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Potential synergism between *Beauveria bassiana* and ether-extract of *Ginkgo biloba* for control of the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae)

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Abstract: The entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuill. is one of the important arthropod pathogens that can play an important role in the regulation of mite populations in agricultural ecosystems. In this study, the combined effect of a native isolate of fungus *B. bassiana* (EUT105) and ether-extract of plant, *Ginkgo biloba* was evaluated against the two-spotted spider mite, *Tetranychus urticae* Koch. At first, effect of three concentrations 5, 10 and 20% of *G. biloba* was evaluated on *B. bassiana* conidial germination and mycelial growth. Then, potential synergism between *B. bassiana* and *G. biloba* was investigated in order to incorporate both in the control of this pest. Only the 20% concentration of plant extract had significantly inhibitive effect on germination and mycelial growth. The mortality of adult female *T. urticae* increased significantly when *B. bassiana* was applied with 5 and 10% concentrations of *G. biloba* extract. Hence, there is a synergistic effect between this native *B. bassiana* and ether-extract of *G. biloba* in controlling the two-spotted spider mite.

Keywords: *Beauveria bassiana*, *Tetranychus urticae*, ether-extract, *Ginkgo biloba*, synergism

Introduction

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is a serious cosmopolitan pest species commonly found on many horticultural and agricultural crops (Bolland *et al*., 1998). Resistance development of *T. urticae* due to the high frequency of acaricide applications led to use alternative strategies and control methods such as using biocontrol agents (Maniania *et al*., 2008). Biological control, including the use of enthomopathogenic fungi, as a part of an integrated pest management (IPM) strategy is expected to reduce the dependence on synthetic acaricides (Wekesa *et al*., 2006). Among these fungi, *Beauveria bassiana* (Bals.) Vuill. may play a major role in the regulation of spider mite populations (Irigaray *et al*., 2003; Wekesa *et al*., 2006; Maniania *et al*., 2008; Bugeme *et al*., 2008; Seiedy *et al*., 2010) and could be used in biological control programs, either as a stand-alone solution in replacement of synthetic acaricides that are currently in use, or as a component of integrated mite management (Maniania *et al*., 2008).

The use of plant-derived chemical compounds for control of spider mites has

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also increased because they are safe for human health and environmental ecosystems (Kumral *et al*., 2009). *Ginkgo biloba* L. (Ginkgoaceae) is a plant with medicinal properties and insecticidal activity too (Kwon *et al*. 1996; Ahn *et al*. 1997; Sun *et al*. 2006). The acaricidal activity of *G. biloba* has been evaluated by Pan *et al*. (2006) on *Panonychus citri* McGregor (Acari: Tetranychidae) and Tork (2011) on *T. urticae*.

A combination of entomopathogenic fungi with plant-based insecticides may provide a more sustainable pest management strategy. Therefore, it is necessary to determine the compatibility of plants extract with entomopathogenic fungi (Sahayaraj *et al*., 2010). There are few studies in this field for example Depieri *et al*. (2005) evaluated compatibility of *B. bassiana* with extracts of Neem and Sahayaraj *et al*. (2010) investigated effect of some extracts of plants and commercial botanicals on conidial germination and mycelial growth of this fungus.

In this research, we demonstrated compatibility of a native isolate of *B. bassiana* with ether-extract of *G. biloba* for their simultaneous application to control *T. urticae*.

Material and Methods

Host plant

The host-plant, bean (*Phaseolus vulgaris* L.) was grown in plastic pots (8 cm in diameter and 8 cm in height) in a greenhouse at 25 ± 3 ºC, 65 ± 20% RH and a photoperiod of 16:8 (L : D).

Mite

Tetranychus urticae population used for the bioassays was collected from a glasshouse in Karaj and reared on host-plant at 25 ± 1 ºC; 70 ± 5% RH and 16: 8 (L : D) photoperiod for several generations before conducting any experiment.

Fungus

A native strain of *B. bassiana* (EUT105, an isolate provided by Entomology section at the University of Tehran) (soil origin) was grown on Sabouraud-Dexterose-Agar (SDA) and maintained at 25 ± 1 °C, 70 ± 5 % RH, and a photoperiod of 16:8 (L : D). Cultures were after sporulation and conidia were obtained through the method of Goettel and Inglis (1997).

Extract of *G. biloba*

Fruits of G. *biloba* were collected from Mazandaran Province in the north of Iran. For extraction, seed kernels removed and dried at were room temperature. Then they were ground into powder using electrical blender, 50 gs dried powder were immersed in 110 ml ethanol on a shaking device at 110 rpm for 144 h, filtrated three times and then the extract was concentrated using a rotary evaporator to one third of the initial volume. This process was repeated three times, ethanol extract was placed into a decanter and 500 ml petroleum ether was added and mixed per 200 ml extract. When two phases were separated, heavier phase containing ethanol extract was removed from the bottom of the container. Ether extract's volume was reduced to one fifth using a rotary evaporator.

Effects of *G. biloba* on *B. bassiana* conidial germination and mycelial growth

SDA medium was autoclaved at 120 °C for 20 min., cooled to 40 ± 5°C and amended with 0.5 g/l streptomycin. Preparing concentrations of *G. biloba* (5, 10 and 20%), these ratios were added to cooled SDA medium. These media were directly inoculated with 150 µl of a conidial suspension of *B. bassiana* containing 10^6 conidia/ml. Twenty four hours post-incubation, percentages of germinated conidia were estimated using a light microscope. Three replicates were considered for each treatment. Control was ether added SDA medium to which ether was added. For determining vegetative growth of fungus, a small plug of *B. bassiana* grown on SDA was placed at the

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center of each Petri dish. The linear growth in excess of the plugs was measured with a ruler at the four cardinal points from the plug on the 8th day following the treatment and the mean value was used in statistical analysis. Both experiments were repeated three times.

Potential synergism between *B. bassiana* and *G. biloba*

To evaluate the potential synergism between *B. bassiana* and *G. biloba*, four different treatments were used on female mites as: (1) *G. biloba*, (2) *B. bassiana*, (3) *G. biloba* + *B. bassiana*, and (4) Control with distilled water and 0.02 % Tween-80. The Concentrations were 5 and 10 % (as sub-lethal concentrations, unpublished data) for *G. biloba* and 10^7 conidia/ml (LC_{50} value, unpublished data) for *B. bassiana* on the female stage. Dry conidia of this isolate were suspended in 0.02 % Tween-80 in sterile distilled water and diluted to a concentration of 10^7 conidia /ml.

A 20-mm diameter leaf disc was placed on wet cotton wool in each 90-mm diameter Petri-dish and 10 adult females of *T. urticae* were placed on each leaf disc. The mites were sprayed with 1.5 ml of each, using a Potter tower (Burkard, UK) by 0.7 kg/cm^2 pressure and controls were treated only by distilled water and 0.02 % Tween-80. Each treatment consisted of three replicates for each concentration. The treated leaf discs were air-dried for 30 min., and then the Petri-dishes were covered. After 24 h, their covers were replaced by the new ones which had a hole of 3 cm in diameter. Petri-dishes were placed in an incubator at 25 ± 1 ºC, 70 ± 5% RH and 16:8h (L : D) photoperiod. Leaf discs were replaced by fresh ones every three days. Mortality was recorded for 7 days. The experiment was repeated twice and pooled data were used in statistical analysis.

Data analysis

Regression analysis was used to analyze the effect of *G. biloba* on *B. bassiana* germination and mycelial growth. Potential synergism between *B. bassiana* and *G. biloba* was tested by one-way analysis of variance (ANOVA) and means were separated by the F-LSD test ($\alpha = 0.05$). The SAS program was used in all cases (SAS Institute, 2002).

Results

Effects of *G. biloba* on *B. bassiana* conidial germination and mycelial growth

The mean percentage of germination for *B. bassiana* conidia at 0, 5, 10 and 20 % of *G. biloba* extract was 95, 94.1, 92.1 and 11.5 %, respectively (Fig. 1). Regression analysis indicated that differences among concentrations were significant ($F_{1, 34} = 57.2$, $P < 0.0001$).

Means for the linear growth of *B. bassiana* on SDA at 0, 5, 10 and 20 % of plant extract after eight days were 1.98, 1.9, 1.9 and 1.5 cm, respectively (Fig. 2). There were significant differences among these concentrations ($F_{1, 34} = 65.7$, $P < 0.0001$).

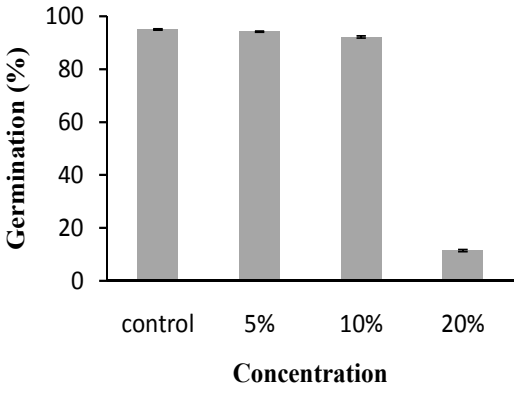

Figure 1 The effect of *Ginkgo biloba* extract concentrations on conidial germination of *Beauveria bassiana.*

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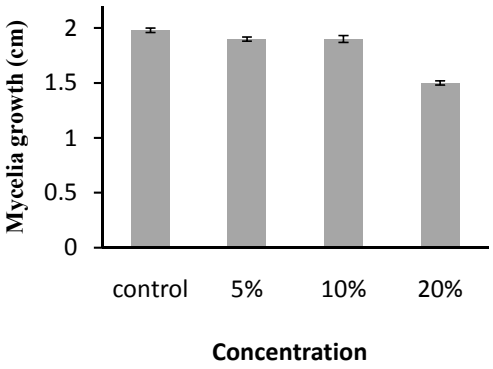

Figure 2 The effect of *Ginkgo biloba* extract concentrations on mycelial growth of *Beauveria bassiana*.

Potential synergism between *B. bassiana* and *G. biloba*

Mortality of mites in treatment *B. bassaina* + *G. biloba* was significantly ($F_{4, 25} = 148.10$, $P < 0.0001$) higher than those in *B. bassiana* or *G. biolba* alone and even higher than additive values of mortality data for *B. bassiana* and *G. biolba*. This result indicated that this plant extract had a synergistic effect on *B. bassiana* for control of *T. urticae* (Table 1).

Table 1 Mean mortality (± SE) of *T. urticae* sprayed with *B. bassiana*, *G. biloba* extract and *B. bassiana* + *G. biloba* extract.

Treatments	Percentage of mortality (Mean ± SE)
G. biloba 5 %	12.76 ± 2.81[a]
G. biloba 10 %	23.66 ± 2.81[b]
B .bassiana	50.93 ± 2.43[c]
G. biloba 5 % + *B. bassiana*	87.28 ± 3.35[d]
G. biloba 10 % + *B. bassiana*	94.55 ± 2.43[d]

Means followed by the same letter did not differ significantly at the 5 % level (ANOVA and F-LSD).

Discussion

Although entomopathogenic fungi have effective role in the management of many arthropod pests, their use will not supersede that of synthetic pesticides in all commercial production systems (Maniania *et al*., 2008). Nevertheless, their effect can be enhanced by the aid of compatible compounds, especially natural compounds that are harmless to agricultural systems. Conidial germination is a very important step in pest control programs by fungi because the beginning of epizootics is conditioned to the capacity of these structures to germinate on the host (Feng *et al*., 1994; De-Oliveira and Neves., 2004). The highest concentration (20 %) of *G. biloba* extract had extremely inhibitive effect on conidial germination and vegetative growth of *B. bassiana* but low and medium concentrations were compatible, conidial germination was about 90% and there was no harmful effect on vegetative growth. Rogerio *et al*. (2005) recorded compatibility of the Neem seed and leaf extracts with *B .bassiana* while Sahayaraj *et al*. (2010) showed *Annona squomosa* L. (Annonaceae) ethanol extract had high inhibiting growth activity on *B. bassiana.*

Inhibition of vegetative growth might be a less representative indication of fung toxicity than the viability of spores or the effect on germination (Loria *et al.,* 1983). There was no previous reports on either the concentration of extraets of G. biloba that would not be deleterious to B. bassiana or its synergists effects and compatibility with the fungus spores. This is the first report regarding the potential acaricidal synergism of *G. biloba* extract with a native isolate of an entomopathogenic fungus. When *B. bassiana* was applied in combination with 5 and 10% *G. biloba* extract, percentage mortality of *T. urticae* increased. Results showed that females *T. urticae* became more susceptible to fungal infection. Previous studies indicated that some compounds of this plant extract had serious erosive effect on the surface of mites' body (Pan *et al*., 2006; Tork 2011). As one of the important steps to successful fungal infection is penetration

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through the cuticle, erosive action of *G. biloba* could be one of the reasons for this synergistic effect. Other researchers also showed the enhancement of fungal infection with plant extracts; (Babu *et al*. (2001) studied the toxicity of Neem seed kernel extract (NSKE) and combination of NSKE and the entomopathogenic fungus *B. bassiana* on *Spodoptera littura* Fabricius (Lepidoptera: Noctuidae) under laboratory trials. They observed that the combination of NSKE and *B. bassiana* significantly increased the mortality over that of each treatment alone. Also Al-Mazraawi *et al*. (2009) reported that using sub-lethal doses of Neem tree extract with *B. bassiana* improved the control of *Thrips tabaci* Lindeman (Thysanoptera: Thripidae).

This study provides useful information on the compatibility between *B. bassiana* (EUT105) and a plant extract. Our results indicated that this native strain of *B. bassiana* can be used with appropriate concentration (5%) of *G. biloba* as a microbiological control agent to control *T. urticae*. Of course, these results are lab-based findings and should be tested under field conditions.

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توانایی تشدیدکنندگی بین قارچ *Beauveria bassiana* و عصاره اتری *Ginkgo biloba* برای کنترل کنه تارتن دولکه‌ای، *Tetranychus urticae*

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چکیده: قارچ بیمارگر حشرات *Beauveria bassiana* یکی از مهم‌ترین بیمارگرها در بندپایان است که می‌تواند نقش مهمی در تنظیم جمعیت کنه‌های گیاهی در کشاورزی داشته باشد. در این مطالعه، ترکیب اثر یک جدایه بومی از قارچ بیمارگر حشرات *B. bassiana* (EUT105) و عصاره اتری از گیاه *Ginkgo biloba* علیه کنه تارتن دولکه‌ای بررسی شد. در ابتدا اثر غلظت‌های ۵، ۱۰ و ۲۰٪ از *G. biloba* روی تندش کنیدی و رشد میسلیومی محاسبه شد. سپس توانایی تشدیدکنندگی بین قارچ *B. bassiana* و *G. biloba* برای کنترل هم‌زمان علیه این آفت سنجیده شد. فقط غلظت ۲۰٪ از عصاره اتری اثر بازدارندگی معنی‌داری روی رشد مسیلیومی داشت. زمانی که قارچ همراه با غلظت‌های ۵ و ۱۰٪ از عصاره استفاده شد، مرگ‌ومیر کنه‌های ماده به‌طور معنی‌داری افزایش پیدا کرد. بنابراین اثر تشدیدکنندگی بین این جدایه بومی از *B. bassiana* و عصاره اتری *G. biloba* برای کنترل کنه تارتن دولکه‌ای وجود دارد.

واژگان کلیدی: *Beauveria bassiana*، *Tetranychus urticae*، عصاره، *Ginkgo biloba*، تشدیدکنندگی

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