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Evaluation of entomopathogenic fungi *Beauveria bassiana* and *Lecanicillium muscarium* on different nymphal stages of greenhouse whitefly *Trialeurodes vaporariorum* in greenhouse conditions

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Abstract. The susceptibility of different larval stages of *Trialeurodes vaporariorum* to the fungal pathogens, *Beauveria bassiana* and *Lecanicillium muscarium* were assessed. Conidial suspensions containing 10^3-10^6 conidia/mL^{-1} were applied to the underside of each leaflet on young and old nymphal stages of *T. vaporariorum* using a sterilized hand sprayer with fine droplet spray nozzle in a randomized complete block design with 6 replications. Results showed the percentages of mortality caused by *B.bassiana* and *L.muscarium* were 63.74% and 62.49% on young nymphs and 71.68% and 87.13% on old nymphs, respectively; the old instars were significantly more susceptible than young instars.

Key words: greenhouse whitefly, entomopathogenic fungi, *Lecanicillium muscarium*, *Beauveria bassiana*.

Introduction

Many crops worldwide are affected by several genera of whiteflies. Whiteflies are tiny, phloem sap-sucking insects that are frequently abundant in vegetable and ornamental plantings. Whiteflies excrete sticky honeydew and cause yellowing or death of leaves. In addition, whiteflies are also a vector of many plant viruses. Whiteflies develop rapidly in warm weather, and population's outbreak when weather is favorable and natural enemies are absent. Chemical control is an important component of whitefly management programs worldwide (Ellsworth & Martinez-Carrillo 2001). However, the use of chemicals has been compromised principally because of rapid emergence of resistance to various classes of insecticides, especially organophosphates, pyrethroids, and cyclodienes (Cahill et al. 1995). The rising costs of traditional chemical pesticides, the increasing resistance of insects to them and their undesirable effects on the environment have led to renewed efforts to search for new tools for controlling insect tests (Scott et al. 1998). As potential alternatives, certain chemicals derived either from microorganisms, termed biopesticides, have been promoted in recent years. Over the past 50 years, the control of insects, weeds, and plant diseases with fungal pathogens has been a very active area of research and has resulted in a large number of commercially-available products (Charudattan 2001, Faria & Wraight 2001, Jackson et al. 2010). Several mycopesticides based on entomopathogenic fungi have already been developed in many countries as alternative control agents (Goettel et al. 2005).

Entomopathogenic fungi invade the host via land on the cuticle and consequent penetration. The host is then killed due to a lack of soluble nutrients in its hemolymph and the release of toxins from the fungi. Therefore, entomopathogenic fungi are potential candidates to control insects such as *Trialeurodes vaporariorum*.

Entomopathogenic fungi parasitizing different life cycle stages of pest regarded as valuable weapons for biocontrol that play an important role in promoting integrated pest management. To date, various strains of entomopathogenic fungi such as *Lecanicillium* (previous name, *Verticillium*) sp. (Steenberg & Humber 1999), *Beauveria bassiana* (Quesada et al. 2006), and *Paecilomyces* (Shia & Feng 2004), have been used to control aphids, lepidopteran larva and other pests. Therefore, for successful establishment of *B. bassiana*, *L. lecanii* and *P. fumosoroseus* to reduce insecticidal treatment in IPM program, the role of these fungi is very important.

The present investigation was conducted for evaluating entomopathogenic fungi *Beauveria bassiana* and *Lecanicillium muscarium* against different nymphal stages of greenhouse whiteflies, *Trialeurodes vaporariorum* as polyphagous insect pests.

Materials and methods

Whiteflies rearing

Adults whiteflies collected from Isfahan University of Technology research greenhouse and reared on cultivated tomato (CH-Flat varieties) in net cages maintained at 24±2°C and 70~80% relative humidity (RH) and under a diurnal day/night cycle of 16/8 hr. 40 adults released in cages from two sexes for 72h, After egg laying had occurred and the adults had been removed. After this time adults removed from the leaf by blowing them off by mouth. After 11 days all nymphal stages in 1st or the 2nd instars, and 20 days after the laying of, all nymphs in 3rd or 4th nymphal instars appeared at the time of inoculation for bioassay experiment.

Entomopathogenic fungi preparation

B.bassiana (DAOM198499 strain separated from codling moth) and *L.muscarium* (Fashand strain separated from soil) selected for culture and concentration preparation. All cultures were grown on potato dextrose agar (PDA) at 24±2 °C. After colonies had grown to a diameter of 5 cm, 5 mL of 0.02% aqueous Tween 80 was dropped on to the colony and the conidia carefully scraped from the medium by means of fine paint brush. The conidial suspension was collected and vortexed to break up clumps or chains of conidia and to achieve a homogeneous suspension. Spores were counted using a haemacytometer and suspensions were diluted with sterile-distilled water to make the basic concentration of approximately 10^6 conidia/mL other concentrations 10^3, 10^4 and 10^5 prepared from basic concentration.

Bioassay

The virulence test was conducted against the 1st to 4th instar nymphs

stages of *T.vaporariorum*. Two entomopathogenic fungi were tested for their ability to kill whitefly nymphs. In control nymphs were treated with water + Tween 80), giving 2 treatments with six replications per treatment. A filter paper wetted with distilled water inserted in petri dish and tomato leaves on it. Tomato leaves petiole covered by cotton enriched by 2 ml (NPK) nitrogen-potassium and phosphorous in order freshing protection of leaves. Conidial suspension was sprayed on leaf surface inserted on filter paper. Two milliliters of the diluted spore suspension in different concentration was applied using a handheld sprayer onto tomato leaves infested with over 35 nymphs of 1-2 and 3-4 instar groups. The leaf was allowed to air dry before it was placed in a plastic container (17.0 × 25.0 × 9.0 cm) with 20 whitefly nymphs and stored at 27 ± 1°C, relative humidity 80-90%, and 12 h light exposure. Dead whiteflies were counted and removed every day for 7 days in different treatments and control.

Statistical analysis

Abbott's formula was used to correct for control mortality (Abbott, 1925) before subjecting mortality data to analysis of variance (ANOVA). The statistical analyses were performed using the Proc GLM procedure (SAS Institute, 2001). Means were separated using Least Significant Difference (LSD) test at 5% level of significance. In addition, probit analysis was performed on the mortality data to determine the time required to kill 50% of the test population (LT50), 95% confidence limit (CL), and regression slope using the program Polo-PC (LeOra Software 1994). Logarithmic distance measured by following formula:

$$\left[a = \frac{\log A - \log E}{N - 1}\right]$$

A=maximum concentration, E=minimum concentration, a=constant coefficient and N=number of concentration.

Other concentration calculated according these relationships:

B= Anti log (log A-a)

C= Anti log (log A-2a)

D= Anti log (log A-3a)

Results

Beauveria bassiana pathogencity on various nymphal instars:

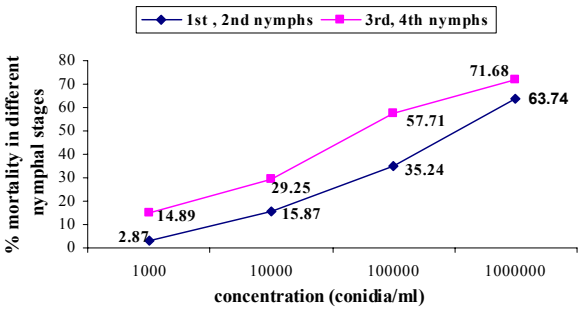

Figure 1. The comparing of mean percentage mortality in bioassay of different concentrations of *Beauveria bassiana* on different nymphal stages of *Trialeurodes vaporariorum*. p<0.001.

This entomopathogic fungus showed suitable control on 1st and 2nd instar of *T.vaporariorum*. Lethal percentage of 10^6 concentration recorded 63.7. This maximum lethal percentage decreased gradually associated with decrease of concentration and minimum of lethal percentage (2.87%) recorded in 10^3 conidia/microlitr, no significant difference with control. LC_{50} of *B.bassina* measured 0.17×10^6 on young nymphs (Fig. 1). Analysis variance of obtained data showed that different concentration of *B. bassiana* had significant effects mortality rate (P<0.01). Higher LC_{50} measured in old nymphal instars compared with young nymphal instars (Tables 1, 2).

L.muscarium pathogencity on various nymphal instars: Mortality rate recorded 63.74 % in 10^6 concentration for 1st and 2nd instars while this concentration showed 71.68 % mortality rate for 3rd and 4th instars (Fig. 1). Percent of pathogencity of this entomopathogenic fungus had significant difference in various concentrations. Significant effects of 10^6 concentration on 3rd and 4th instars did not show positive effect on mortality rate of 1st and 2nd instars. Recorded LC_{50} of *L.muscarium* for young and old instars were 8.7×10^4 and 1.6×10^4 respectively (Tables 3, 4).

Table 1. Lc_{50} and up and down limitation for *Beauveria bassiana* on young nymphal stages of *Trialeurodes vaporariorum*.

Effective dose	Concentration (conidia ml^{-1})	Concentration level	confidence level		
			0.90	0.95	0.99
LC_{10}	2.45×10^3	Low level	1.2×10^3	1.02×10^3	7.07×10^3
		High level	3.8×10^3	4.27×10^3	5.13×10^3
LC_{50}	0.17×10^6	Low level	0.13×10^6	0.13×10^6	0.11×10^6
		High level	0.23×10^6	0.25×10^6	0.28×10^6
LC_{90}	0.13×10^8	Low level	0.72×10^7	0.65×10^7	0.52×10^7
		High level	0.29×10^8	0.35×10^8	0.54×10^8

Table 2. Lc_{50} and up and down limitation for *Beauveria bassiana* on old nymphal stages of *Trialeurodes vaporariorum*.

	Concentration (conidia ml^{-1})	Concentration level	confidence level		
			0.90	0.95	0.99
LC_{10}	2.6×10^2	Low level	81.28	50.25	29.51
		High level	31.6×10^2	7.2×10^2	9.8×10^2
LC_{50}	3.9×10^4	Low level	2.6×10^4	2.6×10^4	1.9×10^4
		High level	5.6×10^4	2.6×10^4	7.1×10^4
LC_{90}	0.56×10^7	Low level	0.28×10^7	0.25×10^7	0.19×10^7
		High level	14×10^8	0.18×10^8	0.32×10^8

Table 3. Lc_{50} and up and down limitation for *Lecanicillium muscarium* on young nymphal stages of *Trialeurodes vaporariorum.*

	Concentration (conidia ml^{-1})	Concentration level	confidence level		
			0.90	0.95	0.99
LC_{10}	3.5×10^{2}	Low level	1.2×10^{2}	95.49	51.28
		High level	7.6×10^{2}	8.7×10^{2}	1.1×10^{3}
LC_{50}	8.7×10^{4}	Low level	6.16×10^{4}	5.8×10^{4}	4.6×10^{4}
		High level	0.12×10^{6}	0.13×10^{6}	0.16×10^{6}
LC_{90}	0.21×10^{8}	Low level	0.68×10^{7}	0.85×10^{7}	0.65×10^{7}
		High level	0.64×10^{8}	0.84×10^{8}	0.16×10^{9}

Table 4. Lc_{50} and up and down limitation for *Lecanicillium muscarium* on old nymphal stages of *Trialeurodes vaporariorum.*

	Concentration (conidia ml^{-1})	Concentration level	confidence level		
			0.90	0.95	0.99
LC_{10}	3.9×10^{2}	Low level	1.9×10^{2}	1.5×10^{2}	1.04×10^{2}
		High level	6.9×10^{4}	7.8×10^{2}	9.5×10^{2}
LC_{50}	1.6×10^{4}	Low level	1.2×10^{4}	1.1×10^{4}	4.7×10^{3}
		High level	2.1×10^{4}	2.3×10^{4}	2.5×10^{4}
LC_{90}	0.69×10^{6}	Low level	0.43×10^{6}	0.43×10^{6}	0.39×10^{6}
		High level	1×10^{7}	0.11×10^{7}	0.15×10^{7}

Discussion

This study showed that *B. bassiana* regarded as one of suitable entomopathogenic fungi for greenhouse whiteflies. Results of this investigation confirmed other researches about pathogencity of these fungi for whiteflies (Faria & Wraight 2001, Jamez & Elzen 2001, Fargus et al. 2003, Malekan et al. 2012, 2013). Results showed that various strains of *B. bassiana* had different range of mortality 3-78% on *Bemisia tabaci* (Quesada-Morga et al. 2006). This mortality rate was different from other researches due to different in pathogencity of various strains on pests (Leland et al. 2005). Molecular investigation and sequencing of studied strains confirmed different pathogencity among them (Andrew et al. 2005).

Host of whiteflies effects on fungi pathogencity significantly. Many researched consider on enthomopathogen pathogencity to host plant, its source and originality (Wang et al. 2004, Torrado-leon et al. 2006). Vicentini et al. (2001) Showed that isolated strains from two order Hemiptera and Homoptera induced more mortality on *B.tabaci* biotype B. Applied *B.bassiana* in this investigation isolated from soil with same results (Vidal et al. 1998).

Andrew et al. (2005) observed higher *B. tabaci* infection on bean compared with tomato due to secondary metabolites availability named tomatin on pathogenic fungi activities in sweet potato whitefly population. Low mortality rate in this studies can related to some these metabolites with negative effects on fungi activities and developments. Same antibioses effects demonstrated in cotton, *B.argentifolii* reared on cotton showed low sensibility to *B. bassiana* and *P.fumosoroseus* compared with pest reared on melon (Wraight et al. 1998).

Pathogen efficiency will change due to plant morphology, enzymatic interaction and physical characteristics between fungi and its part (Miranpuri & Khachatourians 1995). Used tomato variety in this study had more hair compared with others that caused irregular oviposition of whiteflies on it. It's possible that mortality rate of whiteflies changed on other varieties showing host role on population dynamism. Positive effects of entomopathogenic fungi *L.muscarium* on whiteflies demonstrated in many studies (Wraight et al. 1998, Faria & Wraight 2001). Some results in this investigation confirmed before obtained results. Toxic component V3450 and VP28 extracted from *L.muscarium* as antifeedant for *B.tabaci* nymphs (Wraight et al. 1995). Inoculation and death rate related to fungi strain due to different in pathogen characteristics (Moino et al. 2002).

It is surveyed some characteristics of *L.muscarium* on the against the aphid *Macrosiphoniella sanborni* in the laboratory (Jackson et al. 1985). Some of traits were associated with the virulence including fast germination, high sporulation rate, an absence of extracellular amylase activity and high extracellular chitinase activities. They were emphasized that large spore size was not strongly associated with virulence (Jackson et al. 1985). According to this reason and because of *L.muscarium* exhibited high pathogenicity than the *B.bassiana.* Based on these we concluded that probably penetrate of germ tube in *L. muscarium* is could be feasible than the *B.bassiana.* Also if prolonged count of insects infected with *B.bassiana* in the present study the possibility of higher mortality due to prolonged effect of the fungus was present. The intersegmental area of the abdomen is flexible to penetrate of appressoria. So distribution of conidia on nymphal instar effective on the percentage of mortality. According to this fact that the nymphal instar of greenhouse whitefly mobile except 1^{st} instar, if the purpose reaching to high mortality fungal conidia should be entirely covered on nymphs.

Sensibility of different nymphal instar to entomopatogenic fungi were tested in many studies. In present study mortality in 3^{rd} and 4^{th} were higher than 1^{st} and 2^{nd} instar. In some study revealed that mortality reduce by growth of nymphal instar. So that the 1^{st} nymphal instar is most sensibility to entomopathogenic fungi (Van de Veire et al. 1996, Gridin et al. 2000). Some of the entomopathogenic fungi in-

vade to one growth stage of insect. For example the *Zoophthora* sp. naturally infecting only *B. tabaci* adults, whereas other life stages were not attacked (Faria & Wraight 2001). This case is not observed in present study and all of growth instar are infected by entomopathogenic fungi. Malsam and et al. (1998) showed that were not significant different in sensibility of nymphal stages of whiteflies. Paprawski et al. (2000) revealed that the 3rd nymphal stages of *T.vaporariorum* were most sensibility to *B. bassiana*. Smith & Grula (1982) that survey effectiveness of *P.fumosoroseus* and *M.anisopliae* are showed that 1st nymphal instar was most sensitive and 4th nymphal instar was most resistance to these entomopathogenic fungi (Smith & Grula 1982). Vay & Fargues (1977) were found that the nymphal stages and adult of *Leptinotarsa decemlineta* were same sensibility to *P.fumosoroseus* (Vay & Fargues 1977). According to these cases we can result that because of smaller body of 1st and 2nd than the 3rd and 4th, the less conidia/area stand on the cuticle. Van de Veire et al. (1996) declared that the difference in susceptibility among instars correlated to Selection of isolates (Van de Veire 1996). However, it may be different in different studies period for completion of any age. Appears to be unequal in length of nymphal stages, the mortality rates will vary different. In the present study in 22 °C from hatching of eggs until 3th, 8 days and since the beginning of 3rd nymphal stages until 4th lasted for 13 days. Can be said when the conidia exposure on the young nymphal stages, have not enough time for penetration, and destroyed and inoculation of the fungus has been stopped. While in the older nymphs conidia more opportunities to penetrate into the insect's body and the infected older instar nymphs will be more than young instar nymphs. LC_{50} in different bioassay indicative of highly pathogenicity.

Byrne & Thomas (1991) stated that LC_{50} of PF2 isolate of *P. fumosoroseus* on *B. tabaci* was 4.3×10^8 conidia/mL. The LC50 values obtained indicated that the mortality between different nymphs strongly affected by the fungal isolates is used. In present study the mortality rate was higher than the commercial isolates ATCC70874 of *B. bassiana* (which is used in Spain on whiteflies) that has demonstrated high efficiency of strains used in this study. LC_{50} of this isolate reported 3.5×10^8 conidia/mL (Quesada et al. 2006). The LC_{50} in two isolates of *L.muscarium* that have been used by other researcher on *B.tabaci* variable between 2.5×10^5 conidia/mL to 6×10^6 conidia/mL (Wang et al. 2007). In the study of Kim et al., LC_{50} was 2.3×10^6 conidia/mL. In the same study it was found 10^8 and 10^9 conidia/mL that produce 100% mortality that were obtained 7 and 9 respectively (Kim et al. 2004).

It's demonstrated that both local entomopathogenic fungi strains are effective for whiteflies control under greenhouse conditions. It can be formulated after their mass production according toxicology experiments tests. Its novelty aspects are finding best strain for mass production and microbial pesticide formulation.

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