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ORIGINAL ARTICLE

The fungal strain and inoculation method mediate the endophytic activity of *Beauveria bassiana* and its impact on the growth of cucumber plants and the population of *Liriomyza sativae*

Lobna Rajab*, Ahmad M. Dayoub, Ibtisam Gazal, Mohammad Ahmad

Department of Plant Protection, Faculty of Agriculture, Tishreen University, Latakia, Syria

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*Corresponding address:
lobna.h.rajab@tishreen.edu.sy

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Abstract

Endophytic *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin can promote plant growth and health and protect them against herbivores. Two endophytic strains of *B. bassiana*, BS195 (isolated from soil) and BNE20 (isolated from cucumber), were used by foliar spray and root soaking to evaluate *B. bassiana* ability to colonize cucumber plants and promote their growth under stressful greenhouse conditions in two separate experiments, as well as its efficacy against *Liriomyza sativae* Blanchard in a controlled setting. The results showed that the effects significantly depended on the inoculation method and fungal strain. Both *B. bassiana* strains colonized endophytically the tissues of all cucumber plant parts 30 days post-inoculation, with root soaking being significantly better than foliar spray. The present study showed that the application of *B. bassiana* BS195, mainly through root soaking, enhanced many growth and health parameters, including plant height, root length, number of leaves, leaf area, fresh and dry weight, the content of dry matter, and the total phenolic content. Cucumber plant treatment with *B. bassiana* significantly reduced the infestation, severity, number of pupae, and adult emergence of *L. sativae* after 35 and 51 days of adult release with greater efficiency with the root soaking method. We conclude that introducing *B. bassiana* through root soaking seems to be effective in stimulating plant growth, and can be a promising technique in controlling *L. sativae* populations on cucumber plants.

Key words: Cucurbitaceae, endophytes, entomopathogenic fungi, leafminer, plant growth promoting

Introduction

Cucumber (*Cucumis sativus* L.; Cucurbitaceae) is a very popular crop for commercial greenhouse vegetable production in many countries worldwide due to the high value of its fruits (FAOSTAT 2022). Many insect pests attack and damage cucumber plants during the season and cause considerable production losses, including many species of the leafminer genus *Liriomyza* Mik (Diptera: Agromyzidae). The most economically destructive pest species of *Liriomyza* are *L. sativae* Blanchard, *L. huidobrensis* (Blanchard), and *L. trifolii* (Burgess) (Tran *et al.* 2005; Sappanukhro *et al.* 2011; Alaei Verki *et al.* 2020). Female leafminers oviposit eggs inside leaf tissues under the epidermis, and the hatching larvae tunnel and twist through the mesophyll, reducing the area that is actively involved in photosynthesis. The damaged tissues also become more susceptible to plant pathogens and saprophytic organisms (Parrella 1987; Alaei Verki *et al.* 2020). Because of some aspects of the biology of this insect (e.g., egg and larval stages within and protected by leaf tissue, the ability to develop resistance to insecticides, etc.), the application of chemical insecticides may be ineffective in preventing the reduction of cucumber yield, and the use of alternative, more effective management methods is highly warranted (Parrella 1987; Alaei Verki *et al.* 2020).

The application of insect pathogens, such as entomopathogenic fungi (EPFs), is a promising alternative for the protection of crops against herbivorous pests (Inglis *et al*. 2001; Gurulingappa *et al*. 2010). EPFs can be applied against phytophagous insects by traditional spraying with the goal of either directly killing the insect by contact with the inoculum or indirectly when the host comes into contact with the inoculum present on the plant surface (Charnley 1984). However, in traditional use, the inoculum is exposed to harmful UV radiation, fluctuating humidity, and unfavorable temperatures, which could significantly reduce the efficiency of EPFs (Roberts 1989; Kim *et al.* 2013). Therefore, the incorporation of EPFs in plants as endophytes would be a highly interesting and promising approach that could potentially help to avoid the unwanted effects of adverse environmental conditions on the fungal inoculum and to control pests with protected life stages such as leafminers.

Fungal endophytes are commonly defined as fungi that colonize the internal tissues of plants for some or all of their lifecycles without causing any symptoms (Wilson 1995). Plant colonization by fungal endophytes can either be localized or systemic (Vega 2008; Rodriguez *et al*. 2009; Yan *et al*. 2015; Card *et al*. 2016). Recently, it was shown that some EPFs have an ability to live as endophytes (Vega 2018). Endophytic entomopathogenic fungi can protect plants against biotic and abiotic stresses and at the same time promote plant growth (Vega 2008; Ownley *et al*. 2010; Vidal and Jaber 2015). According to Moloinyane and Nchu (2019), they may help protect plants against herbivores either indirectly via induction of plant defenses, or directly via the production of fungal metabolites with insecticidal properties. In addition, an increasing number of plant species have responded to EPFs as plant growth promoters (PGPs) by improving general morphological, yield, and biochemical parameters, in addition to enhancing nutrient uptake by the root system following plant colonization (Lopez and Sword 2015; Begum and Tamilselvi 2016; Bamisile *et al*. 2018; Dash *et al*. 2018; Jaber and Ownley 2018; Liu *et al*. 2022). Ultimately, endophytically induced changes in host plant physiology can alter herbivore population dynamics, creating potentially useful applications in biological pest control (Zahedi *et al*. 2019).

One of the most effective entomopathogens is *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin (Hypocreales: Cordycipitaceae) which is used for the biological control of a wide range of herbivorous pests (Inglis *et al.* 2001; Humber 2012). Colonization efficacy of this entomopathogen, and the end effect on plant health and growth depend on many factors, including inoculation method, fungal strain, plant species and cultivar, environmental conditions and competing rhizosphere and endosphere microorganisms (Vega 2008; Rodriguez *et al.* 2009; Tefera and Vidal 2009; Yan *et al.* 2015; Bamisile *et al*. 2018; Rajab *et al*. 2020; Yerukala *et al*. 2022; Wilberts *et al.* 2023). While previous research sheds light on the ability of *B. bassiana* to colonize and persist in cucumber plants, promote their growth, and increase their tolerance to the destructive piercing-sucking pest *Aphis gossypii* under controlled conditions (Rajab *et al.* 2020; Shaalan *et al*. 2021; Homayoonzadeh *et al.* 2022), the possible endophytic behavior of this fungus and its impact on cucumber plants under the stressful natural greenhouse environment, or even against leafmining pests of cucumber remained unknown.

Our current study examined the ability of the entomopathogenic fungus *B. bassiana* to colonize cucumber crops and promote plant growth under real greenhouse conditions, as well as its efficacy against *L. sativae* in a controlled laboratory set up. In addition, to assess whether these effects are mediated by fungal strain and inoculation method, we tested two strains, one sourced from soil and the other from cucumber tissues, by two different inoculation methods, foliar spray and root soaking.

Materials and Methods

Biological material

Plant material

The cucumber hybrid "Raade F1" was used in this study (Elite Plant-Breeding and Seeds Company, Russia). Prior to each experiment, seeds were surface sterilized by immersing in 2% sodium hypochlorite (NaClO, TM MEDIA®, Titan Biotech Ltd.) for 3 min, 70% ethanol for 1 min, and finally rinsing three times in sterilized distilled water. One hundred µl of the final rinsed water was incubated on Potato Dextrose Agar (PDA; TM MEDIA®, Titan Biotech Ltd., India) plates at 25 ± 1°C in the dark for 2 weeks to confirm the success of the surface sterilization procedure. Also, 15 seeds selected randomly were incubated on PDA plates under the same conditions. Seeds were used when no fungal growth was observed on the last rinsed water plates and when there was no *Beauveria* growth or any saprophytic fungus on the seed check plates.

Fungal strains

Two strains of *B. bassiana* were used. The soil-sourced strain, BS195, was isolated from olive orchard soil at Al-Shabatliyah (Latakia, Syria) using the *Galleria* bait method, which was described by Zimmermann (1986) and Meyling (2007), while the endophytic strain, BNE20, was isolated from the stems of cucumber plants grown in a greenhouse at Al-Kharab (Tartus, Syria) incubated on PDA plates after surface sterilization as described by Rajab *et al.* 2023). Both strains were

identified morphologically and molecularly [the accession numbers are OM302229 (ITS: the nuclear ribosomal internal transcribed spacer region) and OP573422 (TEF: the translation elongation factor 1 alpha) for BS195 and OM302228 (ITS) and OP573421 (TEF) for BNE20], and their ability to colonize cucumber plants after artificial inoculation has been demonstrated under laboratory conditions (Rajab *et al.* 2020, 2023, 2024).

Insects

A laboratory colony was initiated using pupae of *Liriomyza* that were collected from a greenhouse cultivated with cucumber plants in Talsnon, Tartus governorate (34°40'37.4"N, 36°06'00.4"E, 43.8 m a.s.l.). The species was identified as *L. sativae* by Prof. Dr. Hasan Sungur Civelek (Mugla University, Turkey) (Civelek 2002). Insects were reared using cucumber plants in small cages (50 × 50 × 45 cm) for several generations before the start of the experiment. Cotton wool balls soaked in sugar solution (10%) were placed at the bottom corners of the rearing cages for adult feeding.

Inoculum and plant inoculation methods

Fourteen-day-old colonies grown on PDA were flooded with 10 ml of sterile distilled water containing 0.05% Tween 80 and 2% of carboxy methyl cellulose (CMC). The colonies' surface was gently scraped off using a sterile syringe to ensure maximum conidial harvesting, then filtered through sterile muslin to remove any mycelial fragments, and homogenized with a magnetic stirrer for 10 min (Inglis *et al*. 2012). Suspension concentration was calculated using a Malassez counting chamber, then adjusted to 1×10^7 spores · ml^{-1}. Conidial viability for each fungal strain was determined prior to application based on germ tube formation and used if the viability was 90% and above. Conidium was considered to be germinated when it had a germ tube at least two times the length of the conidia.

The fungal spore suspension was applied following two main methods: 1) root soaking (rs); and 2) foliar spray (fs). Surface-sterilized seeds were planted in cork seed trays using potting soil (Floragard®, Germany). For root soaking treatments, seedlings of the first true leaf were uprooted and soaked in the fungal suspension of each strain for 2 h in the dark at room temperature, then transferred to disinfected plastic pots containing potting soil. In foliar spray treatments, first-true-leaf seedlings were uprooted from the cork seed trays, transferred to disinfected plastic pots, and then sprayed with 5 ml per seedling of the fungal suspension using a hand sprayer after covering the pot surface with polyethylene slides to avoid runoff of the conidial spores into the soil. Control plants of each treatment were prepared without the fungus. Potted plants from all treatments were covered with plastic bags for 24 h to maintain a sufficient level of humidity.

Endophytic activity of *Beauveria bassiana* and its impact on cucumber plant growth in a greenhouse

Growth conditions

Plants from all treatments were maintained in an experimental greenhouse (the Faculty of Agriculture, Tishreen University, Latakia, Syria) with natural environmental conditions (temperature, relative humidity, and light/dark cycle). The greenhouse was equipped with four tables (1 × 2 m), and each table was covered with micro-hole mesh to avoid insect attacks during the experiment. Seedlings were watered with tap water as needed, and allowed to grow until the sampling date. There was no fertilization throughout the experiment. Each treatment had a total of 20 plants, five of which were randomly sampled for fungal colonization assessment and plant biochemical parameters 30 days post inoculation (dpi), and the remaining 15 plants were harvested to study plant growth parameters 36 dpi. The treatments were arranged inside the greenhouse in a completely randomized design. The experiment was replicated twice. The first was conducted in September–October 2021, and the second was conducted in July–August 2022. The temperature (min. and max.) and the relative humidity (RH) (min. and max.) were recorded daily using a digital thermo hygrometer (HTC-01, China) (Table S1).

Fungal colonization assessment

To assess the fungal colonization in cucumber plants, six random sections of all the stems, leaves, and roots for each of the five replicates were incubated on PDA plates after the surface sterilization process (described in detail in Rajab *et al.* 2023). The fungal colonization rate (%) was calculated according to Petrini and Fisher (1986):

$$\text{Colonization rate [\%]} = \\ = \frac{\text{number of plant discs showing the fungal growth}}{\text{the total number of plant discs}} \times 100.$$

Plant biochemical parameters

The photosynthetic pigments, total phenolic content, and salicylic acid levels were evaluated to assess the effect of *B. bassiana* on the chemical activity in cucumber plants 30 dpi.

The content of chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Tot_Chl), and carotenoid (Car) were measured according to Lichtenthaler (1987) using pure acetone (PANREAC®) as the extraction solvent. Fresh leaves (0.1–0.12 g) were ground

in Eppendorf tubes (2 ml) and homogenized in pure acetone (1.5 ml) and left in complete darkness for 2 h at 4°C. Chl a, Chl b, and Car absorbencies were measured at 663, 645, and 470 nm, respectively, immediately after extraction using a spectrophotometer (Biochrom Libra S22 Ltd., UK). Pure acetone was used for the blank solution, and measurement units were expressed as μg · g^{-1} of fresh weight using the equations given by Lichtenthaler (1987).

The total phenolic content (TPC) of cucumber leaves was calculated by the Folin-Ciocalteu method (Ainsworth and Gillespie 2007). Fresh cucumber leaves (50–60 mg) were ground with 1 ml of pure methanol. Two hundred μl of the diluted sample were added to 1 ml of Folin-Ciocalteu reagent (10% in sterile distilled water). After 5 min, 3 ml of saturated sodium carbonate, Na_2CO_3 (20%) was added. After 2 h of incubation at room temperature, the absorbance at 750 nm was measured using a spectrophotometer. To calculate the TPC of samples, gallic acid (16–20 mg · ml^{-1}) was used for the standard calibration curve, and the results were reported in μg of gallic acid equivalent per 1 g of fresh weight.

To measure the salicylic acid (SA) content in sampled cucumber leaves, standards of varying concentrations of the SA were prepared as described in Warrier *et al.* (2013), and the extraction was performed according to Warrier *et al.* (2013) with a slightly modified protocol. Five hundred grams of fresh leaves were ground with 10 ml of distilled water. Samples were exposed to ultrasonic waves for 15 min, followed by centrifugation at 5000 rpm for 10 min at 15°C. One hundred μl of the supernatant was mixed with 3 ml of freshly prepared ferric chloride (0.2%). The absorbance of the complex formed between the Fe^{3+} ion and SA was determined at 540 nm using a spectrophotometer and the results were reported in μg · mg^{-1}.

Plant growth parameters

Greenhouse cucumber plants were uprooted 36 dpi, and the number of flowers, fruits, and fully developed leaves was counted for each tested plant. Plant height (the distance from the stem base to its tip), root length, leaf area [calculated using the gravimetric method (Taha and Osman 2018)], fresh shoot weight, and fresh root weight were measured. To determine the weight of dry matter, which includes both shoots and roots, the plant material was placed in individual paper bags and dried in an oven at 65°C for 96 h. The dry matter content (DMC) was expressed in percentage of the fresh shoot weight. Leaf area ratio (LAR) was calculated as the ratio between leaf area and the total plant dry weight (shoots and roots) and reported in $cm^2 \cdot g^{-1}$ (Baligar *et al.* 2020).

Effect of *Beauveria bassiana* application on cucumber infestation with *Liriomyza sativae*

The effects of *B. bassiana* strains on the populations of *L. sativae* on cucumber plants were studied using spore suspension by the following methods: root soaking (rs); foliar spray prior to insect release (fs1); and foliar spray following insect release (fs2), with a control treatment for each method. Details of root soaking and foliar spray methods were described in the "Inoculum and plant inoculation methods" section.

Plants were placed on benches in a large microperforated cage (210 × 120 × 240 cm) at 24 ± 3°C and 60 ± 20% RH. Pupae were collected from rearing cages in small containers (1 l), covered with mesh, and supplied with cotton wool balls soaked in sugar solution (10%) for emerging adults feeding. Containers were put in the middle of the cage at an approximately equal distance from all benches. Two weeks post-fungal treatments, 100 2-day-old adults (mixed females and males of undetermined proportions) were released in the cage and left to mate and oviposit. The treatment of foliar spray after insect release was applied 7 days after insect release. The treatments were arranged in a completely randomized design. Ten plants were planted for each treatment (90 plants in total).

The incidence (the number of infested plants/total number of plants × 100), infestation (the number of the infested leaves/the total number of leaves × 100) and severity, expressed as percentages, were calculated 35 and 51 days post insect release. Severity was evaluated according to the percentage of the leaf area which had tunnels in addition to the number of destroyed leaves (Fig. 1) using a scale of nine scores as described by Singh and Weigand (1994), slightly modified: 1 (no tunnels, leaves free from any damage), 2 (tunnels in less than 10% of the leaves after careful observation), 3 (tunnels in 11–20% of the leaves, no destroyed leaves), 4 (tunnels in 21 to 30% of the leaves, no destroyed leaves), 5 (tunnels in 31 to 40% of the leaves, some destroyed leaves in the lower half of plants), 6 (many tunnels in 41 to 50% of the leaves, destroyed leaves of 10% lower leaves), 7 (many tunnels in 51 to 70% of the leaves, destroyed leaves of 11–20% lower and upper leaves), 8 (many tunnels in 71 to 90% of the leaves, destroyed leaves of 21–30%), and 9 (many tunnels in more than 91% of the leaves and destroyed leaves greater than 31%). Leaves without any damage (score 1) were not included in the calculation.

On each sampling date, pupae were harvested from leaves using a fine brush, counted, placed on sterile, moist filter paper inside Petri dishes, and incubated

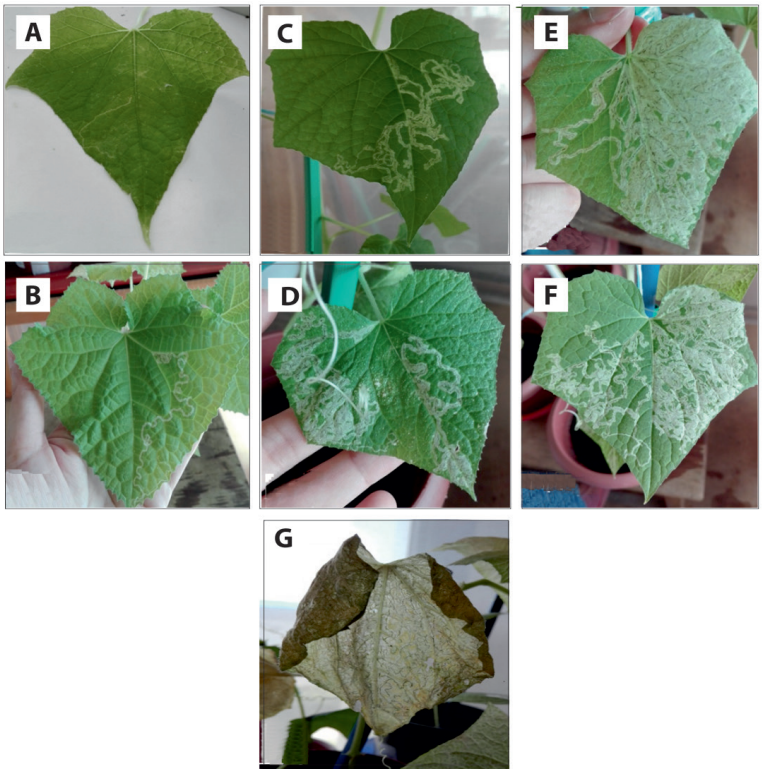

Fig. 1. Severity of cucumber infestation with *Liriomyza sativae* (the area of cucumber leaves which had *L. sativae* tunnels). A – tunnels in less than 10% of the leaves after careful observation, B – tunnels in 11–20% of the leaves, C – tunnels in 31 to 40% of the leaves, D – many tunnels in 41 to 50% of the leaves, E – many tunnels in 51 to 70% of the leaves, F – many tunnels in 71 to 90% of the leaves, and G – many tunnels in more than 91% of the leaves

at 25 ± 1°C until either adult emergence or the appearance of fungal growth on the pupae surface. On the second sampling date (51 days post insect release = 65 dpi), five plants were randomly selected for the fungal colonization assessment as described in the "Fungal colonization assessment" section.

Statistical analysis

Data were tested for normality and homogeneity of the variance using Shapiro-Wilkes and Levine's test, respectively. With the resulting probability of $p \leq 0.05$, data were transformed with a natural logarithm function [ln (y) = Log (y + 1)] to correct for heterogeneity of the variance and produce approximately normally distributed data sets. Data from all experiments were subjected to two-way ANOVA. Means were separated using Tukey's HSD test when a significant *F* test was obtained at $p \leq 0.05$. Statistical analysis of the greenhouse experiments was performed for each dataset separately, as experiments were performed in different years. Data were statistically analyzed using R version 4.3.1 (R core team 2023).

Results

Endophytic activity of *Beauveria bassiana* and its impact on cucumber plant growth in greenhouse

Fungal colonization assessment

Both fungal strains, BS195 and BNE20, of *B. bassiana* were able to colonize the stems, leaves, and roots of cucumber plants 30 dpi under greenhouse conditions, with no *B. bassiana* growth on control plates.

The fungus was more successfully delivered to the plant tissues when the plant roots were soaked in the fungal suspension. In both experiments, the introduction of *B. bassiana* through roots resulted in significantly higher colonization rates in each plant part than the foliar spray ($p \leq 0.0001$), which failed in most treatments to cause substantial fungal colonization (Tables 1, S2). There were no significant differences in the colonization efficacy between the two strains. However, strain BS195 generally caused higher colonization rates than strain BNE20 in the stems and leaves in both experiments, regardless of the application method.

Table 1. The effects of using different methods of *Beauveria bassiana* application (strains BS195 and BNE20) under greenhouse conditions on plant colonization of cucumber, 30 days post-treatment

Treatment	Application method	Colonization rate (Mean ± SE) [%]					
		experiment 1			experiment 2		
		stems	leaves	roots	stems	leaves	roots
BS195	rs	96.67 ± 3.33 a	83.33 ± 7.45 a	96.67 ± 3.33 a	44.67 ± 11.33 a	79.99 ± 9.72 a	49.99 ± 12.91 a
	fs	43.33 ± 11.3 b	36.67 ± 6.24 b	3.33 ± 3.33 b	9.99 ± 6.67 b	19.99 ± 6.23 b	6.67 ± 4.08 b
BNE20	rs	89.99 ± 4.08 a	76.67 ± 10 a	86.67 ± 6.24 a	53.33 ± 6.24 a	76.67 ± 8.49 a	43.33 ± 4.08 a
	fs	13.33 ± 6.24 c	26.67 ± 10 bc	9.99 ± 6.67 b	6.67 ± 4.08 b	9.99 ± 6.67 b	0 b
Control	rs	0 c	0 c	0 b	0 b	0 b	0 b
	fs	0 c	0 c	0 b	0 b	0 b	0 b

Mean followed by the same letter in the same column are not significantly different at $p = 0.05$ (Tukey's HSD test after two-way ANOVA. Abbreviations: rs – root soaking; fs – foliar spray

Plant biochemical parameters

Application of the spore suspension of *B. bassiana*, regardless of the specific strain or the inoculation method used, had no significant effects on the levels of Chl a, Chl b, Tot_Chl, Car, or SA inside cucumber plants. However, the effect of treatment on TPC was significant in both experiments (EXP1: $df = 2$; $F = 7.03$; $P = 0.004$; and EXP2: $df = 2$; $F = 9.67$; $P = 0.0008$), but not the application method nor the interaction between the two factors (Tables 2, S2).

Plant growth parameters

The effects of treatment, inoculation method and their interaction were significant for most of the growth parameters of cucumber plants (Table S3). Inoculating cucumber plants with strain BS195 significantly increased the plant height (rs), the root length (rs), the number of leaves (rs or fs), the leaf area (rs), the fresh shoot weight (rs), the fresh root weight (rs or fs), the dry shoot weight (rs), dry root weight (rs) and the content of DMC (fs) in at least one experiment (Tables 3, 4, S3). Strain BNE20 did not cause any significant enhancement in plant growth compared with the control plants, except for the DMC in the first experiment using the root soaking method.

In both experiments there were no significant differences in the number of leaves, flowers, or the LAR (Tables 3, 4, S3).

Effect of *Beauveria bassiana* application on cucumber infestation with *Liriomyza sativae*

The effects of treatment, inoculation method and their interaction were significant on infestation, severity, number of pupa, and adult emergence of *L. sativae* 35 and 51 days post insect release. However, there were no significant differences in incidence of *L. sativae* regardless of the strain or the application method used (Tables 6, S4).

Cucumber plants treatment with *B. bassiana* using either strain by root soaking or foliar spray following insect release significantly reduced infestation and severity of *L. sativae* 35 and 51 days post insect release, with higher efficiency to the root soaking method ($df = 2$; $F = 4.97$; $P = 0.009$; and $df = 2$; $F = 86.34$; $P \leq 0.0001$, 35 and 51 days after insect release, respectively, for the infestation and $df = 2$; $F = 2.78$; $P = 0.068$; and $df = 2$; $F = 4.04$; $P = 0.02$, 35 and 51 days after insect release, respectively, for the severity) (Table S4). Although the application of *B. bassiana* by foliar spray prior to insect release also reduced the infestation and severity of *L. sativae* on sampling dates, this reduction was not significant when compared to the respective controls (Fig. 2).

Exposure of cucumber plants to either strain of *B. bassiana* by all three inoculation methods significantly reduced the number of pupae 35 and 51 days post insect release, and adult emergence 51 days post insect release. However, only the root soaking method had a significant effect on adult emergence 35 days post insect release regardless of the strain ($df = 2$; $F = 14.75$; $P \leq 0.0001$, 35 days after insect release) (Table S4).

In general, the number of pupae and the percentage of adult emergence were less on plants inoculated through the roots compared to foliar spray, but there were no significant differences between the two fungal strains (Fig. 3, Table S4). No fungal growth was observed on any *L. sativae* individuals in this experiment.

A number of dead larvae were observed in the tunnels on plants sprayed foliarly with fungal suspension (three dead larvae in plants sprayed with BNE20 prior to insect release, two dead larvae in plants sprayed with BNE20 following insect release, and three

Table 2. The effects of *Beauveria bassiana* application (strains BS195 and BNE20) using different methods under greenhouse conditions on biochemical parameters of cucumber, 30 dpi

Treatment	Application method	Mean ± SE											
		experiment 1						experiment 2					
		Chl a	Chl b	Tot_Chl	Car	TPC	SA	Chl a	Chl b	Tot_Chl	Car	TPC	SA
BS195	fs	612.88 ± 25.39 a	369.65 ± 10.12 a	997.35 ± 29.35 a	48.09 ± 3.16 a	34.42 ± 1.13 ab	21.51 ± 0.32 a	615.41 ± 56.82 a	325.79 ± 28.64 a	941.21 ± 82.31 a	78.15 ± 8.92 a	23.08 ± 1.77 abc	18.74 ± 1.23 a
	rs	561.16 ± 39.43 a	334.29 ± 25.97 a	903.95 ± 71.35 a	48.17 ± 5.03 a	35.57 ± 2.29 ab	22.28 ± 0.53 a	549.78 ± 42.8 a	287.43 ± 18.27 a	837.21 ± 31.35 a	67.38 ± 20.26 a	26.64 ± 1.34 a	17.43 ± 0.81 a
BNE20	fs	611.45 ± 32.54 a	340.91 ± 11.43 a	952.36 ± 36.74 a	63.36 ± 16.45 a	35.04 ± 2.19 ab	21.05 ± 0.29 a	624.63 ± 5.36 a	339.94 ± 29.65 a	964.57 ± 32.42 a	70.78 ± 12.49 a	23.71 ± 1.37 abc	17.56 ± 0.92 a
	rs	521.88 ± 46.91 a	339.42 ± 26.62 a	861.29 ± 67.3 a	42.68 ± 10.78 a	38.19 ± 1.76 a	22.05 ± 0.41 a	567.35 ± 65.85 a	319.97 ± 25.26 a	887.32 ± 84.39 a	74.84 ± 14.21 a	26.18 ± 1.45 ab	18.58 ± 0.83 a
Control	fs	574.04 ± 37.27 a	311.66 ± 14.12 a	885.69 ± 46.37 a	73.89 ± 11.33 a	31.42 ± 1.89 ab	22.27 ± 0.51 a	581.87 ± 34.49 a	304.98 ± 16.19 a	886.85 ± 35.45 a	88.27 ± 11.82 a	20.002 ± 0.51 bc	15.55 ± 0.39 a
	rs	516.86 ± 17.97 a	302.03 ± 14.12 a	810.38 ± 20.49 a	30.71 ± 2.48 a	29.16 ± 0.66 b	21.24 ± 0.36 a	583.45 ± 8.37 a	304.16 ± 17.25 a	887.61 ± 22.92 a	78.35 ± 10.45 a	18.54 ± 1.99 c	16.86 ± 1.28 a

Mean followed by the same letter in the same column are not significantly different at $p = 0.05$ (Tukey's HSD test after two-way ANOVA). Abbreviations: rs – root soaking; fs – foliar spray; Chl a – chlorophyll a; Chl b – chlorophyll b; Tot_Chl – total chlorophyll; Car – carotenoid; TPC – total phenolic content; SA – salicylic acid

Table 3. The effects of *Beauveria bassiana* application (strains BS195 and BNE20) using different methods under greenhouse conditions on plant growth parameters of cucumber, 36 dpi in the first experiment

Treatment	Application method	Mean ± SE											
		plant height [cm]	root length [cm]	the number of leaves	the number of fruits	the number of flowers	leaf area [cm^2]	fresh shoot weight [g]	fresh root weight [g]	dry shoot weight [g]	dry root weight [g]	DMC [%]	LAR [$cm^2 \cdot g$]
BS195	fs	93.97 ± 4.81 abc	20.26 ± 1.73 ab	23.67 ± 1.47 a	19.07 ± 0.86 a	12.73 ± 0.76 a	87.202 ± 8.03 b	83.45 ± 10.09 b	30.44 ± 1.97 a	14.37 ± 1.55 abc	1.35 ± 0.12 ab	17.69 ± 0.99 a	7.16 ± 1.52 a
	rs	121.93 ± 9.9 a	26.59 ± 1.88 a	23.4 ± 1.12 a	21.93 ± 2.25 a	16.13 ± 1.38 a	126.1 ± 8.84 a	149.47 ± 13.98 a	37.67 ± 4.11 a	19.36 ± 1.72 a	2.48 ± 0.37 a	13.14 ± 0.67 bc	6.41 ± 0.65 a
BNE20	fs	78.02 ± 7.87 bc	19.59 ± 1.94 ab	18.6 ± 1.27 ab	19.47 ± 0.72 a	13.47 ± 0.72 a	97.96 ± 6.66 ab	92.26 ± 8.78 b	19.03 ± 2.18 b	10.85 ± 1.03 bc	1.63 ± 0.19 ab	11.84 ± 0.39 c	8.88 ± 0.91 a
	rs	108.23 ± 8.09 ab	21.49 ± 2.02 ab	23.47 ± 1.12 a	20.73 ± 0.75 a	16 ± 0.7 a	117.79 ± 6.03 ab	116 ± 12.98 ab	20.54 ± 2.42 b	16.75 ± 1.82 ab	1.76 ± 0.21 ab	15.09 ± 0.89 ab	7.49 ± 0.76 a
Control	fs	77.39 ± 10.9 c	17.63 ± 1.71 b	17.4 ± 0.49 b	18.8 ± 1.01 a	12.8 ± 1.01 a	114.88 ± 7.74 ab	94.06 ± 13.42 b	19.68 ± 1.98 b	10.81 ± 1.59 c	1.37 ± 0.29 b	11.48 ± 0.21 c	11.7 ± 1.62 a
	rs	79.88 ± 3.47 bc	17.59 ± 1.69 b	18.8 ± 2.01 ab	17.27 ± 1.36 a	14.47 ± 2.41 a	95.84 ± 10.93 ab	97.13 ± 4.58 b	16.25 ± 1.56 b	10.85 ± 0.51 bc	1.39 ± 0.13 ab	11.17 ± 0.001 c	7.83 ± 0.88 a

Mean followed by the same letter in the same column are not significantly different at $p = 0.05$ (Tukey's HSD test after two-way ANOVA). Abbreviations: rs – root soaking; fs – foliar spray; DMC – the dry matter content; LAR – leaf area ratio

Table 4. The effects of *Beauveria bassiana* application (strains BS195 and BNE20) using different methods under greenhouse conditions on plant growth parameters of cucumber, 36 dpi in the second experiment

Treatment	Application method	Mean ± SE											
		plant height [cm]	root length [cm]	the number of leaves	the number of fruits	the number of flowers	leaf area [cm^2]	fresh shoot weight [g]	fresh root weight [g]	dry shoot weight [g]	dry root weight [g]	DMC [%]	LAR [$cm^2 \cdot g^{-1}$]
BS195	fs	53.23 ± 2.76 a	17.7 ± 1.91 a	11.13 ± 0.79 bc	8.87 ± 0.67 a	16.07 ± 0.86 a	43.22 ± 3.62 bc	46.69 ± 2.25 ab	14.24 ± 2.22 a	6.88 ± 0.28 ab	1.04 ± 0.19 a	14.82 ± 0.17 a	5.56 ± 0.49 b
	rs	63.06 ± 2.26 a	20.21 ± 1.86 a	17.47 ± 0.76 a	11.4 ± 1.53 a	18 ± 1.64 a	74.02 ± 3.72 a	57.41 ± 4.29 a	21.19 ± 2.36 a	7.52 ± 0.53 a	1.66 ± 0.23 a	13.19 ± 0.33 b	8.603 ± 0.71 a
BNE20	fs	55.99 ± 1.98 a	15.46 ± 1.75 a	12.47 ± 0.76 b	9.53 ± 0.71 a	16.47 ± 0.72 a	54.76 ± 4.43 b	47.18 ± 1.49 ab	21.02 ± 3.12 a	6.93 ± 0.22 ab	1.702 ± 0.25 a	14.7 ± 0.21 a	6.36 ± 0.46 ab
	rs	52.39 ± 2.84 a	15.36 ± 1.38 a	14.07 ± 1.35 b	10 ± 0.73 a	16.8 ± 0.75 a	55.94 ± 6.71 bc	47.25 ± 2.85 ab	19.31 ± 2.74 a	7.04 ± 0.41 ab	1.41 ± 0.25 a	14.95 ± 0.18 a	6.69 ± 0.75 ab
Control	fs	54.39 ± 3.99 a	17.57 ± 1.49 a	8.8 ± 0.43 c	8.8 ± 1.01 a	15.8 ± 1.01 a	38.85 ± 1.95 c	47.48 ± 3.68 ab	22.36 ± 2.64 a	6.86 ± 0.46 b	1.24 ± 0.29 a	14.61 ± 0.2 a	5.12 ± 0.34 b
	rs	55.22 ± 2.16 a	20.25 ± 1.21 a	12.53 ± 0.69 b	10.93 ± 2.37 a	17.47 ± 2.41 a	54.33 ± 3.57 b	41.18 ± 2.19 b	19.47 ± 2.48 a	5.84 ± 0.22 ab	1.44 ± 0.22 a	14.42 ± 0.39 a	7.47 ± 0.38 a

Mean followed by the same letter in the same column are not significantly different at $p = 0.05$ (Tukey's HSD test after two-ways ANOVA). Abbreviations: rs – root soaking; fs – foliar spray; DMC – the dry matter content; LAR – leaf area ratio

Table 5. The effects of application of *Beauveria bassiana* (strains BS195 and BNE20) using different methods on the plant colonization 65 dpi (51 days post-insect-release)

Treatment	Application method	Colonization rate (Mean ± SE) [%]		
		stems	leaves	roots
BS195	rs	33.33 ± 9.13 ab	36.67 ± 12.25 ab	33.33 ± 9.13 a
	fs1	3.33 ± 3.33 c	6.67 ± 4.08 b	0 b
	fs2	9.99 ± 6.67 bc	9.99 ± 4.08 ab	0 b
BNE20	rs	43.33 ± 6.67 a	36.67 ± 11.06 a	26.67 ± 6.67 a
	fs1	9.99 ± 6.67 bc	9.99 ± 6.67 ab	0 b
	fs2	6.67 ± 4.08 c	3.33 ± 3.33 b	6.67 ± 4.08 b
Control	rs	0 c	0 b	0 b
	fs1	0 c	0 b	0 b
	fs2	0 c	0 b	0 b

Mean followed by the same letter in the same column are not significantly different at $p = 0.05$ (Tukey's HSD test after two-way ANOVA). Abbreviations: rs – root soaking; fs1 – foliar spray prior to insect release; fs2 – foliar spray following insect release

Table 6. The effects of application of *Beauveria bassiana* (strains BS195 and BNE20) using different methods on the percentage incidence of *Liriomyza sativae* 35 and 51 days post-insect-release

Treatment	Application method	The incidence (Mean ± SE) [%]	
		35 days post-insect-release	51 days-post-insect release
BS195	rs	80 ± 13.33 a	90 ± 10 a
	fs1	80 ± 13.33 a	100 ± 0 a
	fs2	100 ± 0 a	100 ± 0 a
BNE20	rs	70 ± 15.28 a	80 ± 13.33 a
	fs1	70 ± 15.28 a	90 ± 10 a
	fs2	80 ± 13.33 a	70 ± 15.28 a
Control	rs	100 ± 0 a	100 ± 0 a
	fs1	90 ± 10 a	90 ± 10 a
	fs2	100 ± 0 a	100 ± 0 a

Mean followed by the same letter in the same column are not significantly different at $p = 0.05$ (Tukey's HSD test after two-way ANOVA). Abbreviations: rs – root soaking; fs1 – foliar spray prior to insect release; fs2 – foliar spray following insect release

dead larvae in plants sprayed with BS195 following insect release).

There was no fungal colonization in the control plants, in the roots of plants exposed to foliar spray before insect release using either strain, or in the roots of plants exposed to foliar spray after insect release using BS195. Generally, the fungal colonization rates in the stems and leaves of plants foliarly sprayed with the fungal suspension was less than 10%. The endophytic colonization of stems, leaves, and roots was significantly higher in root soaking treatments 65 dpi ($df = 2$; $F = 15.59$; $P \leq 0.0001$ for stem colonization; $df = 2$; $F = 9.45$; $P = 0.0005$ for leaf colonization, and $df = 2$; $F = 22.46$; $P \leq 0.0001$ for root colonization) (Tables 5, S4).

Discussion

Endophytic hypocrealean entomopathogens have multiple functions that go beyond pest control. This versatility opens the door for new techniques and applications in integrated pest management and crop production for greenhouse crops (Ownley *et al.* 2010; Quesada-Moraga *et al.* 2020). *Beauveria bassiana* is a widely used biological control agent with very low host specificity. It acts through multiple mechanisms of action that are classified into many categories, including antibiosis by releasing a broad spectrum of secondary metabolites, direct parasitism, competition, inducing systemic resistance, and promoting plant

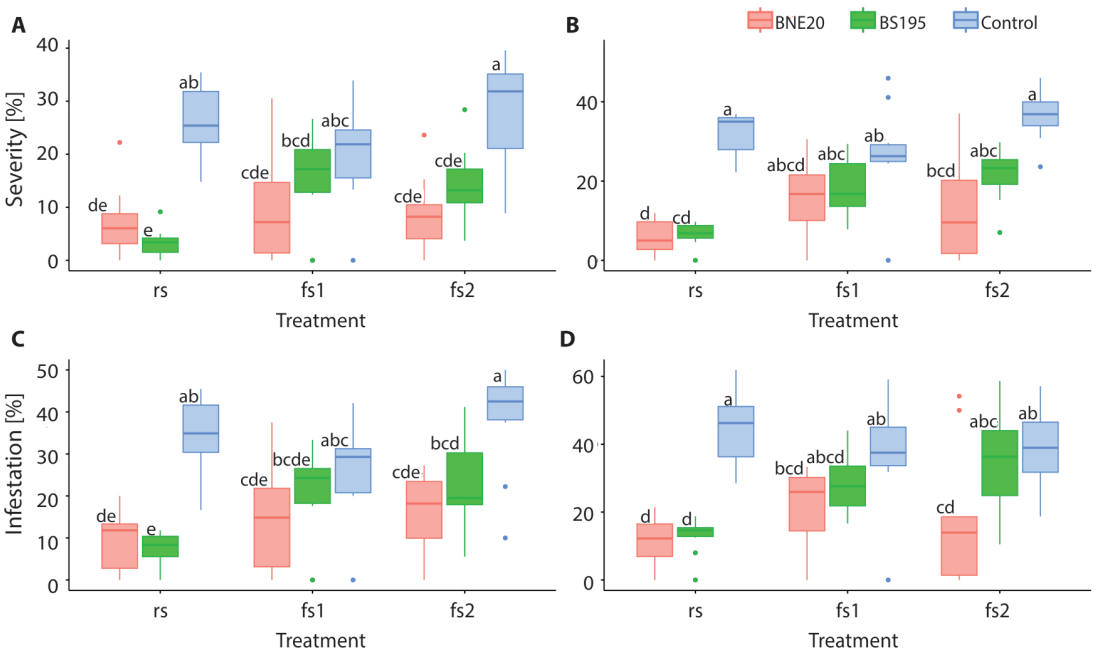

Fig. 2. The effects of *Beauveria bassiana* application (strains BS195 and BNE20) using different methods on the percentage of infestation and severity by *Liriomyza sativae* on cucumber, A and C – severity and infestation (%) after 35 days post-insect-release, respectively; B and D – severity and infestation (%) after 51 days post-insect-release, respectively. Abbreviations: rs – root soaking; fs1 – foliar spray prior to insect release; fs2 – foliar spray following insect release

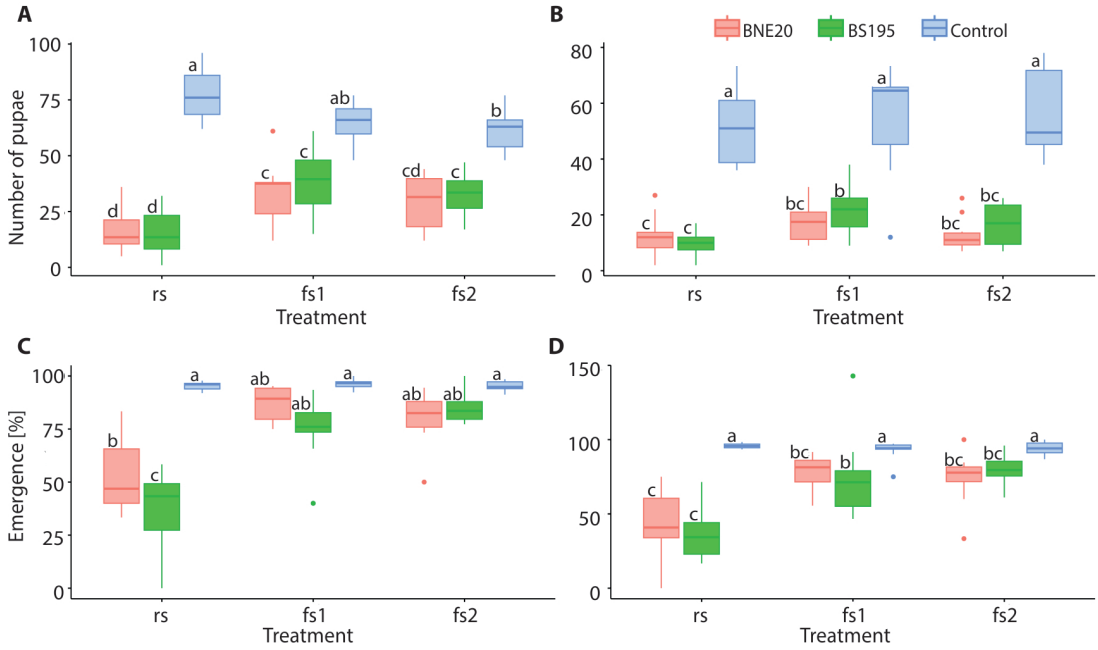

Fig. 3. The effects of *Beauveria bassiana* application (strains BS195 and BNE20) using different methods on the number of pupae and the percentage of adult emergence of *Liriomyza sativae*, 35 and 51 days post-insect-release. Abbreviations: rs – root soaking; fs1 – foliar spray prior to insect release; fs2 – foliar spray following insect release

growth (Vega 2008, 2018; Vega *et al.* 2008; Begum and Tamilselvi 2016; Card *et al.* 2016; Bamisile *et al.* 2018). Our study demonstrated the ability of *B. bassiana* to colonize, promote, and protect cucumber plants from the agromyzid leafminer *L. sativae*. However, the growth and defense enhancement capabilities of this entomopathogen were dependent on the fungal strain and inoculation method used.

Both *B. bassiana* strains colonized the tissues of all cucumber plant parts endophytically 30 dpi by root soaking and foliar spray in an uncontrolled greenhouse microclimate. They moved systemically from the point of inoculation (i.e., roots or leaves) to the other plant parts, with root soaking being significantly better than foliar spray, achieving the highest colonization rates (96.67, 83.33, and 96.67%, for the stems, leaves, and roots, respectively) using the spore suspension of strain BS195. The higher colonization rates with the soil-sourced strain, BS195, compared to the endophytic one, BNE20, could be attributed to the different genetic composition of the fungal strains. Our previous work demonstrated the ability of the same *B. bassiana* strain (BS195) to systemically colonize and persist in cucumber plants through five inoculation methods (seed dusting, seed immersion, soil drench, seedling drench, and foliar spray) under laboratory conditions. Soil drench after sowing provided the highest recovery rates (94.44, 80.25, and 68.26%, for stems, leaves, and roots, respectively), while foliar spray gave the lowest rates (Rajab *et al*. 2020). The high colonization intensity following root soaking compared to other inoculation methods could be due to increased opportunities for infection with *B. bassiana* and may account for the notable differences in promoting plant growth and reducing the agromyzid infestation between application methods tested.

The present study showed that the application of the strain BS195 of *B. bassiana*, mainly through root soaking, increased many morphological growth and biomass parameters (plant height, root length, the number of leaves, leaf area, fresh and dry weight and the content of dry matter) 36 dpi. *Beauveria bassiana* has been reported as a PGP in cucumber plants by other authors. Shaalan *et al*. (2021) examined the number of leaves, flowers, and fruits, and plant height 49 days after fungal seed treatment under natural environmental conditions in non-sterile substrate. Homayoonzadeh *et al*. (2022) studied its effects on plant height, stem diameter, number of nodes/plant, and total yield (kg fresh weight of fruit per plant) of cucumber plants 28 days after foliar application under controlled greenhouse conditions. Both authors reported an enhancement of most of the studied parameters.

Enhanced levels of phenols, hydrogen peroxide, flavonoids, alkaloids, and total chlorophyll have also been reported in cucumber plants after foliar inoculation with *B. bassiana* (Homayoonzadeh *et al*. 2022). In our study, inoculation with *B. bassiana* only raised the total phenolic content in cucumber plants. Other studied chemical parameters such as the content of Chl a, Chl b, Tot_Chl, Car, and SA, were not significantly affected by the application of the fungus. The increase in TPC content in plants after exposure to the fungus is considered a good indicator of plant protection; the higher the level of phenolic compounds present, the better the plant's defense against various threats. The phenolic compounds show antioxidant and antimicrobial activity against a wide spectrum of bacteria and fungi, in addition to protecting plants from the effects of adverse environmental conditions (Silva *et al*. 2007; Vlase *et al*. 2012). The TPC, SA, and the levels of the other chemical compounds in plants are affected by the secondary metabolites produced by the fungus. In general, the chemical activities of fungi seem to be affected by the competitive environment in which they live (Hanson 2008).

In the present study, differences were observed in colonization and plant growth promoting abilities between the two tested *B. bassiana* strains. These differences are expected to be due to the high genetic diversity in populations and communities of *B. bassiana* which may be reflected in their ecological roles including endophytic colonization, the spectrum of metabolites released, and their abilities as PGPs (Meyling and Eilenberg 2007; Rehner *et al*. 2011). The specific source of *B. bassiana* strains and isolates also highly affects their different activities. Plant colonization by *B. bassiana* was highest for isolates collected from insects compared to those isolated from plant and soil substrates (Yerukala *et al*. 2022; Wilberts *et al*. 2023). Our study also indicated differences in colonization rates and several plant growth promoting parameters between the two experiments. These inconsistencies may result from the variance in the respective environmental conditions, especially the high temperature in the second experiment (the maximum temperature ranged between 40.9 and 49.6°C), which does not favor the activity of *B. bassiana* (Hallsworth and Magan 1999; Yeo *et al*. 2003). Endophytic colonization by *B. bassiana* and the net effect on the host plant is influenced by differences in experimental conditions, characteristics of the host plant, and specific interactions between host and fungus as well as abiotic and biotic conditions (Yerukala *et al*. 2022).

The role of *B. bassiana* as a PGP is currently being extensively researched worldwide (Bamisile *et al*. 2018, Tall and Meyling 2018). Many studies show that *B. bassiana* is an affective agent in enhancing growth and productivity parameters of various plant species, such as cotton (Lopez and Sword 2015), common bean (Dash *et al*. 2018), tomato (Barra-Bucarei *et al*. 2020), wheat (Torkaman *et al*. 2023), grape vines (Rondot and Reineke 2019), maize (Liu *et al*. 2022), and sweet pepper (Wilberts *et al*. 2023). However, other studies, such as those conducted on corn (Lewis *et al*. 2001), soybean (Mandasari *et al*. 2015), and tomato (Silva *et al*. 2020) showed no significant effects of *B. bassiana* inoculation on plant growth.

Our results also showed the effective role of *B. bassiana* strains in protecting cucumber plants against

L. sativae infestation. Applying *B. bassiana* (either strain) significantly reduced the infestation and severity of *L. sativae* 35 and 51 days post adult release, in addition to reducing the number of pupae that were harvested from plant leaves and later adult emergence. Results showed that applying *B. bassiana* through root soaking was more effective in reducing the population of *L. sativae* on cucumber leaves than foliar spray. These differences in the effects of inoculation methods could be because of the lifestyle of leafminer larvae inside the tunnels until pupation, which protects them from direct application. On the other hand, the endophytic fungus may act by secreting secondary metabolic compounds and can function using antibiosis and feeding deterrents (Vega 2008; Vega *et al*. 2008). Several secondary metabolites synthesized by *Beauveria* species have shown toxicity against insects of different orders such as beauvericin, bassianolide, and beauverolides (Quesada-Moraga and Vey 2003; Valencia *et al*. 2011; Chebet *et al*. 2021).

Few studies have investigated the role of *B. bassiana* in regulating the populations of *Liriomyza* species. The ability of this entomopathogen to reduce adult longevity, the number of pupae, the infestation rate, and the adult emergence of the pea leafminer, *L. huidobrensis*, in each of the broad bean and common bean plants was demonstrated in the laboratory (Akutse *et al*. 2013) and in common bean in the field for *Liriomyza* spp. (Gathage *et al*. 2016). However, Gathage *et al*. (2016) found that the *B. bassiana*'s capability to colonize plant parts was not a necessary requirement for its ability to protect plants against agromyzid leafminer attacks. Later, Chebet *et al*. (2021) reported the larvicidal effects of the extract of common bean plants colonized by *B. bassiana* on the second instar larvae of *L. huidobrensis* in vitro. Some other leafminer insects with cryptic stages in their lifecycle have been shown to respond to *B. bassiana* as a plant colonizer and protector such as the tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) (Klieber and Reineke 2016; Silva *et al*. 2020; Zheng *et al*. 2023), and the horse-chestnut leafminer, *Cameraria ohridella* Deschka and Dimic (Lepidoptera: Gracillariidae) (Barta 2018).

The protected lifestyle of *L. sativae* larvae may also be the reason for the absence of mycosis and the direct mortality of insects in plants treated with fungal suspension (except a few larvae found dead in their tunnels). However, Akutse *et al*. (2013) reported 100% adult mortality of *L. huidobrensis* on *Vicia faba* plants endophytically colonized by different fungal isolates of *B. bassiana*, but no mycosis was observed. A few authors reported mycosis in different insects after feeding on plants inoculated with *B. bassiana*, such as Bing and Lewis (1993) who detected mycosis in just 2.5% of cadavers of the European corn borer larvae, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae), fed on corn plants colonized by *B. bassiana* applied foliarly, compared to 1.7% mycosis in control pants. Vidal and Jaber (2015) reported 25–85% mortality and 0–100% mycosis of the third instar larvae of the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), fed leaves of the broad bean plants inoculated with *B. bassiana*, as the mortality and mycosis differed depending on the isolates and strains used. Klieber and Reineke (2016) showed a mortality of 0–100% and a mycosis of 0–100% of the different larval stages of the tomato leafminer, *T. absoluta*, fed on tomato leaves sprayed with the fungal suspension of *B. bassiana* (23×10^7 colony forming units $\cdot$ ml^{-1}). Barta (2018) detected mycosis in 5.41–9.23% of cadavers of the horse-chestnut leafminer after exposure to *B. bassiana* treated leaves. Vega (2018) suggested that fungal spores are not usually produced inside plant tissues, because the fungal sporulation of the EPFs inside vascular tissues does not provide any advantage to the fungus, so the fungus could not infect the insect directly and cause mortality or mycosis in most cases. However, it acts through its secondary metabolites that are produced by mycelium and deter feeding.

In conclusion, introducing *B. bassiana* through root soaking seems to be effective in stimulating plant growth, and is a promising technique in controlling *L. sativae* populations on cucumber plants. This inoculation method is simple, practical and helps to avoid the unwanted effects of environmental conditions on the fungal inoculum used in direct application on the plant. It also helps to control pests that have protected life stages such as leafminers. Future research should examine the efficiency of endophytic *B. bassiana* in controlling and managing leaf mining insects on greenhouse and field-grown cucumber plants.

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ORIGINAL ARTICLE

Sweet pepper foliar diseases quantification and identification using an image analysis tool

Vijayanandh Rajamanickam[1]*, Adesh Ramsubhag[2], Jayaraj Jayaraman[2]

[1] Department of Computing and Information Technology, Faculty of Science and Technology, The University of the West Indies, St. Augustine Campus, Trinidad and Tobago
[2] Department of Life Science, Faculty of Science and Technology, The University of the West Indies, St. Augustine Campus, Trinidad and Tobago

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*Corresponding address:
Vijayanandh.Rajamanickam@sta.uwi.edu

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SUPPLEMENTARY MATERIAL

The authors are fully responsible for both the content and the formal aspects of the supplementary material. No editorial adjustments were made.

Table S1. True positive (TP) and false positive (FP) rate of J48 classifiers

	TP Rate	FP Rate	Precision	Recall	F-Measure	MCC	ROC area	PRC area	Class
	0.986	0.000	1.000	0.986	0.993	0.983	0.993	0.994	Late blight
	0.968	0.011	0.968	0.968	0.968	0.956	0.978	0.945	Early blight
	1.000	0.010	0.941	1.000	0.970	0.965	0.995	0.941	Bacterial spot
Weighted Avg.	0.983	0.004	0.984	0.983	0.983	0.973	0.989	0.974	

MCC – Matthews Correlation Coefficient; ROC – Receiver Operating Characteristic; PRC – Precision-Recall Curve

Table S1A. Confusion matrix for J48

a	b	c	Classified as
70	1	0	a = Late blight
0	30	1	b = Early blight
0	0	16	c = Bacterial spot

Table S2. True positive (TP) and false positive (FP) rate of RandomForest tree classifier

	TP rate	FP rate	Precision	Recall	F-Measure	MCC	ROC area	PRC area	Class
	0.972	0.000	1.000	0.972	0.986	0.965	1.000	1.000	Late blight
	0.968	0.046	0.882	0.968	0.923	0.896	0.995	0.988	Early blight
	0.875	0.010	0.933	0.875	0.903	0.889	0.996	0.976	Bacterial spot
Weighted Avg.	0.958	0.013	0.960	0.958	0.958	0.937	0.998	0.993	

MCC – Matthews Correlation Coefficient; ROC – Receiver Operating Characteristic; PRC – Precision-Recall Curve

Table S2A. Confusion matrix for RandomForest

a	b	c	Classified as
69	2	0	a = Late blight
0	30	1	b = Early blight
0	2	14	c = Bacterial spot

Table S3. True positive (TP) and false positive (FP) rate of RandomTree classifier

	TP rate	FP rate	Precision	Recall	F-Measure	MCC	ROC area	PRC area	Class
	0.944	0.021	0.985	0.944	0.964	0.914	0.961	0.964	Late blight
	0.839	0.046	0.867	0.839	0.852	0.801	0.896	0.769	Early blight
	0.938	0.049	0.750	0.938	0.833	0.811	0.944	0.712	Bacterial spot
Weighted Avg.	0.915	0.032	0.922	0.915	0.917	0.870	0.942	0.878	

MCC – Matthews Correlation Coefficient; ROC – Receiver Operating Characteristic; PRC – Precision-Recall Curve

Table S3A. Confusion matrix for RandomTree

a	b	c	Classified as
67	3	1	a = Late blight
1	26	4	b = Early blight
0	1	15	c = Bacterial spot

Table S4. True positive (TP) and false positive (FP) rate of HoeffdingTree classifier

	TP rate	FP rate	Precision	Recall	F-Measure	MCC	ROC area	PRC area	Class
	0.901	0.128	0.914	0.901	0.908	0.771	0.942	0.923	Late blight
	0.871	0.103	0.750	0.871	0.806	0.734	0.926	0.926	Early blight
	0.750	0.000	1.000	0.750	0.857	0.850	0.975	0.931	Bacterial spot
Weighted Avg.	0.873	0.104	0.883	0.873	0.874	0.772	0.942	0.925	

MCC – Matthews Correlation Coefficient; ROC – Receiver Operating Characteristic; PRC – Precision-Recall Curve

Table S4A. Confusion Matrix for HoeffdingTree

a	b	c	Classified as
64	7	0	a = Late blight
4	27	0	b = Early blight
2	2	12	c = Bacterial spot

Table S5. True positive (TP) and false positive (FP) rate of NaiveBayes

	TP rate	FP rate	Precision	Recall	F-Measure	MCC	ROC area	PRC area	Class
	0.887	0.128	0.913	0.887	0.900	0.755	0.935	0.922	Late blight
	0.871	0.115	0.730	0.871	0.794	0.717	0.921	0.867	Early blight
	0.750	0.000	1.000	0.750	0.857	0.850	0.972	0.923	Bacterial spot
Weighted Avg.	0.864	0.107	0.877	0.864	0.866	0.758	0.936	0.908	

MCC – Matthews Correlation Coefficient; ROC – Receiver Operating Characteristic; PRC – Precision-Recall Curve

Table S5A. Confusion matrix for NaiveBayes

a	b	c	Classified as
63	8	0	a = Late blight
4	27	0	b = Early blight
2	2	12	c = Bacterial spot

Table S6. True positive (TP) and false positive (FP) rate of DecisionTable classifiers

	TP rate	FP rate	Precision	Recall	F-Measure	MCC	ROC area	PRC area	Class
	1.000	0.021	0.986	1.000	0.993	0.982	0.988	0.985	Late blight
	0.968	0.011	0.968	0.968	0.968	0.956	0.973	0.941	Early blight
	0.938	0.000	1.000	0.938	0.968	0.964	0.990	0.959	Bacterial spot
Weighted Avg.	0.983	0.016	0.983	0.983	0.983	0.973	0.984	0.970	

MCC – Matthews Correlation Coefficient; ROC – Receiver Operating Characteristic; PRC – Precision-Recall Curve

Table S6A. Confusion matrix for DecisionTable

a	b	c	Classified as
71	0	0	a = Late blight
1	30	0	b = Early blight
0	1	15	c = Bacterial spot