus *F. subglutinans* 12A was investigated. Female individuals of *F. occidentalis* were chosen as hosts in the trials which were conducted under five different scenarios and in each scenario the effects of the fungus on individual thrips was recorded. Demirözer et al. (2016) reported that *F. subglutinans* 12A can cause approximately 70–80% mortality in seven days when the fungus is applied to the individuals directly. Contrarily, the results obtained in experiment 5 using the pepper plants varied. In the single cell cage treatment of the release of uncontaminated thrips onto the pepper plant, the mortality rate was 19.2%, whereas it was 36.3% in Petri dishes on the fifth day. Similarly, on the seventh day, the mortality rate was 34.2% in the

cell cage and 84.9% in Petri dishes. Roy et al. (2001) reported that when *C. septempunctata* infected with entomopathogenic fungus were released into the aphid population, it caused 8% infection in laboratory conditions and 5% in the field.

According to the results of the present study, when *F. subglutinans* 12A was applied to the habitat or when inoculated thrips were released with uncontaminated individuals and if there were cadavers exhibiting mycosis present, higher mortality rates were experienced by the pest population caused by the fungus. Social interactions such as food competition, mating and moreover thigmotactic behaviour (hiding in the same habitats) of *F. occidentalis* are a potential avenues of transmission of fungus. Through this transmission, we believe that entomopathogenic fungus could spread and could be a more effective pathogen against the thrips population. The results from this study should be a basis for future works in this area and should be expanded into field conditions.

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Compliance with ethical standards

Conflict of interest The author declares that he has no conflict of interest.

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