Pathogenicity of *Beauveria bassiana* to Fall Webworm (*Hyphantria cunea*) (Lepidoptera: Arctiidae) on Different Host Plants

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Abstract

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A study on the compatibility of the entomopathogenic fungus *Beauveria bassiana* with two medicinal plants, *Artemisia annua* (0.5%) and *Lavandula stoechas* (0.6%), was conducted against fall webworm, *Hyphantria cunea*, in the presence of three host plants including plane tree (*Platanus orientalis*), boxelder (*Acer negundo*), and mulberry (*Morus alba*). The highest concentration of *B. bassiana* yielded the highest *H. cunea* mortality in all three host plants. The combination of *B. bassiana* and plant extracts caused the highest *H. cunea* mortality in all host plants. The difference could be attributed to the nutritional effects of host plants on total physiological status of larvae. To prove this point, the digestive enzymatic assessments were studied and it was pointed out that a statistical difference of α-amylase, protease, and lipase activities exists among larvae feeding upon different host plants. Thus, a combined application of an entomopathogenic fungus and a botanical insecticide may be beneficial for the control of *H. cunea*.

Keywords: entomopathogenic fungi; *Artemisia annua*; *Lavandula stoechas*

The fall armyworm, *Hyphantria cunea* (Drury, 1770) (Lepidoptera: Arctiidae), is an insect native to North America which has been accidentally introduced to some areas of Europe and Asia (Warren & Tadic 1970). The insect has recently been introduced into Iran resulting in severe damages to trees and agricultural products. It is a multivoltine pest feeding on leaves of host plants and hibernates as pupae in soil around the damaged trees.

The use of chemical insecticides has created many problems such as environmental hazards, resistance in target species, and emergence of secondary pests (Holloman 1993). Wrong use of chemical insecticides can also be harmful to humans (Schwab 1989). One of the important components in pest management is biological suppression of insect pests by employing pathogens like viruses, fungi, bacteria, protozoa, and nematodes (Jayaraj 1986). As potential alternatives, certain chemicals derived either from plants or from microorganisms, termed biopesticides, have been promoted in recent years. These include the entomopathogenic fungi as well as the botanical insecticides.

The entomopathogenic fungus *Beauveria bassiana* (Bal.) Vuillemin (Ascomycota: Hypocreales) is widely regarded as one of the most promising species known for potential developments into practical insect biocontrol agents (Khachatourians 1986; Leathers & Gupta 1993).

Selection of entomopathogenic microorganisms is an important link in the use of effective bio-preparations for protection of plants from insect pests of agricultural crops (Serebrov *et al*. 2003). The fungus *Beauveria bassiana* has a great potential as a mycoinsecticide. In some cases, compatible products may be associated with entomopathogenic fungi to produce higher control efficiency while decreasing the amount of conventional insecticides and minimising risks of environmental contamination and insecticide resistance (Quintela & McCoy 1998). The entomopathogenic filamentous fungus *B. bassiana*

attacks many species of insects including the European corn borer, *Ostrinia nubilalis* (Hubner 1796) (Khachatourian 1986; Feng *et al*. 1994).

Host plants of phytophagous insects can significantly influence host susceptibility to entomopathogenic microorganisms in at least three different ways: (1) by affecting growth rates (Santiago-Álvarez & Ortiz-Garcia 1992), (2) through dietary stress (Mayer *et al*. 2002) or (3) through direct antimicrobial activity of the plant (Costa & Gaugler 1989b; Vega *et al*. 1997; Lacey & Mercadier 1998; Inyang *et al*. 1999a,b; Poprawski *et al*. 2000a,b; Klingen *et al*. 2002). In order to maximise the potentiality of *B. bassiana* as a component of integrated pest management of *H. cunea*, it is necessary to understand the effects of different environmental factors and agricultural practices on fungal efficacy. One of the most important factors that has received just little attention, is the effect of the host plant on the efficacy of *B. bassiana* for the control of insects. While Vidal *et al*. (1998) reported no effect of host plant on the susceptibility of whiteflies to *Paecilomyces fumosoroseous* (Wize), Bolckmans *et al*. (1995), Cuthbertson *et al*. (2005), and Santiago-Alvarez *et al.* (2006) showed an influence of the host plant on insect susceptibility to fungal infection.

The present paper has tried to throw some light on the combined effect of *B. bassiana* and plant extracts on *H. cunea* under the influence of different host plants. A detailed study of compatibility of different treatments of *B. bassiana* with plant extracts would help choosing optimal combination to improve IPM efficiency and enable growers to achieve a higher level of reliability and sustainability in pest management.

MATERIAL AND METHODS

Fungal isolate. Two isolates of *B. bassiana* EUT105 and *B. bassiana* EUT116 (provided by the Laboratory of Biocontrol and Biocoenology, University of Tehran, Iran) were used. These isolates were cultured on Sabouraud's dextrose agar (Merck, Darmstadt, Germany) with 1% yeast extract (SDAY) plates in several Petri dishes (9 cm in diameter), and were grown for 2–3 weeks at 25 ± 1°C under a 16 h/8 h (light/dark) photoperiod and 60 ± 5% RH. Suspension of inoculum was prepared by scraping conidia from the cultures into an aqueous solution of 0.02% Tween 80 (Quesada-Moraga *et al*. 2006).

Preparation of conidial suspensions. Surface of a 14-day-old culture was gently scratched with inoculation needle and transferred to vials containing 5 ml sterile Tween-80 solution (0.1% v/v). The concentration of conidia in stock suspensions was determined by direct count using Neubauer hemocytometer (Science Services GmbH, Munich, Germany). Serial dilutions (10^4–10^{10} conidia/ml) were prepared in sterile distilled water containing Tween 80 and preserved at 5°C until used in the bioassay. The viability of conidia was determined by plate count method on SDA (Sabouraud Dextrose Agar W/Chloramphenicol) (Goettel & Inglis 1997) and was found to be in the range of 90–95%.

Host plants. Host plants belonging to three different botanical families were used: plane tree (*Platanus orientalis* L.), mulberry (*Morus alba* L.), and boxelder (*Acer negundo* L.), from *Platanaceae*, *Moraceae*, and *Sapindaceae* families, respectively.

Insects. *H. cunea* larvae were collected from plane tree (*Platanus orientalis*) in Rasht (University of Guilan, Rasht, north of Iran). They were maintained under laboratory conditions at 27 ± 2°C under a 14 h/10 h (light/dark) photoperiod. These larvae were used to initiate the experiments on plane tree, mulberry, and boxelder and then subsequent generations were used for feeding assays.

Bioassay protocol. Fourth instar larvae were used for two bioassay procedures. Newly emerged larvae were allowed to feed on fresh plane tree, mulberry, and boxelder. Fresh leaves of respected trees were provided daily to the larvae in plastic boxes (15 cm × 15 cm × 15 cm). Leaf discs were changed daily and never allowed to dry or be eaten completely. Insects were maintained as in rearing condition. Leaf dipping and larval dipping were used for bioassay procedures. In leaf dipping method, leaf discs were immersed in a spore suspension (1.0×10^7 conidia/ml) for 10 s and control leaves were immersed in 0.02% Tween 80 instead. The treated and control leaf discs were then provided to the larvae. In larval dipping treatment, fourth instar larvae of *H. cunea* were dipped in the inoculums for 10 s and treated insects were carefully transferred to plastic boxes. The number of the dead larvae was recorded daily. Four replicate infested and treated leaf pieces were used for each combination of dose and host plants. The whole experiment was repeated twice.

***Combined effect of** Beauveria bassiana **and two plant extracts against** H. cunea*. Concentrations of *B. bassiana* and two plant extracts were selected from the individual trials based on the levels of mortality induced by *B. bassiana* and the two plants extracts. We used a concentration of 0.5 and 0.6% for *A. annua* and *L. stoechas*, respectively (based on the previous study by Zibaee *et al*. 2010), *B. bassiana* at 10^7 conidia/ml and a combination of these two treatments for two extracts. Three kinds of leaf discs from three host plants were used in each treatment. At first, leaf discs were dipped in each extract separately, and then the dipped discs were immersed in fungus suspension.

Digestive enzyme assays. Larvae were randomly selected and midguts were removed by dissection under a stereo microscope in ice-cold distilled water. To do so, larval bodies were cut separately by a scalper and midguts were dissected after removing fat bodies and other undesirable organs. They were rinsed in ice-cold distilled water, placed in a pre-cooled homogeniser, and grounded before centrifugation. Equal portions of larval midguts and distilled water were used to have a desirable concentration of the enzymes (w/v). Homogenates were separately transferred to 1.5 ml centrifuge tubes and centrifuged at 13 000 rpm at 4°C for 20 minutes. The supernatants were pooled and stored at 20°C for subsequent analyses.

α-Amylase activity was assayed by dinitrosalicylic acid (DNS) procedure (Bernfeld 1955), using 1% soluble starch (Merck, Darmstadt, Germany) as substrate. 20 µl of the enzyme were incubated at 35°C for 30 min with 100 µl of phosphate buffer (0.02M, pH 7.1) and 40 µl soluble starch. The reaction was stopped by addition of 100 µl DNS and heating in boiling water for 10 min prior to read absorbance at 540 nm. The boiling water stops the α-amylase activity and catalyses the reaction between DNS and starch. One unit of α-amylase activity was defined as the amount of enzyme required to produce 1 mg maltose at 35°C in 30 minutes.

The lipase assays were carried out as described by Tsujita *et al*. (1989). Twenty µl of gut extract and 40 µl of *p*-nitrophenyl butyrate (27mM) as substrate were incorporated in 100 µl of phosphate buffer (0.02mM, pH 7.1), mixed thoroughly, and incubated at 37°C. For negative control tubes, samples were placed in a boiling water bath for 15 min to destroy the enzymatic activity, and then cooled. After 1 min, 100 µl of NaOH (1M) were added to each tube (control and treatment) and absorbance was read at 405 nm. One unit of enzyme would release 1.0 nmol of *p*-nitrophenol per min at pH 7.2 and 37°C when *p*-nitrophenyl butyrate is used as substrate. Standard curve was used to calculate the specific activity of enzyme.

General proteases assay was performed by using hemoglobin (20 mg/ml) as substrate according to Cohen (1993) with slight modifications. 40 µl of hemoglobin solution were added to 100 µl of phosphate buffer (0.02mM, pH 7.1). Reactions were started by adding 40 µl of enzyme extract and incubating at 30°C for 120 minutes. For reaction termination, 100 µl of 30% trichloroacetic acid (TCA) were added to the reaction mixture. Precipitation was achieved by cooling at 4°C for 45 min and the reaction mixture was centrifuged at 13 000 rpm for 10 minutes. Appropriate blanks were used as described before.

Data analysis. POLO-PC software (LeOra 1987) was used to determine mortality and lethal concentration. All data were compared by one-way ANOVA followed by Tukey's studentised test when significant differences were found at $P \leq 0.05$ (SAS 1997). Differences between samplings were considered statistically significant at 5% probability.

RESULTS

B. bassiana and plant extracts treatments used individually as well as together affected larval mortality. Significantly different effects on mortality were observed among different incorporated dosages of *B. bassiana* on *H. cunea* after 11 days. Plant extracts used in this study similarly showed significant mortality, however there was no significant difference between the two fungi isolates used. Different hosts studied influenced the effect of pathogen and the plant extracts used (Figure 1). According to our study, the highest mortality of *B. bassiana* on individual application was 76% on Boxelder plant with insect dipping method for EUT105 isolate and the lowest on plane tree with leaf dipping method for EUT106 isolate. Mortality on mulberry and boxelder was higher than on plane tree. Regarding the integrated effect of *Beauveria bassiana* and plant extracts, mortality of larvae increased due to fungal suspension and plant extracts so that the highest mortality belonged to the combination of *B. bassiana* with *A. annua* on boxelder host plant (90%) in insect dipping method (Figure 2).

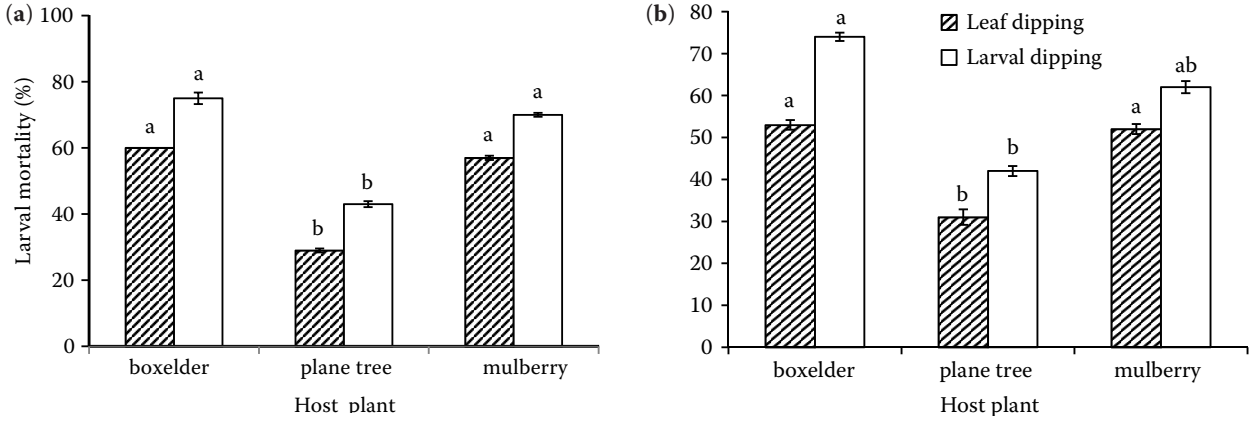

Figure 1. Mean larval corrected mortality of *Hyphamtria cunea* fed on different host plants and exposed to 10×10^7 conidia/ml of *Beauveria bassiana* isolates (a) EUT 105 and (b) EUT 106. The vertical bars represent the standard error of the mean. Within the same treatment and host plant species, mortality data are significantly different

Feeding on different host plants significantly changed the activity of three major digestive enzymes, namely α-amylase, protease, and lipase (Figure 4). α-Amylase assay revealed the highest activity in the larvae fed on plane tree and the lowest one in the larvae fed on boxelder. Similar results were found in the case of protease activity but in lipase assay the highest activity was observed in boxelder and plane tree and the lowest one in mulberry (Figure 4).

DISCUSSION

In this study, individual and combined effects of *B. bassiana* and plant extracts caused increasing mortality of *H. cunea* larvae in a dose- and time-dependent manner. Observed results correspond to previous studies on the impact of *B. bassiana* on caterpilars. In our study, the highest mortality (76%) was observed for 10^7 conidia/ml suspension of *B. bassiana* with insect dipping method. In another isolate of *B. bassiana* (EUT106), mortality of *H. cunea* increased significantly with time and was also significantly affected by the host plant on which the larvae were reared (Santiago-Alvarez *et al.* 2006). *A. annua* and *L. stoechas* treatments increased mortality of *H. cunea* and the highest mortality belonged to *A. annua* in boxelder and mulberry whereas the highest mortality for *L. stoechas* was 67% in host. Integrated combination of *B. bassiana* with plant extracts yielded the highest mortality for *A. annua* and *L. stoechas* on *H. cunea* in insect dipping method whereas in leaf dipping method, the highest mortality was observed in *L. stoechas* and *A. annua* alone, respectively. This difference reveals that plant extracts decrease efficiency of *B. bassiana* in leaf dipping treatment but in insect dipping treatment, the highest mortality was observed. We conclude that this difference resulted from

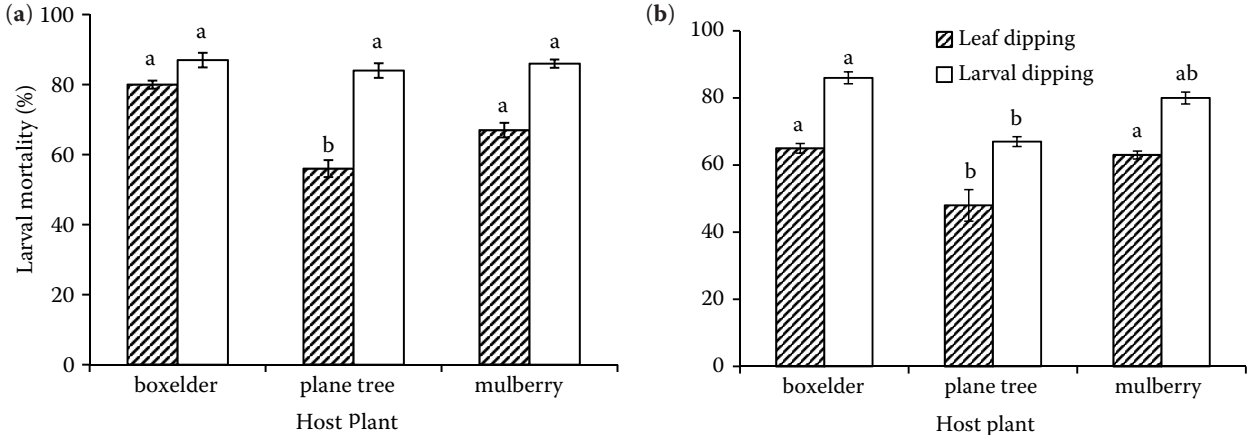

Figure 2. Combined effect of *B. bassiana* spores and *A. annua* extracts on mortality of *H. cunea* larvae. Columns show mean ± SE and statistical differences were marked by different letters

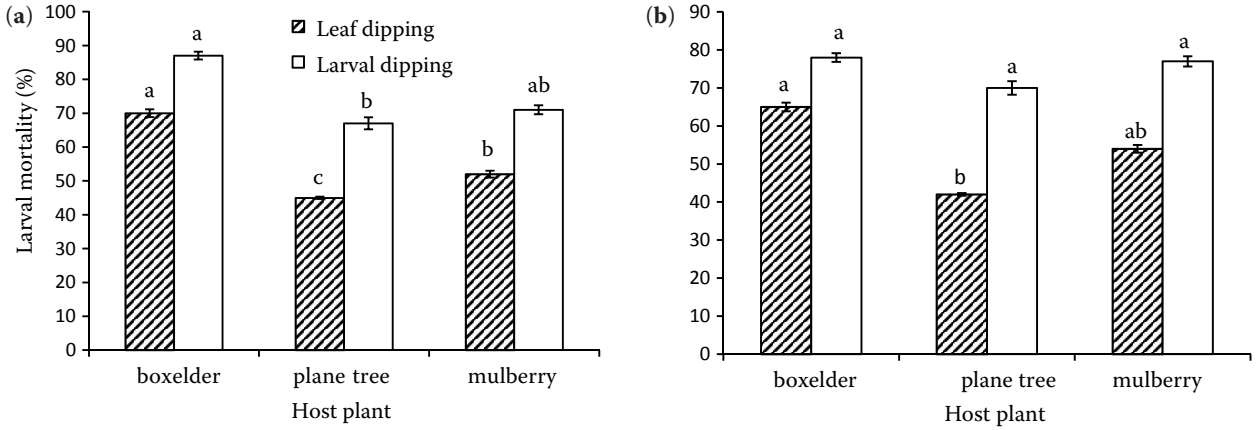

Figure 3. Combined effect of *B. bassiana* spores and *L. stoechas* extracts on mortality of *H. cunea* larvae. Columns show mean ± SE and statistical differences were marked by different letters

direct contact of extracts with *B. Bassiana* and fungi on the host leaves.

Combination of *B. bassiana* with some other materials like endosulfan has been worked out and a 4.9 times greater toxicity against *Spilarctia oblique* has been observed than by endosulfan used alone (Purwar & Sachan 2006). Dayakar *et al*. (2000) found that the combination of insecticides with *B. bassiana* showed a 1.05- and 1.24-fold increase in virulence against *Spodoptera litura* (Fab.) over the sole treatment. Variation in toxicity response of the entomopathogenic fungus *B. bassiana* has also been reported, from synergistic, antagonistic, or neutral to insecticides (Pevling &Weyrich 1992; Mietkiewski & Gorski 1995), fungicides (Olmert &Kenneth 1974), and botanicals (Gupta *et al*. 1999). We have found out that the combination of *B. bassiana* with plant extracts resulted in a greater *H. cunea* mortality than individual treatments of *B. bassiana* and plant extracts, respectively. The correct combination of fungal entomopathogen and a botanical insecticide could be useful for the management of *H. cunea* in different host plants.

In addition, we have found that pathogenicity of *B. bassiana* and two medicinal plant extracts on *H. cunea* is highly dependent on host plants. To our knowledge, there are only few studies on the effect of host plant on the susceptibility of phytophagous insects to fungal infection, and there are great differences in the results obtained. and Hare and Andreadis (1983) found significant differences in susceptibility of *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) to *B. bassiana* when reared on four different Solanaceae species. Bolckmans *et al*. (1995) demonstrated that *P. fumosoroseus* was more efficacious against nymphs of *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae) on cucumber than on tomato. However, cotton, cabbage, eggplant, and bean had no effect on the susceptibility of *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae) to *Nomurea riley* (Farlow) (Fargues & Maniania 1992). Similarly, potato and tomato had no effect on the susceptibility of *Leptinotarsa decemlineata* (Say) to *B. bassiana* (Costa & Gaugler 1989a) and cucumber, cabbage, and tomato had no ef-

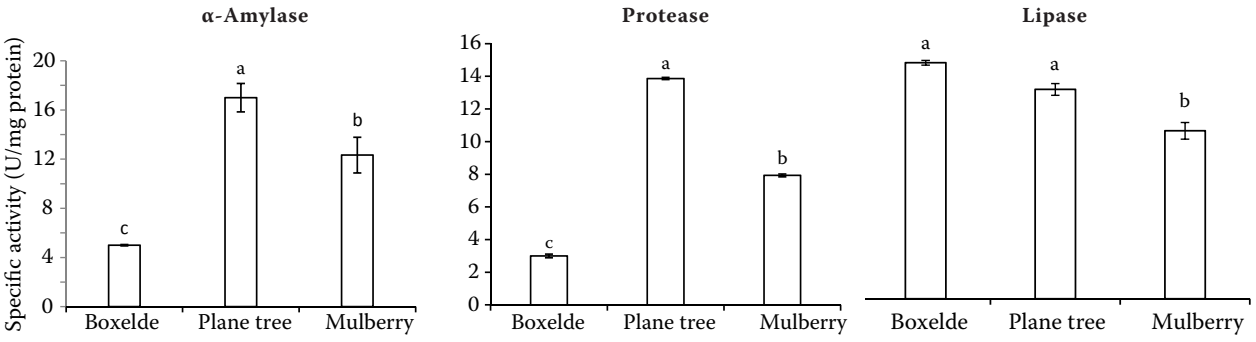

Figure 4. Effect of different host plants on digestive enzymatic activity in the larvae of *H. cunea*. Different letters show the statistical differences among treatments (Tukey, $P \leq 0.05$)

fect on the susceptibility of *Bemisia argentifolii* to *P.fumosoroseous* (Vidal *et al*. 1998). Compared with these studies, our investigation is the first study on efficacy of *B. bassiana* on various host plants.

Insect nutrition is a very important factor regulating the susceptibility of insects to entomopathogens (Steinhaus 1958; Vago 1963). This factor may influence interaction between insect and entomopathogens in several different ways. Inadequate nutrition often leads to increased susceptibility to entomopathogens (Santiago-Alvarez & Ortiz-Garcia 1992), and the use of host plants inducing nutritional stress can substantially enhance efficacy of entomopathogens. *H. cunea* on specific host plants. In the present study mulberry and boxelder make the insect more susceptible to fungal infection in comparison to plane tree which corresponds to the findings of various authors (Bolckmans *et al.* 1995; Hare & Anderadis 1983; Naranjo *et al.* 2003). Host plants can also influence the susceptibility of insect pests to entomopathogenic fungi being secondary plant metabolites. Secondary plant metabolites can inhibit fungal growth and germination (Costa & Gaugler 1989b; Vega *et al*. 1997; Lacey & Mercadier 1998; Inyang *et al*. 1999a,b; Poprawski *et al*. 2000a,b; Klingen *et al*. 2002). Tomatine, a glycoalkaloid produced by many cultivars of tomato, is known to cause inhibition of *P. fumosoroseus* and *B. bassiana* (Costa & Gaugler 1989b; Lacey & Mercadier 1998; Poprawski *et al*. 2000). In this study plane tree was among the host plants causing the highest reduction in mortality of larvae by *B. bassiana.* This is in accordance with the study of Poprawski and Jones (2000) who showed that populations of whiteflies reared on cotton were significantly less susceptible to the infection by *P. fumosoroseus* and *B. bassiana* when reared on melon. In another study, crucifer epicuticular wax has also been shown to have a high fungistatic effect (Inyang *et al*. 1999b). The exact role which secondary compounds can play in the infection process of entomopathogenic fungi is poorly understood. Vega *et al*. (1997) suggested that germination of fungal conidia that come in contact with allelochemicals on the leaf surface, on the insect cuticle, or in the hemolymph could be inhibited. This hypothesis is close to the present study that plane tree caused less mortality on larvae. Antifungal compounds present on the insect host surface or sequestered by the host as a defense against the fungal entomopathogen might have been involved in the inhibition of the fungus at the third trophic level.

In conclusion, we may say that in addition to the many factors known to affect susceptibility of insects to fungal pathogens (Inglis *et al*. 2001), variation among host plant species may also modify the susceptibility of insect pest to entomopathogenic fungi by changing the insect nutrition requirements. This study shows that combination of *B. bassiana* and plant extracts, compared to individual application, increases the effectiveness of control measures. Furthermore, the infectious potential of *B. bassiana* isolates EUT105, EUT106 and effects of plant extracts were affected by the host plant species. So, the utilization and combination of *B. bassiana* and mentioned plant extracts for controlling *H. cunea* is adaptable which should be complemented with further bioassays and in semifield applications. However, further study is required, too.

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