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ARTICLE

Effects of endophytism by *Beauveria bassiana* (Cordycipitaceae) on plant growth, *Fusarium* (Nectriaceae) disease, and Sunn pest *Eurygaster integriceps* (Hemiptera: Scutelleridae) in wheat (Poaceae)

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Abstract
Fungi living within a plant (*i.e.*, endophytic fungi) can directly affect the growth of the host plant or have indirect effects by affecting levels of damage to the plant caused by insect herbivory or plant pathogens. Greenhouse and field trials were conducted to investigate the endophytic effects of the fungus *Beauveria bassiana* on wheat, on the wheat-feeding Sunn pest, *Eurygaster integriceps* (Hemiptera: Scutelleridae), and on the wheat pathogen, *Fusarium culmorum* (Nectriaceae), which causes root and crown rot disease. Conidia of *B. bassiana* were applied to wheat either by foliar spray, soil drench, or seed treatment. Seed treatment with conidia provided the highest re-isolation percentages: 91.7% in leaves, 95.8% in stems, and 91.7% in roots. Inoculated plants were taller and had greater wet and dry weights compared to control plants. In both greenhouse and field studies, *E. integriceps* that were fed inoculated plants laid eggs that were less likely to hatch. Inoculations reduced the incidence of root and crown rot disease by 42% under greenhouse conditions. These results document the potential of using endophytic infections of *B. bassiana* in wheat as a control measure for Sunn pest and root and crown rot disease.

Introduction

Beauveria bassiana (Balsamo) Vuillemin (Cordycipitaceae), a fungus long known for its entomopathogenic properties, causes an insect disease known as white muscardine. Although *B. bassiana* initially was considered only an insect pathogen, it recently has been reported as an endophyte (Jaber and Ownley 2018; Vega 2018). An "endophyte" is an organism that lives inside a plant without causing any apparent symptoms during the plant growth cycle (Wilson 1995; Akello and Sikora 2012).

Although the efficiency of entomopathogenic fungi is influenced by environmental factors such as ultraviolet radiation, low moisture, and temperature (Lacey *et al.* 2015; Jaronski and Mascarin 2017), using *Beauveria* as an endophytic has solved those barriers and demonstrates more cost-efficient strategies to host plants. The reason mainly is that by establishing inside plants, the fungi are protected from environmental abiotic stresses, and this is accompanied

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by longer persistence and less inoculum needed to colonise the host (Mascarin and Jaronski 2016).

Among the entomopathogenic fungi that naturally colonise plants, *B. bassiana* has been reported as an endophyte from a broad range of plants (Vega 2008; Dara 2019). *Beauveria bassiana* has been established as an endophyte in citrus (Rutaceae) (Bamisile *et al.* 2020), coconut seedling (Arecaceae) (Posada and Vega 2005; Gaviria *et al.* 2020), artichoke (Asteraceae) (Guesmi-Jouini *et al.* 2014), banana (Musaceae) (Akello *et al.* 2008a, 2008b), corn (Poaceae) (Wagner and Lewis 2000), sorghum (Poaceae) (Reddy *et al.* 2009; Tefera and Vidal 2009; Mantzoukas *et al.* 2015), and tomato (Solanaceae) (Klieber and Reineke 2015). The endophytic activity of different isolates of *B. bassiana* and their effects on plant growth, height, and tissue nutrient content, the composition of induced plant defensive compounds, and plant volatiles have been previously reported (Tefera and Vidal 2009; Mantzoukas *et al.* 2015; Shrivastava *et al.* 2015; Jaber and Enkerli 2016; Renuka *et al.* 2016; Jaber and Ownley 2018; Sánchez-Rodríguez *et al.* 2018; Tall and Meyling 2018; Vega 2018; Moloinyane and Nchu 2019; Bamisile *et al.* 2020).

The presence of endophytic *B. bassiana* in treated host plants has been effective in controlling pests under lab conditions (Bing and Lewis 1991; Cherry *et al.* 2004; Vega *et al.* 2009; Lopez and Sword 2015). Several studies have reported the impact of endophytic fungi on survival, fitness, development, and fecundity of sap-sucking herbivores (Gurulingappa *et al.* 2010; Akello and Sikora 2012; Lopez *et al.* 2014; Muvea *et al.* 2014; Jaber *et al.* 2018). These studies suggest that the application of endophytic fungi can be integrated with other pest management strategies to provide effective insect pest control. Endophytic fungi also can colonise the rhizosphere and confer protection to the host plant (Ownley and Gwinn 2010; Greenfield *et al.* 2016; Jaber and Ownley 2018) against plant pathogens such as *Rhizoctonia solani* (Ceratobasidiaceae), *Pythium myriotylum* (Pythiaceae) (Ownley *et al.* 2008), *Plasmopara viticola* (Peronosporaceae) (Rondot and Reineke 2019), *Pythium ultimum* (Pythiaceae), *Sphaerotheca fuliginea* (Erysiphaceae), *Fusarium solani* (Nectriaceae) (Jaber and Ownley 2018), and *Magnaporthe grisea* (Magnaporthaceae) (Atugala and Deshappriya 2015).

Wheat (Poaceae) production in the Near and Middle East, Eastern Europe, and North Africa is affected by the Sunn pest, *Eurygaster integriceps* (Hemiptera: Scutelleridae) (Critchley 1998; Hosseininaveh *et al.* 2009; Talaei-Hassanloui *et al.* 2009; Davari and Parker 2018) and the plant pathogen *Fusarium culmorum* (Nectriaceae) (Motallebi *et al.* 2015). Nymphs and adults of *E. integriceps* feed on leaves, stems, and grains (Critchley 1998; Hosseininaveh *et al.* 2009), reducing crop yield. Insects feeding on developing kernels inject salivary hydrolytic enzymes that break down gluten to reduce bread quality (Hosseininaveh *et al.* 2009; Koksel *et al.* 2009). Fungal diseases – namely root and crown rot caused by *F. culmorum* – threaten wheat production in different areas of the world (Grosu *et al.* 2015; Meyer-Wolfarth *et al.* 2017). *Fusarium culmorum* symptoms appear as brown necrotic discolouration, root browning, and necrosis, resulting in stand loss, reduced yield, and decreased grain quality. It can also spread throughout a plant's crown and infect the stems (Beccari *et al.* 2011).

In the present study, we assessed the potential for a native *B. bassiana* isolate for use as an endophyte in wheat to enhance plant growth and confer protection against Sunn pest and *F. culmorum*. We first examined methods to optimise endophytic establishment of the isolate in host plants. We then assessed the direct effect of the endophyte on plant growth. In greenhouse and field studies, we subsequently tested the effect of inoculations on the egg hatch of Sunn pest and the incidence of root and crown disease.

Materials and methods

Fungal isolates and inoculum

Beauveria bassiana TV, isolated from soil in Karaj County, Alborz, Iran in 2017 (Seyedtalebi *et al.* 2017, 2020), was obtained from the collection of entomopathogenic fungi at the Biological

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Control Laboratory, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran. The fungus was cultured on Sabouraud dextrose agar (dextrose (40 g), peptone (10 g), agar (15 g), and distilled water (1 L)) with 1% yeast extract in Petri plates and kept in the dark at 25 ± 1 °C and 65 ± 10% relative humidity for 14 days. Conidia were harvested by scraping the surface of each culture with a sterile spatula and were transferred into a sterilised 100-mL glass beaker containing 50 mL of 0.01% Tween 80 (Merck, Darmstadt, Germany; Gurulingappa *et al.* 2010). The conidial suspension was stirred, filtered, and adjusted to 1×10^7 conidia mL^{-1}, which was used to inoculate the plants and soil, as described below. The viability of conidia was assessed following the method described by Parsa *et al.* (2013).

Fusarium culmorum isolated from wheat in Varamin County, Iran, in 2003 was obtained from the Iranian Research Institute of Plant Protection, Tehran, Iran, cultivated on potato dextrose agar, and kept at 25 ± 1 °C and 65 ± 10% relative humidity in darkness for seven days. The *F. culmorum* inoculum for infecting the test plants was prepared as described by Chekali *et al.* (2011).

Greenhouse experiments

Fungal treatments. Seeds of the wheat cultivar "Pishtaz" were obtained from the Gene Bank, Department of Agronomy and Plant Breeding, University of Tehran, Karaj, Iran. In order to study the possible establishment of *B. bassiana* isolates in wheat, different inoculation methods – soil drench, foliar spray, seed soaking in conidial suspension, and seed treatment in conidial powder – in two sterile *versus* nonsterile conditions were applied. The soil drench and foliar spray treatments were carried out according to Parsa *et al.* (2013), with slight modification. Briefly, 15 mL of fungal suspension at a concentration of 1×10^7 conidia mL^{-1} were used to drench the soil, and 10 mL of the same were used to spray the leaves (foliar spray). The wheat foliage was sprayed using a manual atomiser at 14 days after planting. For the foliar and soil sterile treatments and control, the seeds were surface sterilised before fungal inoculation by immersion in sodium hypochlorite (2% v/v) for two minutes followed by one wash with sterile distilled water, immersion in ethanol (70% v/v) for two minutes, and then three rinses with sterile distilled water. The sterilised seeds then were placed on sterile filter paper to dry, and 20 seeds were planted in each polyvinyl chloride pot (8 and 10 cm in diameter and height, respectively) in sterile soil (400 g per pot). In the nonsterile treatments, the wheat seeds were planted in polyvinyl chloride pots without sterilisation of seeds, soil, and water. In general, eight pots were considered for each treatment, with four pots for sterile treatments and four pots for nonsterile treatments.

For seed inoculation, wheat seeds (approximately 160 seeds) were treated either by soaking in the *B. bassiana* conidial suspension for 12 and 24 hours, depending on the seed-soaking-in-conidial-suspension treatment, or by inoculating the seeds with fungal conidial powder (6 mg, equivalent to 3.1×10^7 conidia/mL). For conidial powder inoculation of seeds, 0.1 g of fungal conidia powder was obtained by scraping the surface of each culture with a sterile spatula and was stirred with 160 seeds for two minutes in a microtube, and then the seeds were planted in eight pots. In the seed-soaking treatment, the inoculated seeds were transferred to an incubator at 25 °C ± 1 °C and 65% ± 5% relative humidity in darkness, dried on sterile tissue paper for 30 minutes under the laminar hood, and then planted in eight pots, as described below.

Re-isolation of *Beauveria bassiana* from tested plants. The presence of *B. bassiana* within the treated wheat plants was verified through fungal isolation from different parts of the plant and plant extract and through detection of *B. bassiana* DNA.

Following Parsa *et al.* (2013), 15 days after inoculation, four wheat plants were removed from each pot (each treatment consisted of eight pots, with four pots for sterile treatments and four pots

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for nonsterile treatments) and washed with running tap water for one minute. For each plant, two parts of the leaf (sampled randomly from the first true leaf), two parts of the root (sampled from the middle of the root and near the root tip), and two parts of the stem (3-cm-long samples taken from the middle of the stem length) were excised, surface sterilised in sodium hypochlorite (2% v/v) and ethanol (70% v/v), and washed three times with sterile distilled water. The six samples of plant material obtained from each plant were then plated together on a 90-mm Petri dish with Sabouraud dextrose agar medium supplemented with the antibiotics tetracycline and penicillin at 2 mg/L each. Plates were sealed with parafilm and incubated in the dark at 25 °C. Every 2–3 days for 2 weeks, fungal outgrowth was recorded and verified as *B. bassiana* morphologically, as described by Humber (1997). To ensure efficacy of the disinfection process, water samples of the last disinfection step were spread onto Sabouraud dextrose agar medium and incubated for 10 days at 25 °C. No fungal growth was observed in any of the last-rinse water from the surface-sterilisation procedure. Ultimate estimation of the presence of endophytic *B. bassiana* was recorded after 14 days and was calculated as colonisation percentage, per the formula:

$$Colonization\ percentage\ = \frac{Number\ of\ plant\ samples\ exhibiting\ fungal\ growth}{Total\ number\ of\ plated\ plant\ samples} \times 100$$

The intensity of endophytic establishment of *B. bassiana* TV applied through the different treatments was evaluated following Abdel-Hafez *et al.* (2015), with some modifications. Fifteen days after the inoculations, three pots were selected, and 10 plants were uprooted from each pot, with tissues separated into aerial (leaf and stem) and ground (root) parts. Ground parts (1 g) and the aerial parts (3 g) were surface sterilised, as described above, and added to sterilised distilled water (10 and 30 mL, respectively). The extract of the plant was prepared by blending the plant material in distilled water (1 mL) with a Waring® blender (Waring Commercial Products, McConnellsburg, Pennsylvania, United States of America) until the solution became homogeneous. The plant extract obtained from each pot was cultured on a Sabouraud dextrose agar medium supplemented with penicillin and tetracycline (2 mg/L each). The numbers of *B. bassiana* colonies were counted and recorded every 2–3 days for 2 weeks.

Detection of *Beauveria bassiana* by two-step nested polymerase chain reaction. To document the presence of infections in wheat plants inoculated with *B. bassiana*, plant tissues were further analysed with polymerase chain reaction. The fungal DNA and genomic plant DNA from aerial parts (leaf and stem) and roots were separately extracted by the cetyltrimethylammonium bromide method provided by Saghai-Maroof *et al.* (1984). Quantity and quality of DNA were determined by the spectrophotometric method, using Thermo Nanodrop (Thermo Fisher Scientific, Waltham, Massachusetts, United States of America) and agarose gel electrophoresis (1%). The amplification process of the first polymerase chain reaction was carried out in a reaction mixture (50 µL) containing universal primers ITS1-F (1 µM; Gardes and Bruns 1993) and ITS4 (1 µM; White *et al.* 1990), *Taq* DNA polymerase (1.0 unit), *Taq* polymerase buffer (1×), deoxynucleoside triphosphate (dNTP; 50 µM), and DNA template (100 ng/µL). The reaction was performed in a C1000 thermocycler (Bio-Rad, Hercules, California, United States of America). The first polymerase chain reaction products were used in two-step nested polymerase chain reaction protocols by designing *B. bassiana*–specific primers BB.fw (5′–GAACCTACCTATCGTTGCTTC–3′) and BB.rv (5′–ATTCGAGGTCAACG TTCAG–3′), as described by Landa *et al.* (2013). The nested polymerase chain reactions were conducted in 25-µL solutions of primers (1.0 µM each), reaction buffer (10×), dNTP (50 µM), DNA polymerase (1.5 units), magnesium chloride (1.5 mM), and the template DNA (1 µL of 20 ng/µL). Polymerase chain reaction was performed with the following cycling profile: 2 minutes of initial denaturation at 95 °C, followed by 35 cycles of amplification with 1 minute

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denaturation at 94 °C, 1 minute annealing at 65 °C, 1 minute extension at 72 °C, and an extra extension step of 5 minutes at 72 °C.

Analysis of plant growth index. To measure the growth index, at 15 days after inoculation of each treatment, wheat plants were removed from each of eight pots per treatment, washed with running tap water, and then dried. The height of the wheat plant from the base to the apical meristem was recorded. The wet weight and the dry weight of the whole plant after drying at 70 °C for 48 hours were also measured.

Sunn pest bioassay. The pathogenicity of the endophyte *B. bassiana* TV isolate was assessed against *E. integriceps*. Based on the results of the endophytic activity of *B. bassiana*, the best wheat plant inoculation method was used in the bioassay experiments. When the wheat plants began the heading stage, the inoculated plants were kept in a cylindrical steel cage (50 cm diameter × 23 cm height), with one opening on top and three openings on the sides covered with fine mesh for ventilation. In each cage, six females and four males of *E. integriceps* were placed on a pot of wheat plants for four days to mate and lay eggs. The insects were then removed, and the numbers of hatched and nonhatched eggs were counted to calculate the percentage of egg hatching. The numbers of nymphs were recorded. This process continued until adult eclosion.

Effect of endophytic *Beauveria bassiana* on Fusarium plant pathogen. Fourteen days after planting the *Beauveria*-inoculated seeds, soil from the crown of each plant was removed, and 4–5 seeds contaminated with *F. culmorum* were placed next to the crown. For controls, five oat (Poaceae) seeds without *Fusarium* inoculation were placed alongside the wheat plants. After two weeks, the emergence of *Fusarium* disease was assessed in the wheat plants that had been treated with *B. bassiana* and in controls, following Yuan *et al.* (2017). Briefly, a scale of 0–4 (0 = healthy plant or plant without symptoms; 1 = 1 – 33% of the plant affected; 2 = 34 – 66% of the plant affected; 3 = 67 – 99% of the plant affected; 4 = dead plant) was used to rank plants according to the percentage of plant tissue affected by chlorosis, leaf necrosis, or defoliation. Disease incidence was then calculated.

Field trial

Field experiments were conducted at the Agricultural Science and Education Farm, University of Tehran, Karaj, Iran during the 2018 and 2019 crop years. An area of approximately 500 m^2 was selected, and operations such as ploughing, tilling, levelling, and irrigation were carried out in the selected area each year before planting. The wheat seeds were cultivated in four blocks, or replicates, in a randomised complete block design. Each block had four plots of 12-m^2 area each. Six rows of wheat were sown in each plot. Distance between rows was 20 cm, and seed spacing in each row was 3 cm. The seeds were treated with *B. bassiana* conidia for treatment plots before planting, as described above, and a similar number of seeds without inoculation were planted in control plots.

The endophytic effect of *B. bassiana* on *E. integriceps* was studied under field conditions. Within each replicate, two plots (a control and a treatment plot) were used to introduce *E. integriceps* at the heading stage of the wheat plants. In each plot, three points were randomly selected, and a steel cage-like net-covered frame was placed over wheat plants. Twelve female and eight male *E. integriceps* were placed in each cage for mating. The field experiments continued, as is described in the Greenhouse experiment section above.

Another plot was used to determine the endophytic effect of *B. bassiana* on *F. culmorum*. After reaching the tillage stage, 4–5 seeds inoculated with *F. culmorum* conidia were planted at the foot

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of each plant. For control plants, the same number of seeds without *Fusarium* inoculation was placed next to the crown. After the plants reached the heading stage, the rate of disease incidence was assessed in the same way as is described in the greenhouse bioassay above.

Statistical analysis

Analysis of variance for each variable was used for data analysis. The endophyte-confirmation test of wheat by *B. bassiana* under greenhouse conditions consigned to a factorial design with three replicates and other greenhouse tests was conducted, based on a completely randomised design with three replicates. Field data for two years were based on a randomised complete block design. A two-sample *t*-test was used to compare the differences between the Sunn pest parameters on control and treated plants. Post analysis of variance means comparisons was done using Tukey's test. SAS version 9.0 software (SAS Institute Inc., Cary, North Carolina, United States of America; https://www.sas.com/en_us/software/iml-sas9.html) was used for statistical analysis.

Results

Re-isolation of *Beauveria bassiana* from plants

The presence of *B. bassiana* as an endophyte in wheat plants was confirmed in all of the tested treatments after 14 days of initial inoculation, and none of the control plants were found to be colonised by the fungus. The recovery of *B. bassiana* as an endophyte from different plant organs – leaf ($F_{9,30} = 88.58$, $P < 0.001$), stem ($F_{9,30} = 40.04$, $P < 0.001$), and root ($F_{9,30} = 35.54$, $P < 0.001$) – differed significantly among the different inoculation methods. The highest percentage of *B. bassiana* re-isolation was obtained in the conidial powder seed inoculation under both sterile and nonsterile conditions from the leaf (91.7% and 62.5%, respectively) and the stem (91.7% and 45.8%, respectively; Fig. 1). In the case of root inoculation, both the conidial powder–inoculated seed treatment (95.8% and 83.3% for sterile and nonsterile conditions, respectively) and foliar spray treatment under nonsterile conditions (58.3%) showed the highest rate of fungal recovery (Fig. 1). *Beauveria bassiana* was not recovered in any of the plant tissues in the conidial suspension–soaked seed treatment after 12 hours under nonsterile conditions. Also, no fungal growth was observed for the foliar spray and seed soaking in conidial suspension 24-hour treatments under nonsterile conditions among leaf samples (Fig. 1). Sterile and nonsterile soil drench, sterile foliar spray, and sterile seed soaking in conidial suspension 24-hour treatments showed the lowest percentages of stem endophytism. As shown in Figure 1, the sterile and nonsterile seeds soaking in conidial suspension 12-hour, sterile foliar spray, nonsterile soil drench, and nonsterile seed soaking in conidial suspension 24-hour treatments showed the lowest endophytic root growth compared to other treatments.

Due to the absence of endophytism in the seed soaking in conidial suspension treatment after six hours and severe fungal contamination in the seed soaking in conidial suspension at 48 hours, these two treatments were excluded from the tests. The root endophyte intensity differed significantly according to the amount of inoculum ($F_{3,12} = 18.73$; $P < 0.001$). According to the colony-forming unit value, the best endophyte was achieved by inoculation of 0.1 g conidia powder with the wheat seeds (equivalent to 3.1×10^7 conidia/mL per seed; data not shown).

In the next step, the intensity of endophytic presence in plant extracts was investigated by counting colony-forming units. An interaction was observed between all three variables, treatment ($F_{4,21} = 567.8$, $P < 0.001$), plant tissues ($F_{4,21} = 259.6$, $P < 0.001$), and experimental conditions ($F_{1,21} = 14.4$, $P < 0.0005$) on the intensity of *B. bassiana* endophyte in wheat plants (Fig. 2). The highest percentage of fungal recovery was obtained in roots after the seed inoculation in conidial powder under sterile and nonsterile conditions. The lowest-intensity

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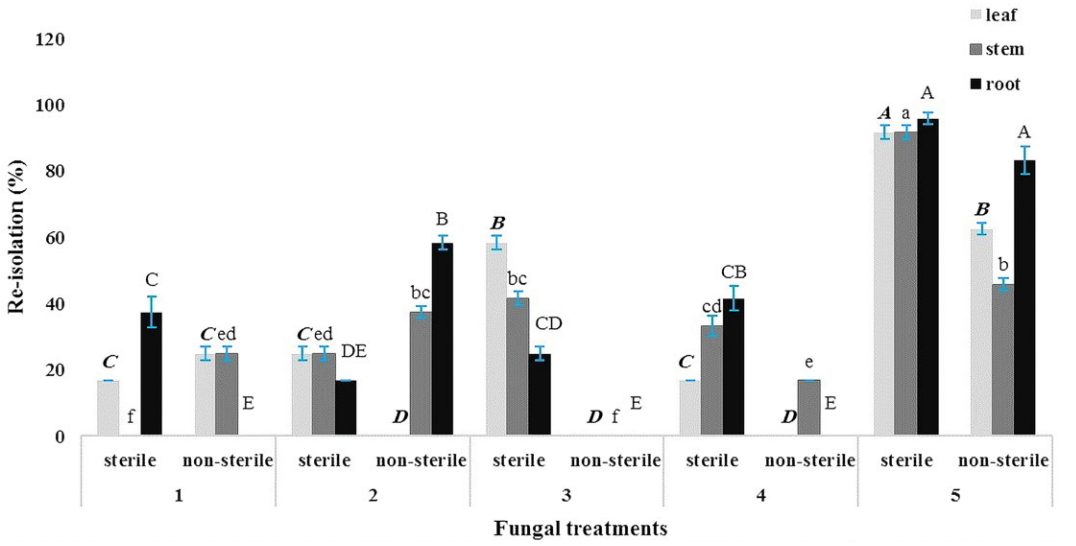

Fig. 1. Mean (± standard error) colonisation percentages of *Beauveria bassiana* TV in different wheat plant parts (leaf, stem, and root) with different inoculation methods under sterile and nonsterile conditions in the greenhouse (**1**, SD; **2**, FS; **3**, SSS 12 hours; **4**, SSS 24 hours; and **5**, SCP treatments). No *Beauveria bassiana* was present in control leaves. SD, soil drench; FS, foliar spray; SCP, inoculation of seed with conidia powder; SSS, seeds soaking in fungal suspension; SSS 12 hours, seeds soaking in fungal suspension for 12 hours; SSS 24 hours, seeds soaking in fungal suspension for 24 hours.

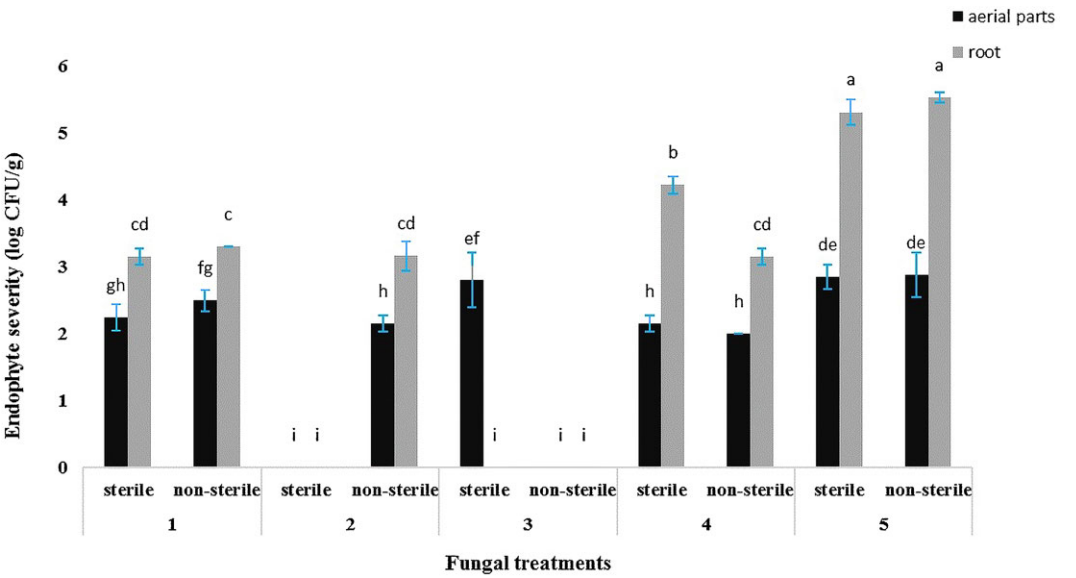

Fig. 2. The effect of endophyte inoculation of *Beauveria bassiana* TV (**1**, SD; **2**, FS; **3**, SSS 12 hours; **4**, SSS 24 hours; and **5**, SCP treatments) on the intensity of fungal colonisation in aerial (leaf and stem) and root tissues of wheat variety Pishtaz under sterile and nonsterile conditions in the greenhouse. SD, soil drench; FS, foliar spray; SCP, inoculation of seed with conidia powder; SSS, seeds soaking in fungal suspension; SSS 12 hours, seeds soaking in fungal suspension for 12 hours; SSS 24 hours, seeds soaking in fungal suspension for 24 hours; CFU, colony-forming units

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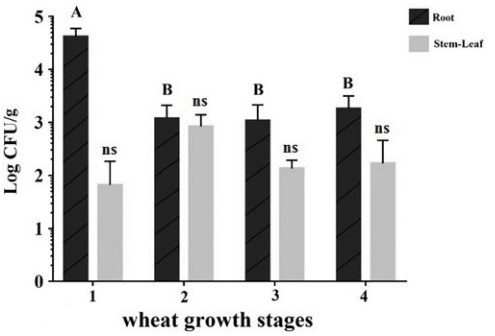

Fig. 3. The effect of inoculation of seed with conidia powder treatment on the intensity of *Beauveria bassiana* TV colonisation in aerial (leaf and stem) and root tissues of wheat variety Pishtaz under different stages of growth (**1**, shooting; **2**, tilling; **3**, stem elongation; and **4**, head emergence) in the field. CFU, colony-forming units

endophytic presence was found in the aerial parts in the seed soaking in conidial suspension 12-hour treatment under both sterile and nonsterile conditions. Moreover, a significant difference occurred between the endophytic intensity in aerial tissues (stem and leaf) and root ($F_{1,21} = 259.6$; $P < 0.001$). Significant differences were also observed among the different fungal treatments. The highest and lowest values for fungal endophytic intensity were observed in the seed inoculation in conidial powder and seed soaking in conidial suspension 12-hour treatments, respectively ($F_{4,21} = 567.8$; $P < 0.001$).

Endophytic intensity in aerial parts and root of wheat plants was affected by inoculation method: the highest amount in roots was obtained by treating seeds in conidial powder, and the lowest value was recorded in the seed soaking in conidial suspension 12-hour treatment ($F_{4,\ 21} = 164.9$; $P < 0.001$).

In the field trials, significant differences were observed in root endophytic intensity among the different growth stages ($F_{4,21} = 201.6$, $P < 0.0001$), but in the aerial plant parts, endophytic intensity was not affected by growth stages (Fig. 3). Endophytic fungus was recovered from all vegetative organs, but its re-isolation from seed was not successful.

The presence of *B. bassiana* in wheat plants was also confirmed by performing polymerase chain reaction and amplifying the ITS1-5.8S-ITS2 sequences. Accordingly, in all treatments, an amplicon with the expected size was observed, but the target sequence was not amplified in the control plants (Fig. 4).

Growth index

The effect of endophytic *B. bassiana* on growth promotion of wheat plants under different inoculation methods in comparison with control plants was analysed under both sterile and nonsterile conditions (Table 1). Height and wet and dry weights differed significantly in the inoculated plants compared to the controls. The most significant differences from the control plants in three parameters were recorded in the seeds treated with conidial powder under nonsterile conditions. The seed treatment in conidial powder under sterile conditions showed a significant difference in height and wet weight. Soil inoculation, or soil drench, led to increased height and dry weight under sterile conditions. Only the wheat plants' height increased in the seed soaking in conidial suspension 12-hour treatment in both sterile and nonsterile conditions.

In contrast to the greenhouse results, no significant differences were observed in the growth factors of inoculated plants compared to that of control plants in the field study.

Bioassay

The endophytic effect of *B. bassiana* was determined on life parameters of Sunn pest (Table 2). The egg-hatch percentages ($t = 3.02$, $df = 6$, $P < 0.05$) and nymph mortality rates ($t = 5.23$, $df = 6$,

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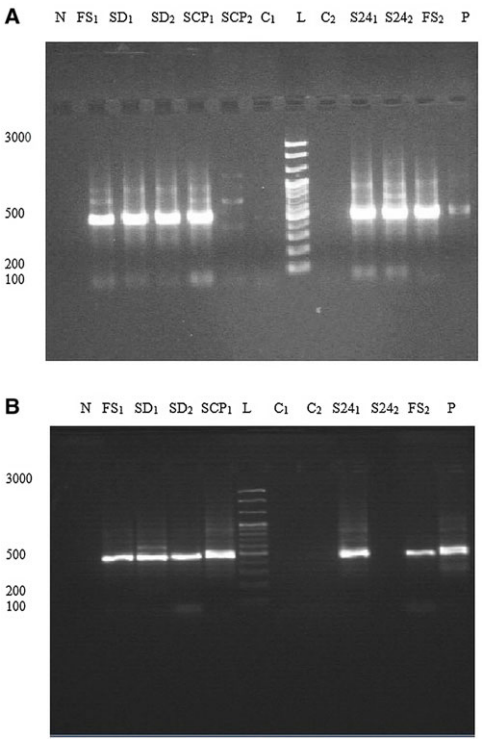

Fig. 4. Detection of *Beauveria bassiana* TV with two-step nested polymerase chain reaction in **A**, wheat variety Pishtaz aerial parts and **B**, in the root. (N = negative control; FS_1 and FS_2 = FS R_1 and R_2; SD_1 and SD_2 = SD R_1 and R_2; SCP_1 and SCP_2 = SCP $R_1$and R_2; C_1 and C_2 = control R_1 and R_2; L = ladder; S24; and $S24_2$ = SSS 24 hours R_1 and R_2; and P = positive control). SD, soil drench; FS, foliar spray; SCP, inoculation of seed with conidia powder; SSS, seeds soaking in fungal suspension; SSS 12 hours, seeds soaking in fungal suspension for 12 hours; SSS 24 hours, seeds soaking in fungal suspension for 24 hours.

$P < 0.01$) were affected significantly by endophytism under greenhouse conditions: 60.9% of the eggs laid by the adults that were fed on the endophyte-treated plants hatched, whereas the hatchability percentage in the controls was an estimated 89.4%. In the treatment under greenhouse conditions, nymphs were unable to complete their life cycle and enter the adult stage.

Interestingly, pooled data from the field trials showed the same results as in greenhouse studies for egg hatching. The mean hatching percentage for adults in control plants was higher than that for adults reared on inoculated plants ($t = 6.49$, $df = 6$, $P < 0.0001$). The percentage of adult emergence from control plants was higher than that from inoculated plants ($t = 5.45$, $df = 6$, $P = 0.001$; Table 2).

Endophytic effect of *Beauveria bassiana* on plant disease

Significant differences between disease incidence in inoculated and control plants were noted ($t = 5.149$, $df = 8$, $P < 0.001$). Disease incidence in inoculated plants was significantly lower (36% ± 4.58%) than that in control plant (63.5% ± 2.55%). Presence of *B. bassiana* as an endophyte in wheat plants decreased 42% of the root and crown rot disease caused by *F. culmorum* in the greenhouse experiment.

Discussion

The wheat variety "Pishtaz" released in 2002 for temperate regions of Iran has good baking properties, shows resistance to yellow rust and wart, and is tolerant to brown rust. Different insect pests, particularly *E. integriceps*, which is the most destructive pest, threaten Iran's wheat production. Another critical agent reducing the production of Pishtaz-variety wheat is *Fusarium* spp. The nonchemical control of these pests *sensu lato* is of particular importance.

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Table 1. The effect of endophytic *Beauveria bassiana* TV on growth promotion of wheat variety Pishtaz plants with different inoculation methods in comparison with control plants under sterile and nonsterile conditions in the greenhouse.

Experiment condition	Treatment[a]	Net increase in height (cm)[b]	Net increase in wet weight (mg)[c]	Net increase in dry weight (mg)[d]
Nonsterile	SD	0.62	0.00	0.00
	FS	0.00	0.00	0.00
	SSS 12 hours	3.25*	0.00	0.01
	SSS 24 hours	0.75	0.01	0.01
	SCP	4.00**	0.08**	0.01*
Sterile	SD	7.75**	0.00	0.04**
	FS	0.88	0.07	0.01
	SSS 12 hours	2.75*	0.02	0.01
	SSS 24 hours	3.12	0.00	0.00
	SCP	3.62**	0.04*	0.01

[a]SD = soil drench; FS = foliar spray; SCP = inoculation of seed with conidia powder; SSS = seeds soaking in fungal suspension; SSS 12 hours = seeds soaking in fungal suspension for 12 hours; SSS 24 hours = seeds soaking in fungal suspension for 24 hours.
[b, c, d]The data show the difference between absolute values of the growth parameter for fungus-treated and control plants.
* The significant difference, $P < 0.05$.
** The significant difference, $P < 0.01$.

Table 2. Mean (± standard error) percentages of Sunn pest egg hatching and adult emergence after feeding on endophyte-treated and control plants in greenhouse and field. Percentage values are based on the recovery of eggs from four cages per treatment as follows: greenhouse (endophyte-treated plants: average 46 eggs/cage; control plants: average 43 eggs/cage); field (endophyte-treated plants: average 33 eggs/cage; control plants: average 35 eggs/cage).

Experimental conditions	Mean (%) ± standard error			
	Greenhouse		Field	
Parameter	Egg hatching	Adult emergence	Egg hatching	Adult emergence
Endophyte-treated plants	60.9 ± 7.8	00.0	22.8 ± 3.4	15.1 ± 3.9
Control plants	89.4 ± 5.2	14.1 ± 2.6	65.5 ± 5.6	31.5 ± 1.6

Taking advantage of the pathogenic potential of naturally occurring *B. bassiana* against insects and its recent discovery for endophytic effects against pests and plant diseases is worth consideration.

The endophytic potential of the *B. bassiana* isolate TV was investigated in wheat plants under greenhouse and field conditions. Of the different inoculation methods tested, the seed treatment in conidial powder showed the highest percentage of *B. bassiana* recovery in all three plant tissues (leaf, stem, and root). Admittedly, soaking seeds in conidial suspension (for 24 hours) and the foliar spray treatment (in all the tested time intervals) showed the lowest percentage of fungal recovery. Achieving the highest recovery rate of *B. bassiana* after inoculation in particular host plants is affected by the fungal inoculation method (Donga *et al.* 2018; Jaber and Ownley 2018). Direct injection of inoculant into coffee (Rubiaceae) (Posada *et al.* 2007) and corn leaves (Wagner and Lewis 2000), stem injection (Bing and Lewis 1991), tomato seed coating (Ownley *et al.* 2008), banana plant immersion (Akello *et al.* 2008b), seed inoculation in other plants (Griffin 2007; Ownley *et al.* 2008), and poppy (Papaveraceae) leaf or seed coating (Quesada-Moraga *et al.* 2006) resulted in high recovery rates of *B. bassiana* after

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inoculation. According to Sánchez-Rodríguez *et al.* (2018), soil inoculation and seed coating were the most effective inoculation methods for *B. bassiana* endophytic establishment in wheat plants, and the foliar spray treatment rarely resulted in fungal recovery from plant tissue. Also, Posada *et al.* (2007) reported that leaves are a poor route for fungal colonisation in the plants. It should be considered that those authors referred to coffee plants, which have stomata within only one surface of their leaves, whereas wheat and other grasses have stomata within both surfaces of leaves. However, we found that the fungus could establish in all parts of wheat by inoculation of seed treatment, soil drench, and foliar spray. *Beauveria bassiana* could successfully be recovered from parts of the plant that were not directly treated with the fungus. This ability may depend on the capacity of *B. bassiana* to reach the plant's xylem and to spread from there throughout the plant (Wagner and Lewis 2000).

The presence of *B. bassiana* in the treated plants was also confirmed using specific primers and two-step nested polymerase chain reaction. Using a combination of different detection methods for the re-isolation of *B. bassiana* leads to accurate detection of endophytic fungus in the plant (Rondot and Reineke 2018). We were able to recover endophytic fungus from all vegetative organs, but we were unsuccessful in re-isolating *B. bassiana* from seed. However, the presence of an endophyte in a specific tissue does not necessarily elicit a measurable effect (Lopez *et al.* 2014).

We demonstrated that *B. bassiana* has potential to improve plant growth parameters such as height and wet and dry weights, and that a relation exists between the inoculation method and plant growth. Plant-associated endophytes can promote plant growth directly through the production of plant hormones or indirectly through antimicrobial activity, ammonia production, and synthesis of deteriorating enzymes that inhibit pathogens (Hassan 2017). Our results support earlier investigations that showed improved wheat growth following inoculation with *B. bassiana* (Gurulingappa *et al.* 2011; Sánchez-Rodríguez *et al.* 2018). In contrast, Mantzoukas *et al.* (2015) found that growth of sorghum was not influenced by the endophytic treatment with *B. bassiana* and *Metarhizium robertsii* (Clavicipitaceae).

In addition to their ability to promote plant growth, entomopathogenic fungal species' insecticidal activity is of special importance. Endophytic *B. bassiana* establishment in wheat is shown to negatively affect mortality and egg hatching of Sunn pest. This effect on insect pests can be attributed to the chemical mechanisms *B. bassiana* induces in the plant (Lopez *et al.* 2014); for example, the destructive effects of endophytic fungi were observed to affect growth and fertility of *Acyrthosiphon pisum* and *Aphis fabae* (both Hemiptera: Aphididae) nymphs. The numbers of nymphs reared on the treated plants were significantly lower than those fed on the control for two continuous generations (Akello and Sikora 2012), which is consistent with our results showing a significant reduction in egg hatching of Sunn pest fed on *B. bassiana*–inoculated plants.

No sign of fungal infection was detected in dead adults and nymphs in the current study, similar to previous studies (Cherry *et al.* 2004; Lopez and Sword 2015). This suggests that the insect-killing mechanism may be due to feeding inhibition, antibiosis, or alteration in host-plant metabolites. Therefore, host-plant quality, not the fungus itself, affects the insects (Quesada-Moraga *et al.* 2009; Akutse *et al.* 2013; Lopez *et al.* 2014; Reddy *et al.* 2014; Mantzoukas *et al.* 2015; Yan *et al.* 2015; Jaber and Ownley 2018; Rondot and Reineke 2018). Reduction in insect populations or damage caused by insect pests such as *Cosmopolites sordidus* (Coleoptera: Curculionidae) (Akello *et al.* 2008a), *Iraella luteipes* (Hymenoptera: Cynipidae) (Quesada-Moraga *et al.* 2009), *Ostrinia nubilalis* (Lepidoptera: Crambidae) (Bing and Lewis 1991), and *Sesamia calamistis* (Lepidoptera: Noctuidae) (Cherry *et al.* 2004) has been attributed to the toxicity of endophytic metabolites of *B. bassiana* for the mentioned insects.

In addition to biocontrol of Sunn pest, endophytic *B. bassiana* was able to reduce *F. culmorum* infection by up to 42%. Similar results have been previously reported: for example, Jaber and Ownley (2018) reported that endophytic colonisation of wheat with *B. bassiana*

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(NATURALIS) resulted in a significant reduction in percentage disease incidence of *F. culmorum*, with up to 51.5% of *B. bassiana*–colonised plants having no visible symptoms of disease, whereas all *Fusarium*-infected noncolonised plants (control) showed visible disease symptoms after 24 days. The interaction between endophytic fungi and plant pathogens could be through fungal parasitism, competition for ecological and nutritional niches, or the production of secondary metabolites (Jaber and Ownley 2018). After seed treatment, the initial endophytic colonisation may induce a defence mechanism in the plant by producing lignin or cell wall, leading to the reduction of pathogen contamination or prevention of the disease occurrence (Jaber and Ownley 2018).

In conclusion, our results demonstrate the potential endophytic activity of an entomopathogenic isolate of *B. bassiana* for promoting growth of wheat and contributing to control of *E. integriceps* and *Fusarium* pathogen. *Beauveria bassiana* can be commercially produced and used in wheat cultivation, along with other elements to successfully control *E. integriceps* and *Fusarium*-caused diseases and to promote wheat growth and performance.

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References

Abdel-Hafez, S.I., Abo-Elyousr, K.A., and Abdel-Rahim, I.R. 2015. Leaf surface and endophytic fungi associated with onion leaves and their antagonistic activity against *Alternaria porri*. Czech Mycology, **67**: 1–22.

Akello, J., Dubois, T., Coyne, D., and Kyamanywa, S. 2008a. Endophytic *Beauveria bassiana* in banana (*Musa* spp.) reduces banana weevil (*Cosmopolites sordidus*) fitness and damage. Crop Protection, **27**: 1437–1441. https://doi.org/10.1016/j.cropro.2008.07.003.

Akello, J., Dubois, T., Coyne, D., and Kyamanywa, S. 2008b. Effect of endophytic *Beauveria bassiana* on populations of the banana weevil, *Cosmopolites sordidus*, and their damage in tissue-cultured banana plants. Entomologia Experimentalis et Applicata, **129**: 157–165.

Akello, J. and Sikora, R. 2012. Systemic acropedal influence of endophyte seed treatment on *Acyrthosiphon pisum* and *Aphis fabae* offspring development and reproductive fitness. Biological Control, **61**: 215–221. https://doi.org/10.1016/j.biocontrol.2012.02.007.

Akutse, K.S., Maniania, N.K., Fiaboe, K.K.M., van den Berg, J., and Ekesi, S. 2013. Endophytic colonization of *Vicia faba* and *Phaseolus vulgaris* (Fabaceae) by fungal pathogens and their effects on the life-history parameters of *Liriomyza huidobrensis* (Diptera: Agromyzidae). Fungal Ecology, **6**: 293–301. https://doi.org/10.1016/j.funeco.2013.01.003.

Atugala, D. and Deshappriya, N. 2015. Effect of endophytic fungi on plant growth and blast disease incidence of two traditional rice varieties. Journal of the National Science Foundation, **43**: 173–187. https://doi.org/10.4038/jnsfsr.v43i2.7945.

https://doi.org/10.4039/tce.2022.43 Published online by Cambridge University Press

Bamisile, B.S., Akutse, K.S., Dash, C.K., Qasim, M., Carlos, L., Aguila, R., *et al.* 2020. Effects of seedling age on colonization patterns of *Citrus limon* plants by endophytic *Beauveria bassiana* and *Metarhizium anisopliae* and their influence on seedlings growth. Journal of Fungi, **6**: 29. https://doi.org/10.3390/jof6010029.

Beccari, G., Covarelli, L., and Nicholson, P. 2011. Infection processes and soft wheat response to root rot and crown rot caused by *Fusarium culmorum*. Plant Pathology, **60**: 671–684. https://doi.org/10.1111/j.1365-3059.2011.02425.x.

Bing, L.A. and Lewis, L.C. 1991. Suppression of *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae) by endophytic *Beauveria bassiana* (Balsamo) Vuillemin. Environmental Entomology, **20**: 1207–1211. https://doi.org/10.1093/ee/20.4.1207.

Chekali, S., Gargouri, S., Paulitz, T., Nicol, J.M., and Rezgui, M. 2011. Effects of *Fusarium culmorum* and water stress on durum wheat in Tunisia. Crop Protection, **30**: 718–725. https://doi.org/10.1016/j.cropro.2011.01.007.

Cherry, A.J., Banito, A., Djegui, D., and 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*. International Journal of Pest Management, **50**: 67–73. https://doi.org/10.1080/09670870310001637426.

Critchley, B.R. 1998. Literature review of Sunn pest *Eurygaster integriceps* Put. (Hemiptera, Scutelleridae). Crop Protection, **17**: 271–287.

Dara, S.K. 2019. Non-entomopathogenic roles of entomopathogenic fungi in promoting plant health and growth. Insects, **10**: 277.

Davari, A. and Parker, B.L. 2018. A review of research on Sunn pest (*Eurygaster integriceps* Puton (Hemiptera: Scutelleridae)) management published 2004–2016. Journal of Asia–Pacific Entomology, **2**: 352–360. https://doi.org/10.1016/j.aspen.2018.01.016.

Donga, T.K., Vega, F.E., and Klingen, I. 2018. Establishment of the fungal entomopathogen *Beauveria bassiana* as an endophyte in sugarcane, *Saccharum officinarum*. Fungal Ecology, **35**: 70–77. https://doi.org/10.1016/j.funeco.2018.06.008.

Gardes, M. and Bruns, T.D. 1993. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Molecular Ecology, **2**: 113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x.

Gaviria, J., Parra, P.P., and Gonzales, A. 2020. Selection of strains of *Beauveria bassiana* and *Metarhizium anisopliae* (Ascomycota: Hypocreales) for endophytic colonization in coconut seedlings. Chilean Journal of Agriculture and Animal Science, **36**: 3–13. https://doi.org/10.4067/S0719-38902020005000101.

Greenfield, M., Gómez-Jiménez, M.I., Ortiz, V., Vega, F.E., Kramer, M., and Parsa, S. 2016. *Beauveria bassiana* and *Metarhizium anisopliae* endophytically colonize cassava roots following soil drench inoculation. Biological Control, **95**: 40–48. https://doi.org/10.1016/j.biocontrol.2016.01.002.

Griffin, M.R. 2007. *Beauveria bassiana*, a cotton endophyte with biocontrol activity against seedling disease. Ph.D. dissertation. University of Tennessee, Knoxville, Tennessee, United States of America.

Grosu, A.I., Sicuia, O.A., Dobre, A., Voaideş, C., and Cornea, C.P. 2015. Evaluation of some *Bacillus* spp. strains for the biocontrol of *Fusarium graminearum* and *F. culmorum* in wheat. Agriculture and Agricultural Science Procedia, **6**: 559–566. https://doi.org/10.1016/j.aaspro.2015.08.085.

Guesmi-Jouini, J., Garrido-Jurado, I., López-Díaz, C., Ben Halima-Kamel, M., and Quesada-Moraga, E. 2014. Establishment of fungal entomopathogens *Beauveria bassiana* and *Bionectria ochroleuca* (Ascomycota : Hypocreales) as endophytes on artichoke *Cynara scolymus*. Journal of Invertebrate Pathology, **119**: 1–4. https://doi.org/10.1016/j.jip.2014.03.004.

https://doi.org/10.4039/tce.2022.43 Published online by Cambridge University Press

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 Protection, **30**: 349–353. https://doi.org/10.1016/j.cropro.2010.11.017.

Gurulingappa, P., Sword, G.A., Murdoch, G., and McGee, P.A. 2010. Colonization of crop plants by fungal entomopathogens and their effects on two insect pests when in planta. Biological Control, **55**: 34–41. https://doi.org/10.1016/j.biocontrol.2010.06.011.

Hassan, S.E. 2017. Plant growth–promoting activities for bacterial and fungal endophytes isolated from medicinal plant of *Teucrium polium* L. Journal of Advanced Research, **8**: 687–695. https://doi.org/10.1016/j.jare.2017.09.001.

Hosseininaveh, V., Bandani, A., and Hosseininaveh, F. 2009. Digestive proteolytic activity in the Sunn pest, *Eurygaster integriceps*. Journal of Insect Science, **9**: 70. https://doi.org/10.1673/031.009.7001.

Humber, R.A. 1997. Fungi: identification. *In* Manual of techniques in insect pathology. *Edited by* L. Lacey. Academic Press, Cambridge, Massachusetts, United States of America. Pp. 153–185. https://doi.org/10.1016/B978-012432555-5/50011-7.

Jaber, L.R., Araj, S., and Qasem, J.R. 2018. Compatibility of endophytic fungal entomopathogens with plant extracts for the management of sweet potato whitefly *Bemesia tabaci* Gennadius (Homoptera: Aleyrodidae). Biological Control, **117**: 164–171. https://doi.org/10.1016/j.biocontrol.2017.11.009.

Jaber, L.R. and Enkerli, J. 2016. Effect of seed treatment duration on growth and colonization of *Vicia faba* by endophytic *Beauveria bassiana* and *Metarhizium brunneum*. Biological Control, **103**: 187–195. https://doi.org/10.1016/j.biocontrol.2016.09.008.

Jaber, L.R. and Ownley, B.H. 2018. Can we use entomopathogenic fungi as endophytes for dual biological control of insect pests and plant pathogens? Biological Control, **116**: 36–45. https://doi.org/10.1016/j.biocontrol.2017.01.018.

Jaronski, S.T. and Mascarin, G.M. 2017. Mass production of fungal entomopathogens. *In* Microbial control of insect and mite pests: from theory to practice. *Edited by* L. Lacey. Academic Press, Cambridge, Massachusetts, United States of America. Pp. 141–155. https://doi.org/10.1016/B978-0-12-803527-6.00009-3.

Klieber, J. and Reineke, A. 2015. The entomopathogen *Beauveria bassiana* has epiphytic and endophytic activity against the tomato leaf miner, *Tuta absoluta*. Journal of Applied Entomology, **140**: 580–589. https://doi.org/10.1111/jen.12287.

Koksel, H., Ozderen, T., Olanca, B., and Ozay, D.S. 2009. Effects of suni bug (*Eurygaster* spp.) damage on milling properties and semolina quality of durum wheats (*Triticum durum* L.). Cereal Chemistry, **86**: 181–186. https://doi.org/10.1094/CCHEM-86-2-0181.

Lacey, L.A., Grzywacz, D., Shapiro-Ilan, D.I., Frutos, R., Brownbridge, M., and Goettel, M.S. 2015. Insect pathogens as biological control agents: back to the future. Journal of Invertebrate Pathology, **132**: 1–41 https://doi.org/10.1016/j.jip.2015.07.009.

Landa, B.B., López-Díaz, C., Jiménez-Fernández, D., Montes-Borrego, M., and Muñoz-Ledesma, F.J. 2013. In-plant detection and monitorization of endophytic colonization by a *Beauveria bassiana* strain using a new-developed nested and quantitative PCR-based assay and confocal laser scanning microscopy. Journal of Invertebrate Pathology, **114**: 128–138. https://doi.org/10.1016/j.jip.2013.06.007.

Lopez, D.C. and Sword, G.A. 2015. The endophytic fungal entomopathogens *Beauveria bassiana* and *Purpureocillium lilacinum* enhance the growth of cultivated cotton (*Gossypium hirsutum*) and negatively affect survival of the cotton bollworm (*Helicoverpa zea*). Biological Control, **89**: 53–60. https://doi.org/10.1016/j.biocontrol.2015.03.010.

https://doi.org/10.4039/tce.2022.43 Published online by Cambridge University Press

Lopez, D.C., Zhu-Salzman, K., Ek-Ramos, M.J., and Sword, G.A. 2014. The entomopathogenic fungal endophytes *Purpureocillium lilacinum* (formerly *Paecilomyces lilacinus*) and *Beauveria bassiana* negatively affect cotton aphid reproduction under both greenhouse and field conditions. PLOS One, **9**: e103891. https://doi.org/10.1371/journal.pone.0103891.

Mantzoukas, S., Chondrogiannis, C., and Grammatikopoulos, G. 2015. Effects of three endophytic entomopathogens on sweet sorghum and on the larvae of the stalk borer *Sesamia nonagrioides*. Entomologia Experimentalis et Applicata, **154**: 78–87. https://doi.org/10.1111/eea.12262.

Mascarin, G.M. and Jaronski, S.T. 2016. The production and uses of *Beauveria bassiana* as a microbial insecticide. World Journal of Microbiology and Biotechnology, **32**: 1–26. https://doi.org/10.1007/s11274-016-2131-3.

Meyer-Wolfarth, F., Schrader, S., Oldenburg, E., Weinert, J., and Brunotte, J. 2017. Biocontrol of the toxigenic plant pathogen *Fusarium culmorum* by soil fauna in an agroecosystem. Mycotoxin Research, **33**: 237–244. https://doi.org/10.1007/s12550-017-0282-1.

Moloinyane, S. and Nchu, F. 2019. The effects of endophytic *Beauveria bassiana* inoculation on infestation level of *Planococcus ficus*, growth and volatile constituents of potted greenhouse grapevine (*Vitis vinifera* L.). Toxins, **11**: 72. https://doi.org/10.3390/toxins11020072.

Motallebi, P., Alkadri, D., Pisi, A., Nipoti, P., Tonti, S., Niknam, V., *et al.* 2015. Pathogenicity and mycotoxin chemotypes of Iranian *Fusarium culmorum* isolates on durum wheat, and comparisons with Italian and Syrian isolates. Phytopathologia Mediterranea, **54**: 437–445.

Muvea, A.M., Meyhöfer, R., Subramanian, S., Poehling, H.M., Ekesi, S., and Maniania, N.K. 2014. Colonization of onions by endophytic fungi and their impacts on the biology of *Thrips tabaci*. PLOS One, **9**: e108242. https://doi.org/10.1371/journal.pone.0108242.

Ownley, B.H., Griffin, M.R., Klingeman, W.E., Gwinn, K.D., Moulton, J.K., and Pereira, R.M. 2008. *Beauveria bassiana*: endophytic colonization and plant disease control. Journal of Invertebrate Pathology, **98**: 267–270. https://doi.org/10.1016/j.jip.2008.01.010.

Ownley, B.H. and Gwinn, K.D. 2010. Endophytic fungal entomopathogens with activity against plant pathogens: ecology and evolution. *In* The ecology of fungal entomopathogens. *Edited by* H.E. Roy, F.E. Vega, M.S. Goettel, D. Chandler, J.K. Pell, and E. Wajnberg. Springer Netherlands, Heidelberg, Germany. Pp. 113–128. https://doi.org/10.1007/978-90-481-3966-8.

Parsa, S., Ortiz, V., and Vega, F.E. 2013. Establishing fungal entomopathogens as endophytes: towards endophytic biological control. Journal of Visualized Experiments, **74**: e50360. https://doi.org/10.3791%2F50360.

Posada, F., Aime, M.C., Peterson, S.W., and Rehner, S.A. 2007. Inoculation of coffee plants with the fungal entomopathogen *Beauveria bassiana* (Ascomycota: Hypocreales). Mycological Research, **111**: 748–757. https://doi.org/10.1016/j.mycres.2007.03.006.

Posada, F. and Vega, F.E. 2005. Establishment of the fungal entomopathogen *Beauveria bassiana* (Ascomycota: Hypocreales) as an endophyte in cocoa seedlings (*Theobroma cacao*). Mycologia, **97**: 1195–1200. https://doi.org/10.3852/mycologia.97.6.1195.

Quesada-Moraga, E., Landa, B., Muñoz-Ledesma, J., Jiménez-Diáz, R., and Santiago-Alvarez, C. 2006. Endophytic colonisation of opium poppy, *Papaver somniferum*, by an entomopathogenic *Beauveria bassiana* strain. Mycopathologia, **161**: 323–329. https://doi.org/10.1007/s11046-006-0014-0.

Quesada-Moraga, E., Munoz-Ledesma, F., and Santiago-Alvarez, C. 2009. Systemic protection of *Papaver somniferum* L. against *Iraella luteipes* (Hymenoptera: Cynipidae) by an endophytic strain of *Beauveria bassiana* (Ascomycota: Hypocreales). Environmental Entomology, **38**: 723–730. https://doi.org/10.1603/022.038.0324.

Reddy, G.V.P., Zhao, Z., and Humber, R.A. 2014. Laboratory and field efficacy of entomopathogenic fungi for the management of the sweetpotato weevil, *Cylas formicarius* (Coleoptera: Brentidae). Journal of Invertebrate Pathology, **122**: 10–15. https://doi.org/10.1016/j.jip.2014.07.009.

https://doi.org/10.4039/tce.2022.43 Published online by Cambridge University Press

Reddy, N.P., Khan, A.P.A., Devi, U.K., Sharma, H.C., and Reineke, A. 2009. Treatment of millet crop plant (*Sorghum bicolor*) with the entomopathogenic fungus (*Beauveria bassiana*) to combat infestation by the stem borer, *Chilo partellus* Swinhoe (Lepidoptera: Pyralidae). Journal of Asia Pacific Entomology, **12**: 221–226. https://doi.org/10.1016/j.aspen.2009.06.001.

Renuka, S., Ramanujam, B., and 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 Journal of Microbiology, **56**: 126–133. https://doi.org/10.1007/s12088-016-0574-8.

Rondot, Y. and Reineke, A. 2018. Endophytic *Beauveria bassiana* in grapevine *Vitis vinifera* (L.) reduces infestation with piercing-sucking insects. Biological Control, **116**: 82–89. https://doi.org/10.1016/j.biocontrol.2016.10.006.

Rondot, Y. and Reineke, A. 2019. Endophytic *Beauveria bassiana* activates expression of defence genes in grapevine and prevents infections by grapevine downy mildew *Plasmopara viticola*. Plant Pathology, **68**: 1719–1731.

Saghai-Maroof, M.A., Soliman, K.M., Jorgensen, R.A., and Allard, R. 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proceedings of the National Academy of Sciences, 81: 8014–8018. https://doi.org/10.1073/pnas.81.24.8014.

Sánchez-Rodríguez, A.R., Raya-Cíaz, S., María, Á., García-Mina, J.M., Carmen, M., and Quesada-Moraga, E. 2018. An endophytic *Beauveria bassiana* strain increases spike production in bread and durum wheat plants and effectively controls cotton leafworm (*Spodoptera littoralis*) larvae. Biological Control, **116**: 90–102. https://doi.org/10.1016/j.biocontrol.2017.01.012.

Seyedtalebi, F.S., Safavi, S.A., Talaei-Hassanloui, R., and Bandani, A.R. 2017. Quantitative comparison for some immune responses among *Eurygaster integriceps*, *Ephestia kuehniella* and *Zophobas morio* against the entomopathogenic fungus *Beauveria bassiana*. Invertebrate Survival Journal, **14**: 174–181. https://doi.org/10.25431/1824-307X/isj.v14i1.174-181.

Seyedtalebi, F.S., Safavi, S.A., Talaei-Hassanloui, R., and Bandani, A.R. 2020. Variable induction of cuticle-degrading enzymes of *Beauveria bassiana* isolates in the presence of different insect cuticles. Journal of Crop Protection, **9**: 563–576. http://dorl.net/dor/20.1001.1.22519041.2020.9.4.13.6.

Shrivastava, G., Ownley, B.H., Augé, R.M., Toler, H., Dee, M., Vu, A., *et al.* 2015. Colonization by arbuscular mycorrhizal and endophytic fungi enhanced terpene production in tomato plants and their defense against a herbivorous insect. Symbiosis, **65**: 65–74. https://doi.org/10.1007/s13199-015-0319-1.

Talaei-Hassanloui, R., Kharazi-Pakdel, A., and Hedjaroude, G.A. 2009. Transmission possibility of the fungus *Beauveria bassiana* KCF102 by mating behavior between Sunn pest, *Eurygaster integriceps* (Hem.: Scutelleridae) adults. Journal of Entomological Society of Iran, **28**: 1–6.

Tall, S. and Meyling, N.V. 2018. Probiotics for plants? Growth promotion by the entomopathogenic fungus *Beauveria bassiana* depends on nutrient availability. Microbial Ecology, **76**: 1002–1008. https://doi.org/10.1007/s00248-018-1180-6.

Tefera, T. and Vidal, S. 2009. Effect of inoculation method and plant growth medium on endophytic colonization of sorghum by the entomopathogenic fungus *Beauveria bassiana*. BioControl, **54**: 663–669. https://doi.org/10.1007/s10526-009-9216-y.

Vega, F.E. 2008. Insect pathology and fungal endophytes. Journal of Invertebrate Pathology, **98**: 277–279. https://doi.org/10.1016/j.jip.2008.01.008.

Vega, F. 2018. The use of fungal entomopathogens as endophytes in biological control: a review. Mycologia, **110**: 4–30. https://doi.org/10.1080/00275514.2017.1418578.

Vega, F.E., Goettel, M.S., Blackwell, M., Chandler, D., Jackson, M.A., Keller, S., *et al.* 2009. Fungal entomopathogens: new insights on their ecology. Fungal Ecology **2**: 149–159. https://doi.org/10.1016/j.funeco.2009.05.001.

https://doi.org/10.4039/tce.2022.43 Published online by Cambridge University Press

Wagner, B.L. and Lewis, L.C. 2000. Colonization of corn, *Zea mays*, by the entomopathogenic fungus *Beauveria bassiana*. Applied and Environmental Microbiology, **66**: 3468–3473. https://doi.org/10.1128/AEM.66.8.3468-3473.2000.

White, T.J., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications. Part three: genetics and evolution. Academic Press, Cambridge, Massachusetts, United States of America. Pp. 315–322.

Wilson, D. 1995. Endophyte: the evolution of a term, and clarification of its use and definition. Oikos, **73**: 274–276. https://doi.org/10.2307/3545919.

Yan, J.F., Broughton, S.J., Yang, S.L., and Gange, A.C. 2015. Do endophytic fungi grow through their hosts systemically? Fungal Ecology, **13**: 53–59. https://doi.org/10.1016/j.funeco.2014.07.005.

Yuan, Y., Feng, H., Wang, L., Li, Z., Shi, Y., Zhao, L., *et al.* 2017. Potential of endophytic fungi isolated from cotton roots for biological control against verticillium wilt disease. PLOS One, **12**: e0170557. https://doi.org/10.1371/journal.pone.0170557.

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