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Article

The entomopathogenic fungus *Beauveria bassiana* and its compatibility with *Phytoseiulus persimilis* (Acari: Phytoseiidae): Effects on *Tetranychus urticae* (Acari: Tetranychidae)

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ABSTRACT
Two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) is an economically important pest that devastates varieties of crops worldwide and develops significant resistance to common chemical pesticides. Ovicidal effects of two isolates of entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin (isolates *F* and *J.B.*) were evaluated on the eggs of *T. urticae* using a spray method at 25 ± 2°C, 60–70٪ RH and a photoperiod of 16:8 h (L: D). Egg mortality was determined by using different concentrations of conidia (1×10^3, 1×10^4, 1×10^5, 1×10^6, 1×10^7, and 1×10^8 conidia/ml). Mortality was found to increase with time and was dependent on time and conidia concentration, ranging from 4.06% on day 3 at a concentration of 1×10^3 to 84.37% on day 7 at a concentration of 1×10^8 for the *F* isolate and from 1.87 to 65% for the *J.B.* isolate, respectively. This study also investigated the direct spray of the entomopathogenic fungus, *Beauveria bassiana* (isolates *F* and *J.B.*) on the predatory mite, *Phytoseiulus persimilis* (Athias-Henriot) under laboratory conditions. The predator adults were susceptible to isolate *F* (total mortality ± SE: 35 ± 1.97% on the seventh day) but least susceptible to isolate *J.B.* (total mortality ± SE: 27.25 ± 1.33% on the seventh day). Moreover, viability of *P. persimilis* after spraying fungal isolate of *J.B.* on bean leaf discs at three time intervals (0, 24 and 48 hours) showed no significant difference. Further knowledge is needed to adjust the timing of various releases of both biological control agents to obtain maximum effectiveness in the greenhouse with minimum impact of the fungus on the predator.

KEY WORDS: Biological control; mortality; ovicidal effects; side effect; two-spotted spider mite.

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INTRODUCTION

Two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is a cosmopolitan and polyphagous species feeding on more than 900 plants including field crops, horticultural crops and ornamental plants, with great economic importance for crops in greenhouses and in the field (Janssen *et al*. 1997; Zhang 2003; Kumar *et al.* 2010; Clotuche *et al*. 2011).

Biocontrol of the mite with fungal agents has long been considered because of the development of insecticide resistance and undesirable side effects, such as death of non-target organisms (e.g. predators) and residue concerns (Stumpf and Nauen 2001; Van Leeuwen *et al.* 2010). The use of

entomopathogens to control certain pests may be a future alternative for solving problems of chemical resistance (Omoto *et al.* 1994) and environmental contamination (Alves *et al.* 2005). The entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales) is widely distributed in nature (St. Leger *et al.* 1992). It has been tested in the laboratory and field against numerous insect and mite pests. *Beauveria bassiana* has proved to be a potential biocontrol agent against spider mites (Dresner 1949; Andreeva and Shternshis 1995; Alves *et al.* 1998, 2002; Faria and Wraight 2001; Irigaray *et al.* 2003; Chandler *et al.* 2005; Seiedy *et al*. 2010). However, the success of fungal entomopathogens as biological control agents depends not only on the high efficacy against pests, but also on its impact on beneficial arthropods (i.e. parasitoids and predators) (Thungrabeab and Tongma 2007; Shipp *et al.* 2012). In fact, it is necessary to determine their effects on non-target organisms prior to their release (Seiedy *et al.* 2012a, b). Several laboratory methods are designed to evaluate the effects of pathogens by exposing predatory mites to pathogen (e.g. Ludwig and Oetting 2002; Chandler *et al.* 2005; Koike *et al.* 2005; Donka *et al.* 2008; Pozzebon and Duso 2009, Seiedy *et al.* 2012a, b; 2014, Zhang *et al.* 2015; Dogan *et al.* 2017).

The aim of this study was to evaluate the lethal effect of the entomopathogenic fungus *B. bassiana* against eggs of two spotted spider mite, *T. urticae* and determine whether the entomopathogenic fungus would disrupt biological control program by interfering with *P. persimilis*.

MATERIALS AND METHODS

Preparation of conidia

The *B. bassiana* isolates *F* and *J.B.* were obtained from the School of Biology and Center of Excellence in Phylogeny of Living Organisms, College of Science, University of Tehran, Tehran, Iran. In order to prepare conidial suspensions for bioassay, fungal isolates were cultured on SDAY at 25 ± 2°C, 60–70% RH and in dark for 14 days to obtain conidia. Conidia of each isolate were harvested by washing down with 10 ml sterile water containing 0.02% Tween 80 and filtered through moist filter papers (Whatmann® No. 1).

The conidial suspension was vortexed for 5 min to produce a homogenous suspension. Spore concentrations were determined using a haemocytometer. Suspensions were prepared at concentrations of 1×10^3, 1×10^4, 1×10^5, 1×10^6, 1×10^7, and 1×10^8 conidia/ml through dilution in the surfactant Tween 80 (0.02%); the spore suspensions used were less than four hours old and had been stored in a refrigerator.

Mite colony

Tetranychus urticae were originally collected from a farm in Karaj City, Alborz Province (35° 48′ 04.6″ N, 50° 57′ 39.6″ E, at an altitude of 1315 m a.s.l.), Iran. The mites were reared in laboratory on bean plants (*Phaseolus vulgaris* L. var. Alamoti) at 25 ± 2°C, 60–70% RH and photoperiod of 16L:8D h.

To obtain same-aged eggs for bioassays, adult females were collected from the mite culture and put on leaf discs placed on wet cotton wool in Petri dishes (9 cm diameter) and allowed to freely lay eggs for 5 h. Subsequently, all the adults were removed from the leaf by fine brush and only eggs were left on leaf surfaces.

Phytoseiulus persimilis were obtained from Koppert Biological Systems Inc., The Netherlands. The colony was fed on a mixture of various stages of *T. urticae.* The detailed experimental protocol for rearing the predator was described by Seiedy *et al*. (2015). To obtain females of the same age, we transferred about 10 gravid females from the stock colony to a *T. urticae*-infested bean leaf in each of Petri dishes. The females were allowed to lay eggs for 8 h and then were removed. Newly emerged predators (same-aged) were used for the experiments.

Experimental procedures

Pathogenicity evaluation of the entomopathogenic fungus, B. bassiana *on* T. urticae *eggs*

To inoculate the mite eggs at each concentration (1×10^3, 1×10^4, 1×10^5, 1×10^6, 1×10^7, and 1×10^8 conidia/ml), 16 uncovered experimental arenas (a bean leaf disk, 30 mm in diameter excised from bean plants on top of a layer of cotton in a 6 cm Petri dish filled with sterile distilled water) each carrying 20 eggs were sprayed with 1 ml of conidial suspension. All eggs on Petri dish were maintained in germinator after spray and checked daily until they hatched. Unhatched eggs were recorded until the counts had no change for seven consecutive days. In the present study, it was impossible to determine whether an egg was alive or dead at the time of observation unless fungal outgrowth was visible. Therefore, all unhatched eggs were transferred to moist filter paper for 7 day maintenance for fungal outgrowth, which was evidence to egg death attributable to fungal infection. Also, the control was treated by sterile distilled water and adhesive spreader (Tween 80, 0.02% ml/l).

Pathogenicity evaluation of the entomopathogenic fungus, B. bassiana *on* P. persimilis *adults*

For the bioassay, 20 *P. persimilis* females (3 day old, after maturation) were individually placed on experimental unit (fresh bean leaf disc with 30 mm in diameter). The leave were placed upside down on cotton saturated with water in Petri dishes (9 cm diameter) (Seiedy *et al.* 2012a). The leaf disc was sprayed with 1 mL of 1×10^6 conidia/mL; there were 10 replicates for each isolate. The control treatments received only sterile distilled water and Tween 80-0.02% mL/L. Mortality was recorded daily for 7 days. Dead mites were separately transferred to Petri dishes lined with moist filter paper and mycosis was confirmed by microscopic examination of the hyphae and spores on the surface of the dead mite body.

Viability of P. persimilis adult after spraying of fungal isolate F on bean leaf discs at 3 time intervals (0, 24 and 48 hours)

Fresh bean leaf discs (mentioned in second experimental units) were prepared and then were sprayed with 1 mL of 1×10^6 conidia/mL. Three time intervals (0, 24 and 48 h post-inoculation of bean leaf discs by fungal isolate of *F*) were considered for introducing the predators as different treatments. Thirty replicates were conducted for each time intervals and 20 adults from uniform *P. persimilis* mites (3 day old, after maturation) were placed on each fresh cucumber leaf disc. The control treatments received only sterile distilled water and adhesive spreader (Tween 80-0.02% mL/L). Viability of each predator was noted daily until it died. Mycosis was confirmed as mentioned above.

Data analysis

Differences of mortality between two isolates on *T. urticae* and *P. persimilis* were evaluated using by *t*-test procedure, SAS software (SAS version 6.12, Cary, NC). Viability of *P. persimilis* adults was analyzed using the ANOVA and GLM procedures of SAS (SAS Institute 1990). Average values were separated using Fisher's Least Significant Difference (LSD) test.

RESULTS

Fungal infection to mite eggs

The healthy eggs of *T. urticae* were smooth, whitish and translucent after laying on the bean leaves but egg surface became obviously collapsed after one week exposure to high conidial concentrations. Fungal outgrowths appeared on the unhatched eggs after maintenance on moist

filter paper at 25 ± 2°C for 7 days. When the unhatched eggs were individually mounted on slides and examined under microscope, the fungal outgrowths were identified as *B. bassiana*.

The pathogenicity of tested isolates of entomopathogenic fungi against eggs at six different conidial concentrations is shown in Table 1. Both tested isolates showed mortality at all tested conidial concentrations on eggs of *T. urticae*. Among the tested isolates, *F* showed the highest mortality percentages (84.37 ± 1.19%) at the concentration of 1×10^8 conidia /ml. In addition, there was a significant difference ($P < 0.001$) between the mortality induced by all fungal concentrations (Table 1).

Table 1. Total mortality (% ± SE) of *Tetranychus urticae* after application of *Beauveria bassiana* conidia suspension at various concentrations after 3, 5, and 7 days.

		Concentration 1×10^3	Concentration 1×10^4	Concentration 1×10^5	Concentration 1×10^6	Concentration 1×10^7	Concentration 1×10^8
F	Day 3	4.06 ± 0.93	4.6 ± 0.55	8.43 ± 0.99	13.12 ± 0.89	15 ± 0.67	16.87 ± 0.62
	Day 5	17.81 ± 1.04	23.75 ± 1.25	34.06 ± 1.38	45.93 ± 1.04	48.75 ± 1.25	57.18 ± 1.36
	Day 7	35.62 ± 1.43	45.31 ± 1.16	54.06 ± 1.04	77 ± 0.91	75.62 ± 1.28	84.37 ± 1.19
J.B.	Day 3	1.87 ± 0.62	2.66 ± 0.64	5.62 ± 0.77	10 ± 0.88	12.18 ± 0.78	13.43 ± 0.88
	Day 5	13.12 ± 1	17.5 ± 0.91	28.12 ± 1.19	36.56 ± 1.62	42.18 ± 1.36	45.31 ± 1.73
	Day 7	26.25 ± 1.33	35.62 ± 1.10	41.25 ± 1.07	58.12 ± 1.87	58.75 ± 1.16	65 ± 1.58
t		4.79	6.04	8.57	5.69	9.75	9.77
P		P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001

Phytoseius persimilis *mortality trends*

Early mycosis-caused death of the predatory mites usually began from day 3 after fungal treatment. The infected females looked shrunken and dark. Table 2 showed mite mortalities caused by 1×10^6 conidia /mL concentration after seven days.

Compared to other days, there was a significant difference in mortality of *P. persimilis* at 1×10^6 conidia/mL in two fungus isolates on fourth ($P = 0.02$), fifth ($P = 0.002$), sixth ($P = 0.0004$) and seventh ($P = 0.003$) days (Table 2). Comparing total mortality among these isolates, we found that isolate *F* had a higher mortality than *J.B.* (35±1.97%) on the seventh day.

Table 2. Total mortality (% ± SE) of *Phytoseiulus persimilis* after application of *Beauveria bassiana* isolates *F* and *J.B.* (conidia suspension at 1×10^6 conidia/mL) in 7 days post-inoculation.

	Day 3	Day 4	Day 5	Day 6	Day 7
F	5.5 ± 0.89	13 ± 1.10	20 ± 1.29	28.5 ± 1.67	35 ± 1.97
J.B.	3.5 ± 1.06	9.5 ± 0.89	14.5 ± 0.89	20 ± 1.05	27 ± 1.33
t	t = 1.43	t = 2.46	t = 3.50	t = 4.29	t = 3.36
P	P = 0.16	P = 0.02	P = 0.002	P = 0.0004	P = 0.003

Viability of the predatory mite P. persimilis *adult at 3 time intervals after spraying*

The average (± SE) viability of the predatory mites at three time intervals after treatment (0, 24 and 48 h) and control were 25.03 ± 0.55, 24.33 ± 0.44, 24.1 ± 0.41 and 25.53 ± 0.61 days, respectively; there was no significant difference among them ($F = 1.78$; $P = 0.15$).

DISCUSSION

The pathogenicity of two isolates of *B. bassiana* against the eggs of *T. urticae* revealed that both isolates of *F* and *J.B.* were highly infective on spider mite eggs and displayed surprising ovicidal activity against the pest in bioassays. The mean mortality values of host individuals on the seventh day for the lowest concentration were 35.62% and 26.25% and for the highest concentration were

84.37% and 65.1% for *F* and *JB,* respectively. The egg mortality caused by isolate *F* was higher than *JB*, suggesting its stronger ovicidal activity. Shi and Feng (2004) reported that *B. bassiana* and *Paecilomyces fumosoroseus* were capable of infecting *T. urticae* (Syn.: *T. cinnabarinus*) eggs at the concentrations of 58, 298 and 1306 conidia/mm^2, but the ovicidal activity of the *B. bassiana* isolate was greater than that of the *P. fumosoroseus* isolate. Erler *et al.* (2013) compared *M. anisopliae F52* with one isolate of *B. bassiana* and reported that *B. bassiana* was highly effective against *T. urticae* eggs.

In our results, using different fungal isolates could be the reason of significant differences in ovicidal activities ($P < 0.0001$). Other researchers have also reported ovicidal activities are due to fungal species as well as host species (Lacey *et al.* 1999; Samuels *et al.* 2002; Irigaray *et al.* 2003; Shi and Feng 2004; Erler *et al*. 2013; Bugeme *et al*. 2014; Dogan *et al.* 2017). Dogan *et al.* (2017) noted that fungal virulence was not just dependent upon the fungal strain, but also depended on the formulation, dose and frequency of application.

Another objective was to investigate the susceptibility of predatory mites to pathogen application time. Because methods of integrated pest management are consistent with the consumers' demand to reduce health and environment risks. Screening for eligible biocontrol agents is a step in developing new or improving existing environment-friendly strategies offering an alternative to conventional pest control. Our understanding about the possibility of concomitant use of both species in biocontrol programs of *T. urticae* will enhance with this experiment. In our experiments, the predatory species was susceptible to isolate *F* (35 ± 1.97% mortality after 7 days) than *J.B.* (27 ± 1.33% mortality after 7 days) (Table 2) when conidia were applied directly on the mites. Dogan *et al.* (2017) found that the susceptibility of *Neoseiulus californicus* (McGregor) and *P. persimilis* to entomopathogenic fungi appears to be dependent upon the strain and dose.

Further research must conduct into the survival of predatory mites on entomopathogenic fungus-contaminated surfaces at different times because adjust the timing of various releases of both biological control agents to obtain maximum effectiveness in the greenhouse with minimum impact of the fungus on the predator is needed. Our research showed that there was no significant difference in viability of *P. persimilis* after spraying of fungal isolate of *F* on bean leaf discs at three time intervals (0, 24 and 48 hours) ($F = 1.78$; $P = 0.15$).

Some factors can affect the outcome of intraguild interactions (Huang *et al.* 2012; Seiedy 2014). Predatory mites can remove fungal spores on the cuticles of its body by grooming behavior (using legs to clean the body); this behavior may be a defense mechanism to improve survival (Okuno *et al.* 2012). It can be a possible reason for the absence of a significant difference in the viability of predatory mites after spraying of fungal isolate of *F* on bean leaf discs at three time intervals (0, 24 and 48 hours) in the present study. Similar results were obtained by Wekesa *et al.* (2007) and Seiedy *et al.* (2015) who demonstrated that the behavior of phytoseiids can be affected by the presence of an excessive number of the entomopathogenic fungus, *Neozygites floridana* (Weiser and Muma) Remaudiere and *B. bassiana*. According to their results, *P. longipes* Evans and *Amblyseius swirskii* (Athias-Henriot) detected and removed most conidia attached to the body by self grooming.

More recently, ultrastructural experiments have showed that *B. bassiana* conidia adhered to the cuticle of predatory mites but were ineffective because the conidia were either shed or shriveled (Wu *et al*. 2016).

Moreover some of the behavioral factors such as prey preference, functional response of predator, life table of predator and the predator activity were altered by the presence of fungi in the patch or on treated hosts (Pell and Vandenberg 2002; Algaze 2006; Meyling and Pell 2006; Pourian *et al.* 2011; Seiedy *et al.* 2012a, 2012b; Seiedy 2014).

Donka *et al.* (2008) demonstrated spore density of *Lecanicillium muscarium* (Petch) and grade of contact of *P. persimilis* could affect the predator survival. On the hand, Jacobson *et al.* (2001)

found that *B. bassiana* had no detrimental effect on *A. cucumeris* Oudemans when sprayed onto excised cucumber leaves in a laboratory bioassay, or when sprayed onto greenhouse-grown cucumbers. Butt *et al.* (2016) reported that fatty acids in the epicuticular waxes often affect spore adhesion, germination and penetration of the host cuticle.

Successful use of entomopathogenic fungi as microbial control agents of mites will ultimately depend on how carefully the strains are selected (virulence, persistence), on the scale of the production, formulation, compatibility with other control agents and on the efficacy of the delivery system, and the timing of the application. Isolate *J.B.* of *B. bassiana* has lower virulence than *F* on predatory mite, so this isolate would be most efficient for use in the control of *T. urticae* because its compatibility with predatory mites.

However, complementary studies should be carried out to assess the potential of this isolate in pest management programs in greenhouse and field conditions.

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قارچ بیمارگر *Beauveria bassiana* و سازگاری آن با کنه شکارگر *Phytoseiulus persimilis* (Acari: Phytoseiidae): تاثیر روی کنهٔ تارتن دولکه‌ای *Tetranychus urticae* (Acari: Tetranychidae)

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چکیده

کنهٔ تارتن دولکه‌ای *Tetranychus urticae* Koch (Acari: Tetranychidae) آفت مهم اقتصادی است که به محصولات زیادی در سراسر جهان آسیب می‌زند و مقاومت زیادی نسبت به آفت‌کش‌های معمول شیمیایی دارد. در این پژوهش، در مرحلهٔ نخست زهراگینی دو جدایه (*F* و *J.B.*) از قارچ *Beauveria bassiana* در شش غلظت 10^3 تا 10^8 کنیدی در میلی‌لیتر با استفاده از روش پاششی روی تخم کنهٔ تارتن دولکه‌ای بررسی و مشاهده شد که بیشینهٔ مرگ ایجاد شده توسط این جدایه‌ها روی کنه‌های بالغ بین ۶۵ تا ۸۴/۳۷ درصد به ترتیب برای دو جدایهٔ *J.B.* و *F* در دمای ۲±۲۵ درجهٔ سلسیوس، رطوبت نسبی ۶۰ الی ۷۰ درصد و شرایط نوری ۱۶ ساعت روشنایی و ۸ ساعت تاریکی است. هم‌چنین در این مطالعه به بررسی تاثیر مستقیم کاربرد قارچ بیمارگر روی کنه شکارگر *Phytoseiulus persimilis* در شرایط آزمایشگاهی پرداخته شد. کنه‌های بالغ شکارگر به جدایه *F* (۱/۹۷±۳۵ درصد مرگ و میر) نسبت به جدایه *J.B.* (۱/۳۳±۲۷/۲۵ درصد مرگ و میر) حساس‌تر بودند. افزون بر این، زنده‌مانی کنه شکارگر بعد از پاشش قارچ بیمارگر روی دیسک‌های برگ لوبیا در سه بازه زمانی؛ ۰، ۲۴ و ۴۸ ساعت پس از پاشش قارچ، تفاوت معنی‌داری را نشان نداد. پژوهش‌های بیشتری برای رهاسازی هم‌زمان دو عامل کنترل بیولوژیک برای به دست آوردن تاثیر بیشتر در گلخانه در کنترل آفت با کمترین اثر بیماری‌زایی روی کنه شکارگر لازم است.

واژگان کلیدی: مهار زیستی؛ مرگ و میر؛ اثرات تخم‌کشی؛ اثر جانبی؛ کنهٔ تارتن دو لکه‌ای.

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