Acta Biologica 27/2020 | www.wnus.edu.pl/ab | DOI: 10.18276/ab.2020.27-14 | strony 149–160

Endophytic colonization of tomato by *Beauveria bassiana* for control of the greenhouse whitefly, *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae)

Eliza Pourtaghi,[1] Reza Talaei-Hassanloui,[2] Fatemeh Nasibi,[3] Khalil-Berdi Fotouhifar[4]

[1] Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran
[2] Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran, ORCID: 0000-0002-2960-7135
[3] Department of Biology, Faculty of Science, Shahid Bahonar University of Kerman
[4] Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran

Corresponding author e-mail: rtalaei@ut.ac.ir

Keywords colonization, endophyte, entomopathogenic fungus, leaf spray, Whitefly

Abstract We evaluated the efficacy of four different methods for endophytic inoculation of entomopathogenic fungus *Beauveria bassiana* in tomato plants. Fourteen days after inoculation, root dipping and leaf spraying allowed recovery of *B. bassiana* from leaves, while the fungus was recovered from roots in all inoculation methods, except soil drenching. Significant increases in mortalities of nymphs and adults of the greenhouse whitefly, 36 to 52%, were recorded on *B. bassiana*-endophyt plants. The total phenolic and protein contents of tomato plants were increased by endophyte colonization. Results of this study confirm the efficiency of foliar spraying of *B. bassiana* for colonization in tomato plants and its insecticidal activity against whitefly. The persistence of *B. bassiana* as endophyte was confirmed up to 56 days after inoculation. Given the insecticidal activity and beneficial effects of the endophyte on plant growth, *B. bassiana* could be considered as a suitable element in integrated pest management.

Endofityczna kolonizacja pomidora przez Beauveria bassiana w celu zwalczania mączlika szklarniowego, *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae)

Słowa kluczowe kolonizacja; endofit; grzyb entomopatogenny; dolistny aerosol; mączlik

Streszczenie Oceniliśmy skuteczność czterech różnych metod endofitycznej inokulacji entomopatogennego grzyba *Beauveria bassiana* do pomidorów. 14 dni po zaszczepieniu stwierdzono obecność *B. bassiana* w liściach w przypadku inokulacji poprzez zanurzanie korzeni i oprysk liści, natomiast obecność grzyba w korzeniach stwierdzono przy wszystkich metodach inokulacji, z wyjątkiem zwilżania gleby. Na pomidorach zainfekowanych *B. bassiana* odnotowano znaczny wzrost śmiertelności nimf i osobników dorosłych mączlika szklarniowego, wynoszący od 36 do 52%. Całkowita zawartość fenoli i białka w tkankach pomidora wzrosła w wyniku kolonizacji endofitów. Wyniki badań potwierdzają skuteczność oprysku dolistnego *B. bassiana*

w kolonizacji pomidora i jego owadobójcze działanie na mączlika szklarniowego. Trwałość *B. bassiana* jako endofitu została potwierdzona do 56 dni po inokulacji. Biorąc pod uwagę aktywność owadobójczą i korzystny wpływ endofitu na wzrost roślin, *B. bassiana* można uznać za odpowiedni element zintegrowanej ochrony roślin przed szkodnikami.

Introduction

Fungal endophytes inhabit the internal tissues of a plant for at least part of their life cycle without causing any apparent symptoms in the host (Wilson, 1995). These endophytes have been reported to benefit plant health and performance by enhancing growth, protecting plant tissues against pathogens, parasitic nematodes, and herbivorous pests, and direct translocation of nitrogen from insect cadavers to the host plant (Saikkonen et al., 2004; Behie, Bidochka, 2014; Vega, 2018). The endophytic association of fungi has been reported in many plants, including some important agricultural crops such as wheat, soybeans, maize, and tomatoes (Vega et al., 2008; McKinnon et al., 2018).

Beauveria bassiana (Balsamo) Vuillemin (Hypocreales: Cordycipitaceae) is a soil borne fungus that attacks an extensive host range of insects at all stages of development (Ownley et al., 2004). In addition to insecticidal activity, *B. bassiana* has been found to protect plants against certain plant pathogens (Ownley et al., 2004; 2008) as well as to function as a biofertilizer (Vega, 2018; Afandhi et al., 2019). Different isolates of *B. bassiana* have been reported as naturally occurring endophytes in a variety of plant species such as tomato, potato, maize, cotton, cucumber, melon, and rice (Vega, 2008; Brownbridge et al. 2012; Vidal, Jaber, 2015). The insecticidal and antimicrobial activity of endophytic *B. bassiana* has been well documented in some previous studies (Cherry et al., 2004; Ownley et al., 2008; Gurulingappa et al., 2010; Akello, Sikora, 2012). *B. bassiana* has been successfully inoculated into tomato plants using different application methods and their insecticidal activity has been confirmed against two chewing-tunneling lepidopteran pests (*i.e.* the tomato fruitworm, *Helicoverpa armigera* (Hübner) (Lep: Noctuidae) and the tomato leafminer, *Tuta absoluta* (Meyrick) (Lep: Gelechiidae) (Qayyum et al., 2015; Allegrucci et al., 2017).

In this study, we evaluated the efficacy of different methods for establishment of *B. bassiana* as endophyte in tomato plants. Then, the *in planta* efficiency of fungal endophyte against a sucking insect, greenhouse whitefly *Trialeurides vaporariorum*, Westwood (Hem: Aleyrodidae), was investigated under greenhouse conditions. As whitefly management programs are currently dependent on the use of chemical insecticides (Schlaeger et al., 2018) on the freshly consumed tomato in most countries, our results could be important in considering alternative pest control methods.

Materials and methods

Plant

Seeds of tomato plants, *Solanum lycopersicum* L. cv. Falat, were cultivated in plastic pots (15 cm height, 20 cm diameter) filled with a mixture of soil, peat and perlite. Plants were grown in a research greenhouse (26 ±3°C, 60 ±10% RH, and 16L:8D photoperiod) and a nutrient solution (NPK) was applied according to manufacturer recommendations.

Fungus

Beauveria bassiana TV isolate, was obtained from the Biological Control Laboratory at Department of Plant Protection, University of Tehran (Karaj, Iran). The virulence of this isolate has been previously reported against insect species in the laboratory (SeyedTalebi et al., 2016; Kosari et al., 2016). The fungus was cultured on Sabouraud dextrose agar (SDA) medium and kept in a growth chamber at 25 ±1°C, 70 ±10% RH, and 16L:8D photoperiod. For preparation of fungal suspension, the conidia of *B. bassiana* were collected from 14-day-old cultures, suspended in distilled water, and filtered through sterile filter paper in a Buchner funnel. The conidia were quantified using a hemocytometer and a light microscope. The required fungal suspension (10^8 conidia ml^{-1}) was adjusted with regards to conidial viability according to the method described by Goettel and Inglis (1997).

Insect

Trialeurodes vaporariorum was collected from tomato greenhouses in Karaj County (Alborz Province, Iran) and maintained on tomato plants. The whiteflies were kept into wooden cages (60 × 40 × 40 cm) covered by net in a growth chamber at 26 ±1°C, 70 ±10% RH, and 16L:8D photoperiod.

Plant inoculation

Tomato plants were treated with *B. bassiana* concentration 10^8 conidia ml^{-1} using four inoculation methods; seed dressing with fungal suspension, rhizosphere inoculation, foliar application of conidial suspension, and soil drenching. For all inoculation methods, tomato seeds were immersed in 0.5% sodium hypochlorite for 1 min and washed in sterile distilled water for one min. The seeds were then surface sterilized by placing them in 70% ethanol for 2 min, and rinsed two times in sterile distilled water for one min. Treated seeds were placed on sterile paper towels to dry for 30 min. For seed inoculation, the treated seeds were immersed in 10 ml of the conidial suspension of *B. bassiana*. After 48 hours of incubation, the seeds were sown in pots in the greenhouse with above-mentioned condition. For rhizosphere and foliar application methods, tomato seeds were individually sown in plastic pots and the 14-day old seedlings were used for inoculation. For rhizosphere inoculation, 10 ml of the conidial suspension was added to the soil around tomato stems. For foliar application, tomato leaves were sprayed with 10 ml of the fungal suspension using a glass hand sprayer. To avoid conidial runoff to the soil, the top of each pot was covered with aluminium foil. In soil drenching method, 50 ml of the conidial suspension was added to one kg of soil in pots and one tomato seed was sown in each pot after 24 hours of inoculation. In control, tomato leaves were sprayed with 10 ml of distilled water.

Evaluation of endophytic establishment

For all application methods, the colonization of tomato plants by *B. bassiana* was evaluated 7 and 14 days after inoculation. For this purpose, the plants were removed from the soil and their root was washed with distilled water. For each plant, one gr of roots and one gr of leaves were cut and surface-disinfected by immersion in 0.5% sodium hypochlorite for 2 min, followed by one min in distilled water, and 2 min in 70% ethanol and rinsed with sterile distilled water three times in a laminar flow cabinet. The samples were then dried on sterile paper towels for 30 min.

Subsamples of the surface sterilized leaves and roots were placed on SDA media to ensure that residual conidia have not retained their germination potential. The samples were finely crushed in 10 ml distilled water and one ml of the resultant extract was spread over dishes containing SDA media. The dishes were daily checked and the number of fungus colonies was determined after 14 days of incubation at 25°C. A total of 8 plants were used for each application method. After determination of the most efficient technique, this experiment was repeated to explore the changes in frequency of fungal colonies at days 7, 14, 21, 28, and 56 following inoculation. This experiment was also performed with four replicates.

Insecticidal effect

After selection of the most efficient inoculation method based on the number of colonies on foliar and root samples, the insecticidal activity of endophytic *B. bassiana* against *T. vaporariorum* adults was evaluated using laboratory bioassays. To explore the capability of *B. bassiana* to systemically colonize the entire plant and protect the plant parts other than the treated parts, two spraying procedures (*i.e.* spraying of the fungal suspension on either a single leaf or the whole plant) were applied. For this purpose, tomato plants were sprayed with 10 ml of the fungal suspension using hand sprayer either thoroughly or on one randomly selected leaf. In control, the plants were treated with the same volume of distilled water. After 7 days of plant inoculation, treated plants were placed in cages covered by insect net and 15 pairs of adult *T. vaporariorum* were released on each plant. After 48 hours of oviposition, adult whiteflies were removed and an equal number of eggs was retained on each plant. The plants were then maintained under controlled conditions in a greenhouse and the mortality of different life stages of was recorded until adult emergence. The percentage of egg mortality was determined by dividing the number of unhatched eggs by the total egg number. The early (first and second) and late (red-eye) nymphal stage mortality was estimated by counting the number of dead nymphs in each life stage. The percentage of adult mortality was estimated by knowing the number of emerged pupa and the total number of pupae. The dead whiteflies were incubated at room temperature and monitored for presence of mycosis. All bioassays were performed with 4 replicates.

Plant biochemical changes

The effect of endophytic colonization of tomato plants by *B. bassiana* on total phenols and total protein content of leaves was evaluated in either absence or presence of whitefly infestation. Tomato plants were sprayed with 10 ml of the fungal suspension using glass hand sprayer. In control, the plants were treated with the same volume of distilled water. On the seventh day after fungal inoculation, half of the plants in both control and treated groups were exposed to 15 pair of adult *T. vaporariorum* for 48 hours to provide a total of four treatments including i) endophyte-free plants without whitefly infestation, ii) endophyte-free plants with whitefly infestation, iii) plants with endophyte, but without whitefly infestation, and iv) plants with both endophytic fungi and whitefly infestation. Twenty-four days after whitefly infestation, the leaves were removed and freeze-dried at –80°C for further analyses. Assessment of total phenolic content was performed according to the Folin-Ciocalteu method (Slinkard, Singleton, 1977) with slight modifications. Briefly, 0.1 gr of the freeze-dried samples were homogenized in 5 mL of 95% EtOH and incubated at darkness for 24 hours. The mixture was then centrifuged at 4,000 rpm for 10 min and the clear supernatant was collected as crude extract. One hundred µl of the crude extract were mixed with 0.2 ml of Folin-Ciocalteu reagent and 2 ml of distilled water. After 3 min, the reaction

was neutralized by addition of 1 ml of sodium carbonate (Na_2CO_3) (15%) and incubating at room temperature for 2 hours. The absorption was read at 765 nm in a spectrometer. Gallic acid was used as a standard and the total phenolic content was expressed as gallic acid equivalents (GAE) in $mg \cdot g^{-1}$ of fresh weight.

To measure the amount of protein, proteins were extracted from the foliar part of the plant at 0 to 4°C on ice. For this purpose, one gram of plant freeze dried tissue in a mortar containing three milliliters of buffer phosphate of (50 milimolar, pH = 7.2), containing ethylene diamine tetra-acetic acid (EDTA), phenyl methane sulfonyl fluoride (PMSF), and polyvinyl pyrrolidone (PVP) 1% were shed. The extract was placed in refrigerated centrifuge for 4 minutes at 4,000 g and 4°C for 15 minutes. A supernatant solution was used to study the protein assay (a solution of each sample was stored in several microtubes and stored at –80°C). The total protein content was measured according to the procedure described by Bradford (1976). To test tubes, 0.1 ml of protein extract, 5 ml of biuret was added and rapidly vortexed. After two minutes, the absorption was read at 595 nm in a spectrometer. The protein concentration was calculated using bovine serum albumin. Protein content was expressed as $mg.g^{-1}$ of fresh weight.

Data analysis

Statistical analyses for data of assays were performed using ANOVA in SAS software version 9.1 (SAS Institute, 2002). For mean comparisons, F-LSD test was used as post-ANOVA. T-test was used for two-sapmle comparisons. Percentages data were normalized by arcsine square root transformation before being analyzed.

Results and discussion

Endophytic establishment

Seven days after inoculation by all techniques, no colonization was obtained in roots, while the establishment of *B. bassiana* in leaf tissues was confirmed only in plants treated with foliar spraying, but not other inoculation techniques (Table 1).

Table 1. The frequency of *Beauveria bassiana* colonies (±SE) recovered from roots and leaves of tomato plants 7 and 14 days after inoculation with four different techniques

Inoculation method	Leaf tissue		Root tissue	
	day 7	day 14	day 7	day 14
Control	00.00 ±00.00^{b}	00.00 ±00.00^{c}	00.00 ±00.00^{a}	00.00 ±00.00^{c}
Soil drenching	00.00 ±00.00^{b}	00.00 ±00.00^{c}	00.00 ±00.00^{a}	00.00 ±00.00^{c}
Seed inoculation	00.00 ±00.00^{b}	00.00 ±00.00^{c}	00.00 ±00.00^{a}	8.50 ±0.25^{b}**
Root inoculation	00.00 ±00.00^{b}	15.00 ±3.48^{b}**	00.00 ±00.00^{a}	37.75 ±5.76^{a}**
Leaf spraying	2.25 ±0.63^{a}	133.75 ±25.93^{a}*	00.00 ±00.00^{a}	30.50 ±5.36^{a}**

Different letters within each column show significant differences at $P < 0.05$ level (LSD test); * and ** show significant difference between day 7 and 14 of each inoculation technique and each tissue sample at $P < 0.05$ and $P < 0.01$ levels, respectively (*t*-test).

Two weeks after inoculation, endophytic colonization of the fungus was detected in plant roots when they were inoculated using foliar spraying, seed treatment and rhizosphere inoculation. Similarly, foliar spraying and rhizosphere inoculation resulted in successful establishment of *B. bassiana* in leaf tissues 14 days after inoculation. These results are in consistent with previous studies indicating that inoculated fungi have the potential to move across the vascular tissues and systemically colonize the entire plant (Wagner, Lewis, 2000; Mantzoukas et al., 2015). The highest frequency of fungal colonies (133 CFU/ml) was obtained using foliar spraying at day 14 after inoculation, followed by rhizosphere inoculation (37 CFU/ml) and foliar spraying (30 CFU/ml) at day 7 after inoculation, respectively (Table 1). No evidence of fungal establishment was detected in soil drenching method and control (Table 1). Consistent with these results, Allegrucci et al. (2017) reported significant difference in endophytic colonization of *B. bassiana* in tomato plants when they used different techniques (leaf spraying, root dipping, and seed inoculation), with the highest efficiency was obtained from leaf spraying. However, these authors detected the highest percentage of colonization at day 7 post inoculation that was different from our current study. In another study, Afandhi et al. (2019) reported that the endophytic colonization of *B. bassiana* in common bean plants varies significantly depending on inoculation technique, parts of plant from which samples are taken (root, stem, or leaves), and the time elapsed after inoculation. Similarly, it was shown in coffee plants that foliar sprays favors leaf colonization whereas soil drenches favors root colonization (Parsa et al., 2013). A variety of factors, such as plant species/cultivar, fungal species/isolate, plant age at the time of inoculation, inoculation method, concentration of the inoculum, tissue specificity of endophytic fungus, and plant growing condition, have been argued to affect the capability of a fungus to establish as an endophyte (Parsa et al., 2013; Qayyum et al., 2015; Afandhi et al., 2019).

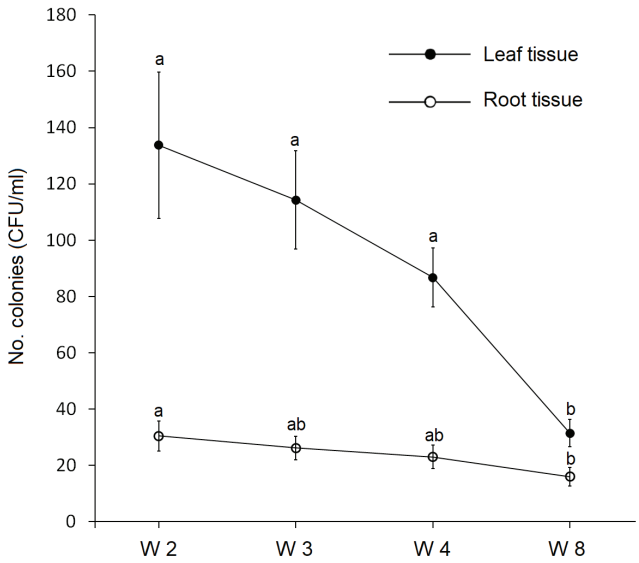

Figure 1. The frequency of *Beauveria bassiana* colonies (±SE) recovered from roots and leaves of tomato plants 2, 3, 4, and 8 weeks after foliar spraying; different letters on each curve show significant differences among weeks at $P < 0.05$ level (F-LSD test)

We used the leaf spraying technique to study the changes in frequency of fungal colonization in leaf and root tissues at days 7, 14, 21, 28, and 56. As expected from the previous experiment, the root and leaf tissues hosted 00.00 and 3.11 CFU/ml of fungal colony at day 7. The frequency of fungal colonies in both root and leaf samples maximized at week 2 and exhibited a descending pattern towards week 8 (Figure 1).

Consistent with these findings, the results of previous studies indicate that fungal colonization following foliar spraying maximizes between days 7 and 14 and decreases over time (Russo et al., 2015; Allegrucci et al., 2017; Afandhi et al., 2019). In different studies, *B. bassiana* has been shown to retain its endophytic association for up to three months with citrus lemon (Bamisile et al., 2019), jute plants (Biswas et al., 2012) and maize (Renuka et al., 2016), up to nine months with pine trees (Brownbridge et al., 2012), and up to eight months with coffee (Posada and Vega, 2006). A possible explanation for declines in fungal colonization over time can be the competition with other fungi and bacteria in the system, resulting in inhibition of *B. bassiana* growth (Russo et al., 2015).

Insecticidal effect

Significant differences were found in mortality percentages of whiteflies among different life stages and treatments. Although, the mortality rates of different life stages were not statistically different in control, an ascending pattern in mortality was observed by increase in whitefly age, with the highest mortality rate for the adult stage (Figure 2).

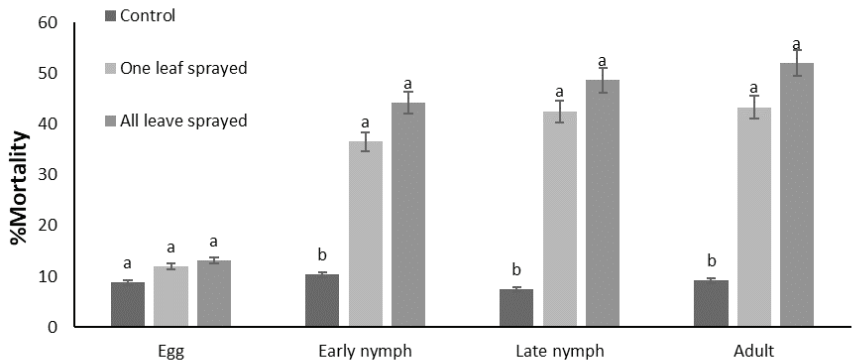

Figure 2. Mortality percentage of different life stages of *Trialeurodes vaporariorum* when reared on endophyte-free tomato plants (control) or plants sprayed with *B. bassiana* suspension on either a single leaf or the entire plant; different letters show significant difference among treatments in the same life stage at $P < 0.05$ level (F-LSD test)

The egg mortality was not affected by fungal inoculation. However, a significant increase in mortality of nymphal and adult stages was recorded on plants inoculated with *B. bassiana* (Figure 2). Inoculation of the entire plant with fungal suspension caused a higher mortality in all developmental stages of *T. vaporariorum* in comparison with inoculation of a single leaf, though the difference was not statistically significant (Figure 2). Garrido-Jurado et al. (2017) showed that the nymphal mortality of the sweet potato whitefly *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) ranges between 84–100% in melon leaves that are directly sprayed with *B. bassiana* suspension, but decreases (66–88%) in leaves of the same plant that are not directly sprayed. The mortality of nymphal and adult stages ranged from 36 to 52% in plants inoculated with *B. bassiana*. No mycosis was detected on any of dead whiteflies. These results are in line with some previous studies, indicating that endophytic fungi can cause mortality in herbivorous insects not only by direct contact of the conidia with the target insect, but also by expressing a variety of fungal metabolites either *in planta* or on plant surfaces (Arnold, 2008; Gurulingappa et al., 2011; Garrido-Jurado et al., 2017; Bamisile et al., 2019).

Plant biochemical changes

We measured the total phenolic and protein contents of tomato leaves following endophytic colonization by *B. bassiana* and/or infestation with *T. vaporariorum*. The results revealed significant increase in both phenolic and protein content of leaves following endophyte colonization regardless of whitefly infestation (Figure 3). The highest contents of both phenols and proteins were detected in plants simultaneously inoculated with fungal endophyte and infested with whiteflies. In absence of fungal endophyte, whitefly infestation had no significant effect on phenolic content; however, a significant increase in the total protein content was recorded following whitefly infestation (Figure 3). These results may highlight the promoting effect of fungal endophytes on protein and phenolic content of plants, which may be intensified following herbivore attack. In agreement with this results, previous studies also reported increased accumulation of compounds such as reducing sugar, total flavonoids, total phenolic compounds, trans-resveratrol, as well as increased activity of enzymes such as phenylalanine ammonia-lyase following endophyte colonization (Shoresh, Harman, 2008; Gomez-Vidal et al., 2009; Yang et al., 2018).

Compounds such as alkaloids, terpenoids, proteins, flavonoids, and phenols may be produced *in planta* either directly by endophytes themselves (Gunatilaka, 2006; Ownley et al., 2010) or indirectly by influencing on host plant (Shoresh, Harman, 2008; Gomez-Vidal et al., 2009; Gasoni, Gurfinkel, 2009). For example, colonization of date palm trees (*Phoenix dactylifera* L.) by *B. bassiana* and some other endophytic fungi resulted in increased production of proteins involved in plant defense, stress response, energy metabolism and photosynthesis (Gomez-Vidal et al., 2009). Different proteins were expressed following colonization by the fungal endophyte *Trichoderma harzianum* Rifai in maize (Shoresh, Harman, 2008) which were involved in carbohydrate metabolism, defense, and photosynthesis. In another study, the total phenlic content of 15-day-old cotton seedlings was reported to be about two times higher than that of 5-day-old seedlings following colonization by the endophytic fungus, *Cladorrhinum foecundissimum* Sacc. & Marchal (Gasoni, Gurfinkel, 2009).

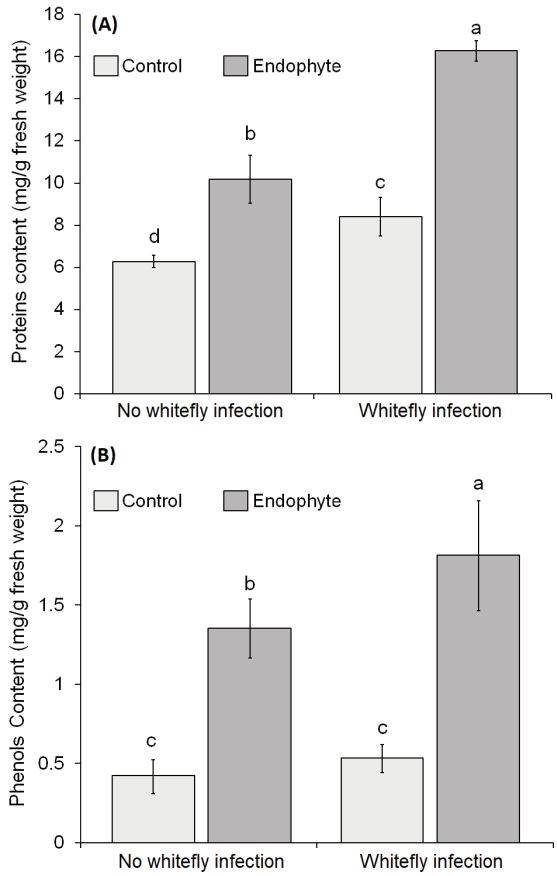

Figure 3. Effect of endophytic colonization by *Beauveria bassiana* or/and infestation by *Trialeurodes vaporariorum* on the total protein (A) and total phenol (B) content of tomato leaves; different letters show significant differences at $P < 0.05$ level (F-LSD test)

Conclusions

The successful establishment of *B. bassiana* (isolate TV) as an endophyte in tomato plant (cv. Falat) and its negative effects on different developmental stages of *T. vaporariorum* were reported for the first time in this study. Additionally, the fungal endophyte was found to promote the total phenolic and protein contents of tomato plants, especially when the plants were infested by *T. vaporariorum*. The leaf spraying was considered as the most efficient inoculation technique and fungal colonization was confirmed up to 56 days after inoculation. The persistence of entomopathogenic fungus as endophyte in plant tissues provides an opportunity to include these beneficial organisms in pest management programs (Lewis et al., 1996; Jaber et al., 2018; Gonzalez-Mas et al., 2019) and can be also used in integrated management programs of both insect pests and plant diseases (Ownley et al., 2008; Bamisile et al., 2019). Future studies may

focus on exploring the mechanisms underlying the systemic effect of endophytic *B. bassiana* on *T. vaporariorum* as well as assessing the potential sub-lethal effects of the endophyte on life parameters of the whitefly.

Acknowledgement

Financial support by College of Agriculture and Natural Resources, University of Tehran of this project No. 73132800/6/28 and technical help by Dr. A.A. Kosari are acknowledged.

References

Afandhi, A., Widjayanti, T., Emi, A.A.L., Tarno, H., Afyanti, M., Handoko, R.N.S. (2019). Endophytic fungi *Beauveria bassiana* Balsamo accelerates growth of common bean (*Phaeseolus vulgaris* L.). *Chem. Boil. Technol. Agric*, *6*, 11. DOI: 10.1186/s40538-019-0148-1.

Akello, J., Sikora, R. (2012). Systemic acropedal influence of endophyte seed treatment on *Acyrthosiphon pisum* and *Aphis fabae* offspring development and reproductive fitness. *Biol. Control*, *61*, 215–221.

Allegrucci, N., Velazquez, M.S., Russo, M.L., Perez, E., Scorsetti, A.C. (2017). Endophytic colonisation of tomato by the entomopathogenic fungus *Beauveria bassiana*: the use of different inoculation techniques and their effects on the tomato leafminer *Tuta absoluta* (Lepidoptera: Gelechiidae). *J. Plant Prot. Res*., *57*, 331–337.

Arnold, A.E. (2008). Endophytic fungi: hidden components of tropical community ecology. Tropical Forest Community Ecology (pp. 254–271), S.A. Schnitzer, W.P. Carson (eds). New York: John Wiley & Sons.

Bamisile, B.S., Dash, C.K., Akutse, K.S., Qasim, M., Aguila, L.C.R., Wang, F., Keppanan, R., Wang, L. (2019). Endophytic *Beauveria bassiana* in foliar-treated citrus limon plants acting as a growth suppressor to three successive generations of *Diaphorina citri* Kuwayama (Hemiptera: Liviidae). *Insects*, *10*, 176. DOI: 10.3390/insects10060176.

Behie, S.W., Bidochka, M.J. (2014). Ubiquity of insect-derived nitrogen transfer to plants by endophytic insect-pathogenic fungi: an additional branch of the soil nitrogen cycle. Appl. Environ. *Microbiol*., *80*, 1553–1560.

Biswas, C., Dey, P., Satpathy, S., Satya, P. (2012). Establishment of the fungal entomopathogen *Beauveria bassiana* as a season long endophyte in jute (*Corchorus olitorius*) and its rapid detection using scar marker. *BioControl*, *57*, 565–571.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem*., *72*, 248–254.

Brownbridge, M., Reay, S.D., Nelson, T.L., Glare, T.R. (2012). Persistence of *Beauveria bassiana* (Ascomycota: Hypocreales) as an endophyte following inoculation of radiata pine seed and seedlings. *Biol. Control*, *61*, 194–200.

Cherry, A.J., Banito, A., Djegui, D., Lomer, C. (2004). Suppression of the stem-borer *Sesamia calamistis* (Lepidoptera; Noctuidae) in maize following seed dressing, topical application and stem injection with African isolates of *Beauveria bassiana*. *Int. J. Pest Manage.*, *50*, 67–73.

Garrido-Jurado, I., Resquín-Romero, G., Amarilla, S., Ríos-Moreno, A., Carrasco, L., QuesadaMoraga, E. (2017). Transient endophytic colonization of melon plants by entomopathogenic fungi after foliar application for the control of *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae). *J. Pest Sci*., *90*, 319–330.

Gasoni, A.B., Gurfinkel, B.S. (2009). Biocontrol of *Rhizoctonia solani* by the endophytic fungus *Cladorrhinum foecundissimum* in cotton plants. *Australas. Plant Pathol*., *38*, 389–391.

Goettel, M.S., Inglis, G.D. (1997). Fungi: Hyphomycetes. In: L.A. Lacey (ed.), *Manual of Technique of Insect Pathology* (pp. 213–249). London: Academic Press.

Gomez-Vidal, S., Salinas, J., Tena, M., Lopez-Llorca, L.V. (2009). Proteomic analysis of date palm (Phoenix dactylifera L.) responses to endophytic colonization by entomopathogenic fungi. *Electrophoresis*, *30*, 2996–3005.

Gonzalez-Mas, N., Cuenca-Medina, M., Gutiérrez-Sánchez, F., Quesada-Moraga, E. (2019). Bottom-up effects of endophytic *Beauveria bassiana* on multitrophic interactions between the cotton aphid, *Aphis gossypii*, and its natural enemies in melon. *J. Pest Sci*., *92*, 1271–1281.

Gunatilaka, A.A. (2006). Natural products from plant-associated microorganisms: distribution, structural diversity, bioactivity, and implications of their occurrence. *J. Nat. Prod*., *69*, 509–526.

Gurulingappa, P., Sword, G.A., Murdoch, G., McGee, P.A. (2010). Colonization of crop plants by fungal entomopathogens and their effects on two insect pests when *in planta*. *Biol. Control*, *55*, 34–41.

Gurulingappa, P., Mcgee, P.A., and Sword, G. (2011). Endophytic *Lecanicillium lecanii* and *Beauveria bassiana* reduce the survival and fecundity of *Aphis gossypii* following contact with conidia and secondary metabolites. *Crop Prot*., *30*, 349–353.

Jaber, L.R., Araj, S.E., Qasem, J.R. (2018). Compatibility of endophytic fungal entomopathogens with plant extracts for the management of sweetpotato whitefly *Bemesia tabaci* Gennadius (Homoptera: Aleyrodidae). *Biol. Control*, *117*, 164–171.

Kosari, A.A., Sahragard, A., Talaei-Hassanloui, R. (2016). Effect of host plant on the virulence of entomopathogenic fungus *Beauveria bassiana* against *Tetranychus urticae* and predatory bug *Orius niger*. *Biol. Control Pest Plant Dis*., *5* (2), 129–138.

Lewis, L.C., Berry, E.C., Obrycki, J.J., Bing, L.A. (1996). Aptness of insecticides (*Bacillus thuringiensis* and carbofuran) with endophytic *Beauveria bassiana*, in suppressing larval populations of the European corn borer. *Agr. Ecosys. Environ*., *57*, 27–34.

Mantzoukas, S., Chondrogiannis, C., Grammatikopoulos, G. (2015). Effects of three endophytic entomopathogens on sweet sorghum and on the larvae of the stalk borer *Sesamia nonagrioides*. *Entomol. Exp. Appl*., *154*, 78–87.

McKinnon, A.C., Glare, T.R., Ridgway, H.J., Mendoza-Mendoza, A., Holyoake, A., Godsoe, W.K., Bufford, J.L. (2018). Detection of the entomopathogenic fungus *Beauveria bassiana* in the rhizosphere of wound-stressed *Zea mays* plants. *Front. Microbiol*., *9*, 1161.

Ownley, B.H., Gwinn, K.D., Vega, F.E. (2010). Endophytic fungal entomopathogens with activity against plant pathogens: ecology and evolution. *BioControl*, *55*, 113–128.

Ownley, B.H., Griffin, M.R., Klingeman, W.E., Gwinn, K.D., Moulton, J.K., Pereira, R.M. (2008). *Beauveria bassiana*: Endophytic colonization and plant disease control. *J. Invertebr. Pathol*., *98*, 267–270.

Ownley, B.H., Pereira, R.M., Klingeman, W.E., Quigley, N.B., Leckie, B.M. (2004). *Beauveria bassiana*, a dual purpose biocontrol organism, with activity against insect pests and plant pathogens, 255–269. In: R. Lartey, A. Caesar, *Emerging Concepts in Plant Health Management* (pp. 255–269). Research Signpost, Kerala, India.

Parsa, S., Ortiz, V., Vega, F.E. 2013. Establishing fungal entomopathogens as endophytes: towards endophytic biological control. *J. Vis. Exp*., *74*, e50360.

Posada, F., Vega, F.E. (2006). Inoculation and colonization of coffee seedlings (*Coffea arabica* L.) with the fungal entomopathogen *Beauveria bassiana* (Ascomycota: Hypocreales). *Mycoscience*, *47*, 284–289.

Qayyum, M.A., Wakil, W., Arif, M.J., Sahi, S.T., Dunlap, C.A. (2015). Infestation of *Helicoverpa armigera* by endophytic *Beauveria bassiana* colonizing tomato plants. *Biol. Control*, *90*, 200–207.

Renuka, S., Ramanujam, B., Poornesha, B. (2016). Endophytic ability of different isolates of entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuillemin in stem and leaf tissues of maize (*Zea mays* L.). *Indian J. Microbiol*., *56*, 126–133.

Russo, M.L., Pelizza, S.A., Cabello, M.N., Stenglein, S.A., Scorsetti, A.C. (2015). Endophytic colonisation of tobacco, corn, wheat and soybeans by the fungal entomopathogen *Beauveria bassiana* (Ascomycota, Hypocreales). *Biocontrol Sci. Technol*., *25*, 475–480.

Saikkonen, K., Wali, P., Helander, M., Faeth, S.H. (2004). Evolution of endophyte–plant symbioses. *Trends. Plant Sci*., *9*, 275–280.

SAS Institute (2002). SAS/STAT Software. version 9.1. SAS Institute Inc., Cary, NC, USA.

Schlaeger, S., Pickett, J.A., Birkett, M.A. (2018). Prospects for management of whitefly using plant semiochemicals, compared with related pests. *Pest Manag. Sci*., *74*, 2405–2411.

SeyedTalebi, F., Safavi, A., Talaei-Hassanloui, R., Bandani, A. (2016). Study of the virulence and conidial germination types for some *Beauveria bassiana* isolate. *Biol. Control Pest Plant Dis*., *7* (1), 65–73.

Shoresh, M., Harman, G.E. (2008). The molecular basis of shoot responses of maize seedlings to *Trichoderma harzianum* T22 inoculation of the root: a proteomic approach. *Plant Physiol*., *147*, 2147–2163.

Slinkard, K., Singleton, V.L. (1977). Total phenol analysis: Automation and comparison with manual methods. *Am. J. Enol. Vitic*., *28*, 49–55.

Vega, F.E. (2008). Insect pathology and fungal endophytes. *J. Invertebr. Pathol*., *98*, 277–279.

Vega, F.E. (2018). The use of fungal entomopathogens as endophytes in biological control: a review. *Mycologia*, *110*, 4–30.

Vega, F.E., Posada, F., Aime, M.C., Pava-Ripoll, M., Infante, F., Rehner, S.A. (2008). Entomopathogenic fungal endophytes. *Biol. Control*, *46*, 72–82.

Vidal, S., Jaber L.R. (2015). Entomopathogenic fungi as endophytes: plant-endophyte-herbivore interactions and prospects for use in biological control. *Curr. Sci*., *109*, 46–54.

Wagner, B.L., Lewis, L.C. (2000). Colonization of corn, *Zea mays* by the entomopathogenic fungus *Beauveria bassiana*. *Appl. Environ. Microbiol*., *66*, 3468–3473.

Wilson, D. (1995). Endophyte: the evolution of a term, and clarification of its use and definition. *Oikos*, *73*, 274–276.

Yang, M.Z., Huang, L.H., Ao, X.J., Ren, A.Y., Yuan, M.Q., Zhang, H.B. (2018). Endophytic Fungal strains Specifically Modified the Biochemical Status of Grape Cells. J. *Plant Biol*., *61*, 210–216.

Cite as: Pourtaghi, E., Talaei-Hassanloui, R., Nasibi, F., Fotouhifar, K.-B. (2020). Endophytic colonization of tomato by *Beauveria bassiana* for control of the greenhouse whitefly, *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae). *Acta Biologica*, *27*, 149–160. DOI: 10.18276/ab.2020.27-14.