

Fungal endophytes as promising tools for the management of bean stem maggot *Ophiomyia phaseoli* on beans *Phaseolus vulgaris*

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Abstract Common bean, *Phaseolus vulgaris*, is an important food and cash crop in Africa. Its production is seriously affected by the bean stem maggot (BSM), *Ophiomyia* spp., which attacks seedlings. We evaluated the ability of eleven fungal isolates to colonize bean plants and the effects of inoculation on BSM feeding and oviposition, pupation, and adult emergence. All fungal isolates were able to colonize different bean plant parts (root, stem, and leaves), except isolates of *Metarhizium anisopliae* and *Beauveria bassiana* isolate ICIPE 273. Colonization was generally higher on the roots than on the stem and leaves and varied significantly between the fungal isolates. BSM feeding and oviposition were significantly reduced in all

the fungus-inoculated bean plants which in turn affected pupation and adult emergence as compared to the control. *Metarhizium anisopliae* ICIPE 20 outperformed the other isolates in interfering with BSM lifecycle. Although *M. anisopliae* ICIPE 78 recorded a high number of punctures similar to the control, a significant reduction in the number of pupae and adult emergence was observed, suggesting possible BSM growth inhibition. This study clearly demonstrates that fungal endophytes can be considered as promising tools for the management of BSM in East Africa.

Keywords Entomopathogenic fungi · Agromyzidae · Colonization · Feeding · Oviposition · Adult emergence

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Key message

- The bean stem maggot (BSM) is an important pest in East Africa; due to its cryptic feeding and its ability to pupate in stem, its management is extremely difficult.
- It is hypothesized that fungal entomopathogens as endophytes can be effectively be used to control leaf mining pests such as BSM.
- Our results showed the ability of some fungal endophytes to colonize bean plant parts and their effect in reducing BSM feeding and oviposition as well as pupation and adult emergence as compared to the control.
- *Metarhizium anisopliae* isolates which did not colonize the bean plants were also able to reduce BSM feeding, oviposition, pupation, and emergence.
- The outcomes of this study suggest the possibility to use fungal endophytes as tools for the management of BSM in East Africa.

Introduction

The common bean, *Phaseolus vulgaris* L. (Fabaceae), is an important food and cash crop in Africa, particularly in the Eastern, Southern, and Great Lakes regions of the continent where it is considered as an important source of human food in terms of calories (Pachico 1993), protein, oil, and micronutrients (Singh 1990; Pyndji and Trutmann 1992; Blair et al. 2007). It is also a source of income for resource-poor households where a significant proportion is exported to European markets (Abate and Ampofo 1996; Wortmann et al. 1999). Annual production of beans in Kenya is estimated to be over 90,000 MT covering around 150,000 hectares. However, serious decline due to insect pests have been observed over the years (USAID 2010). Among the various insect pests of bean, the bean stem maggot (BSM), *Ophiomyia* spp. (Diptera: Agromyzidae), and bruchids are the most important field and storage pests, respectively (Greathead 1969; Karel 1985; Abate 1991). BSM is typical of dry conditions and low fertility soils (Karel and Autrique 1989; Abate and Ampofo 1996; Songa and Ampofo 1998). Bean flies oviposit on young seedlings and larval feeding and tunneling interfere with nutrient transport and creates avenues for entry of disease organisms (Ampofo and Massomo 1998). The presence of BSM pupae in the plant results in the swelling and rotting and plant suffers from premature leaf fall which causes up to 100 % loss (Wickramasinghe and Fernando 1962; Ochilo and Nyamasyo 2011).

The management of BSM is difficult because of the cryptic behavior of the pest. Generally, the control is achieved through the use of a traditional IPM approach that consists of appropriate sowing dates, optimum plant density, resistant varieties, intercropping, and good crop husbandry (Abate and Ampofo 1996). Chemical control is also commonly used, however, it is discouraged due to environmental and health risks, and resistance development by the BSM. There is the need therefore to look for alternative strategies that are safe, environmentally friendly, and cost-effective.

In recent years, there is a shift in emphasis towards utilization of entomopathogenic fungi (EPF) for the management of crop insect pests such as leaf miners (Migiro et al. 2010), thrips (Ekesi and Maniania 2002), and aphids (Jandricic et al. 2014). Some EPF have versatile attributes; they can be used as biopesticides applied in inundative approach or as endophytes. As endophytes, EPF can directly and indirectly promote plant growth and development through plant defense against herbivorous insects (Vega et al. 2009). For instance, fungal endophytes have been reported to deter feeding, oviposition, and performance of sap-sucking insects such as thrips

(Muvea et al. 2014, 2015) and leaf mining insects (Akutse et al. 2013). On the other hand, the effect of entomopathogenic endophytes *Beauveria bassiana* (Bals.-Criv.) Vuill. and *Purpureocillium lilacinum* (Thom) Luangsa-ard, Houbaken, Hywel-Jones, and Samson (Ascomycota: Hypocreales) in enhancing plant growth has been reported by Lopez and Sword (2015). However, no studies have been conducted on BSM. The objective of this study was therefore initiated to screen fungal isolates for endophytic colonization of bean plants and assess the effect of inoculation on BSM feeding and oviposition, pupation, and adult emergence.

Materials and methods

Ethical considerations

The study was carried out at the International Centre of Insect Physiology and Ecology (*icipe*) laboratories in Nairobi, Kenya (S 03.35517°, E 037.33861°, and 1616 m.a.s.l.). Bean plants, fungal isolates, and the pest (BSM) used in this study are not endangered species. The fungal isolates were obtained from the *icipe*'s Arthropod Germplasm Centre and no permission was required since *icipe* operates under a Headquarters' agreement with the Kenyan Government. The study was not undertaken in protected areas of land therefore no special permits were required to undertake the study. Permission was only obtained from the farmers' field prior to sampling.

Bean plant

Phaseolus vulgaris, variety Brown Rose Coco was used in this study. Bean seeds were sowed in 15-cm pots (5–8 plants per pot) and maintained at room temperature (25 ± 3 °C and 60 % R.H.) using a mixture of manure and soil in a ratio of 1:5. The substrate was sterilized in an autoclave for 2 h at 121 °C and allowed to cool for 72 h prior to planting. Pots were maintained immediately in a screen house (2.8 m length \times 1.8 m width \times 2.2 m height) at 25 ± 3 °C, for 2 weeks. Seedlings were thinned to three per pot after germination and were watered once per day in the afternoon.

BSM colony establishment

The initial stock of BSM originated from Kabaru (0.2833°S, 37.1667°E, 2309 m.a.s.l.) around Mt Kenya region. Bean plants showing symptoms of BSM attacks were uprooted and kept in cages for a period of 3–4 weeks for emergence of bean flies. The newly emerged adults

were collected using an aspirator and transferred to the infestation cage which contained clean bean plants to allow females to oviposit. After emergence, specimens of adult bean flies were placed in 95 % alcohol for taxonomical identification. Based on Spencer's key (Spencer 1973), samples were identified as *Ophiomyia phaseoli* (Tryon) (Diptera: Agromyzidae) on morphological features using taxonomic keys. This was later confirmed using molecular tools by amplification of COI (Cytochrome Oxidase 1) region. The purified PCR products were sent to Macrogen Inc, Europe Laboratories, Netherlands, for sequencing. DNA sequences were edited using bio-edit software and subjected to a Basic Local Alignment Search Tool (BLAST) algorithm search (NCBI). Gene accession numbers for the all the characterized specimens were EF104664.1 with 98 % percent identity and E = 0.

Insects were reared on 1-week-old fresh potted bean plants in a Plexiglas cage (50 × 50 × 45 cm) in screen house to allow female BSM to oviposit. Bean plants were changed every 48 h and infested plants were transferred into clean cages for larval development until pupation and adult emergence. The newly emerged flies were again placed in the infestation cage (30 × 30 × 25 cm) with fresh bean plants as previously described. The colony was maintained at 27 ± 2 °C with a photoperiod of 12L: 12D and relative humidity of approximately 40 %. Flies were fed on a 10 % natural honey solution provided in cotton balls placed at the bottom corner of the infestation cage.

Fungal cultures

The list of fungal species used in this study and their origin are presented in Table 1. Fungal isolates were mainly from the genera *Metarhizium* (5), *Beauveria* (3), *Hypocrea* (1), and *Trichoderma* (2). The *Metarhizium* and *Beauveria* isolates were obtained from the International Centre of Insect Physiology and Ecology (icipe)'s Arthropod

Germplasm Centre. All the isolates were cultured on potato dextrose agar (PDA), except *Metarhizium* isolates which were cultured on Sabouraud dextrose agar (SDA). They were maintained at 25 ± 2 °C in complete darkness. Conidia were harvested by scraping the surface of 2–3 week-old sporulating cultures with a sterile spatula. The harvested conidia were then mixed in 10 mL sterile distilled water containing 0.05 % Triton X-100 in universal bottles containing 3 mm glass beads. The conidial suspensions were vortexed for 5 min to produce a homogeneous suspension. Conidial counts were done using an improved Neubauer Hemocytometer (Goettel and Inglis 1997). A final concentration of 1 × 10⁸ conidia mL⁻¹ was used for the inoculation of bean seeds.

The viability of conidia was assessed before any bioassay by spread plating 0.1 mL of 3 × 10⁶ conidia mL⁻¹ onto 90-mm Petri dishes containing SDA or PDA (Goettel and Inglis 1997). The plates were incubated at 25 ± 2 °C and were examined after 16–20 h under a light microscope at a magnification of 400X. Conidia were considered as germinated when the germ tube was twice the diameter of the conidium. Four replicate plates were used per isolate. In viability tests, >88 % of conidia of all the isolates germinated (Table 1).

Inoculation of bean plants

For each treatment, five bean seeds were surface sterilized in 70 % ethanol for 2 min followed by 1.5 % sodium hypochlorite for 3 min and rinsed with sterile distilled water three times. The last rinse water was plated out to assess the reliability of the surface sterilization procedure (Schultz et al. 1998). Inoculation was done by soaking bean seed in conidial suspensions (1 × 10⁸ conidia mL⁻¹) for 2 h (Akutse et al. 2013). For the control, sterilized seeds were soaked in sterile distilled water for 2 h. Seeds were then dried on sterile paper towel for 30 min and planted in

Table 1 List of fungal isolates and their origin used in this study and percentage of germination 12 DAI on SDA and PDA plates at 25 ± 1 °C

Fungal species	Isolates	Locality (country)	Source	Year of isolation	Germination (%) X ± SE
<i>Metarhizium anisopliae</i>	ICIPE 20	Migori (Kenya)	Soil	1989	88 ± 4.6
	ICIPE 78	Ungoye (Kenya)	<i>Temnoschoita nigroplagiata</i>	1990	96 ± 2.8
	ICIPE 69	Matete (DRC)	Soil	1990	88 ± 4.6
	GZP	Kendubay (Kenya)	<i>Busseola fusca</i> Larvae	1989	94 ± 3.4
	ICIPE 30	Kendubay (Kenya)	<i>Busseola fusca</i> Larvae	1989	88 ± 4.6
<i>Beauveria bassiana</i>	ICIPE 279	Mbita (Kenya)	Soil	2005	90 ± 4.2
	ICIPE 273	Kericho (Kenya)	Coleopteran larvae	2004	94 ± 3.4
	GILU3	Kenya	Monocots	2012	92 ± 3.8
<i>Trichoderma asperellum</i>	M2RT4	Kenya	Maize, Akello (2012)	2012	90 ± 4.2
<i>Trichoderma atroviride</i>	F5S21	Loitokitok (Kenya)	(Onion, Muvea et al. 2014)	2014	94 ± 3.4
<i>Hypocrea lixii</i>	F3ST1	Kenya	Maize, Akello (2012)	2012	92 ± 3.8

plastic pots containing the planting substrate (mixture of manure and red soil 1:5). The substrate was sterilized in an autoclave for 2 h at 121 °C and then allowed to cool for 72 h prior to planting. Five seeds were sowed per pot and then maintained at 25 ± 3 °C, 60–80 % R.H. and with a 12-h photoperiod in a screen house for 2 weeks. Seedlings were thinned to three per pot and were watered twice per day (morning and afternoon).

Evaluation of the endophytic colonization

Two weeks after inoculation of the seeds, three bean plants per treatment and per replicate were carefully uprooted and washed with tap water. The seedlings were cut into different sections, i.e., leaves, stems, and roots. Five randomly selected leaf, stem, and root sections from each plant were surface sterilized as already described above. The different plant parts were aseptically cut under a laminar flow hood into 1×1 cm pieces before placing them, 4 cm apart, onto PDA plates supplemented with a 0.05 % solution of streptomycin sulfate (Istifadah and McGee 2006; Gurulingappa et al. 2010). The plates were incubated at 25 ± 1 °C for 10 days, after which the presence of endophytes was observed. The last rinse water was also plated out in order to assess the reliability of the surface sterilization procedure as described earlier. The colonization of the different plant parts was recorded by counting the number of pieces of the different plant parts that showed the presence of inoculated fungal growth/mycelia (Petrini and Fisher 1987). Only the presence of endophytes that were inoculated was scored. Microscope slides were prepared from the mother plates and used for morphological identification. Fungal structures such as conidia and conidiogenous cells obtained from fungal cultures were mounted in lactophenol cotton blue (0.01 % w/v) and observed under a Leica microscope (EZ4 HD, Germany) equipped with a digital camera microscope. The treatments were randomized in complete block design and the experiment replicated five times at three different occasions.

Effects of fungus-inoculated bean plants on BSM

The effects of fungus-inoculated bean plants on feeding, oviposition punctures on leaves, pupation, and adult emergence were evaluated. Thirty (30) two-day-old presumably mated adult female BSM flies were exposed for 72 h to 2-week-old fungus-inoculated host plant seedlings in Plexiglas cages ($30 \times 30 \times 25$ cm) and maintained at 26 ± 1 °C, 50–70 % RH and 12L: 12D photoperiod. After 72 h post-exposure, plants were replaced with fresh non-inoculated seedlings to prevent excessive oviposition and feeding damage by adult flies.

For feeding and oviposition, inoculated plants were maintained until the leaves were well developed (approximately 5–8 days after exposure). In the control, plants were not inoculated with fungal pathogens. Two leaves were cut from each pot and stained in boiling lactophenol-acid fuchsin solution for 5 min (Nuessly et al. 1995). After staining, the leaves were placed in 90-mm Petri dishes for 1 h before destaining. The stain was removed by immersing the leaves in warm water for 3 min. The number of feeding and oviposition punctures were counted under a stereomicroscope and recorded as described by (Muvea et al. 2014).

For pupation and fly emergence, bean plants were maintained until larvae reached the pupation stage. The number of pupae on the stem and the number of emerged adults were recorded under a dissecting microscope. All the treatments were randomized and experiments were replicated five times at three different occasions.

Data analysis

Colonization frequency (CF) was calculated as described by (Petrini and Fisher 1987) using the formula: Colonization (%) = $(PF/TP) \times 100$, where PF = Number of plant pieces colonized, TP = Total number of plant pieces. The proportion of fungal colonization per plant part was arcsine-transformed before subjecting it to ANOVA and means were separated using SNK (Student–Newman–Keuls) and multiple comparison tests. The number of oviposition and feeding punctures, number of pupae, and adult emergence data were log transformed before being subjected to same procedure (ANOVA/SNK). R statistical software, 2.15.4 version (R Development Core Team 2013), was used for all the analysis. The level of significance was fixed at 95 % confidence interval.

Results

Endophytic colonization of *P. vulgaris* by fungal isolates

The endophytic colonization of bean plants varied significantly between the fungal isolates

($F_{11, 165} = 18.7$, $P < 0.0001$) and plants parts (root, stem, and leaves) ($F_{2, 312} = 8.5$, $P = 0.0002$). The interaction between fungal isolates and plant parts was significant ($F_{22, 165} = 4.1$, $P < 0.0001$). For instance, *T. atroviride* (F5S21) showed the highest percentage colonization of the stem (86.6 %); whereas *T. asperellum* (M2RT4) exhibited high colonization of the roots (85.3 %) (Fig. 1). *Beauveria bassiana* ICIPE 279 and *H. lixii* F3ST1 had the lowest percentage colonization of the leaves (10.8 and 10.7 %, respectively) (Fig. 1). All the isolates of *M.*

anisopliae and *B. bassiana* ICIPE 273 failed to colonize any of the bean plant parts.

Effects of fungus-inoculated bean plants on BSM feeding and oviposition

The feeding and oviposition punctures differed between fungal isolates ($F_{11, 165} = 19.82$, $P < 0.0001$) (Fig. 2). The number of feeding and oviposition punctures by BSM was significantly lower in all the fungus-inoculated plants, except *M. anisopliae* ICIPE 78 which was similar to the control (Fig. 2).

Effects of fungus-inoculated bean plants on BSM pupation and adult emergence

Fewer BSM pupae were produced in fungus-inoculated plants compared to the control ($F_{11, 165} = 17.28$, $P < 0.0001$) (Fig. 3). *Metarhizium anisopliae* ICIPE 20 recorded the lowest number of pupae; but was not significantly different from the other fungal isolates, except *B. bassiana* ICIPE 273, *T. atroviride* F5S21, and *M. anisopliae* GZP (Fig. 3). More adult BSM emerged from the control than from the fungus-treated plants ($F_{11, 165} = 10.46$, $P < 0.0001$) (Fig. 4). *Metarhizium anisopliae* isolates ICIPE 20 and ICIPE 30 had significantly lower numbers of adult emergence than *T. atroviride* F5S21, *M. anisopliae* GZP, and *B. bassiana* ICIPE 273 as observed with the number of pupae (Fig. 4).

Discussion

Endophytic colonization differed between the fungal species and isolates, and the bean plant parts. For instance, *T. asperellum* isolate M2RT4, *T. atroviride* isolate F5S21, *H.*

lixii isolate F3ST1, and two *B. bassiana* isolates GILU3 and ICIPE 279 were able to colonize different parts of the bean plant, except *B. bassiana* isolate ICIPE 273. On the other hand, *M. anisopliae* isolates failed to colonize any of the parts of bean plant. The level of colonization of different parts of the bean plant varied according to fungal isolates. For example, inoculation of *H. lixii* F3ST1 resulted in higher colonization of roots and stem than leaves while the one of *T. atroviride* F5S21 resulted in higher colonization of the stem and leaves than roots (Fig. 1). Similar results have been reported on other host plants (Vega et al. 2008; Tefera and Vidal 2009; Gurulingappa et al. 2010; Akutse et al. 2013; Muvea et al. 2014). The variation in colonization rate could be due to the fact that fungal endophytes display preferential tissue colonization within their plant hosts as reported by Behie et al. (2014). Similar observations were made on other bean plants such as French bean, Faba bean, and onion (Akutse et al. 2013; Muvea et al. 2014). All the isolates of *M. anisopliae* failed to colonize any of the bean plant parts. Similar observation was reported earlier with *M. anisopliae* ICIPE 30 (the same used in the present study) on French bean and Faba bean (Akutse et al. 2013). On the other hand, endophyte colonization of *Metarhizium* sp. has been reported elsewhere. For instance, Akello (2012) reported that *M. anisopliae* could be endophyte inside maize and bean tissues. Sasan and Bidochka (2012) and Behie et al. (2014) reported endophyte colonization of *Metarhizium* in haricot bean plants while García et al. (2011) reported the same in tomato plants. The technique for *M. anisopliae* recovery used in our study might not have allowed the detection of this fungal species which is typically found in the plant rhizosphere. Plant tissues were homogenized using a tissue homogenizer before plating (Sasan and Bidochka 2012; Behie et al. 2014), while whole pieces of plant parts

Fig. 1 Colonization of different bean plant parts (root, stem, and leaves) by fungal pathogens, *Beauveria bassiana* (Bb) GILU3, 279 and 273, *Trichoderma atroviride* (Trich) F5S21, *T. asperellum* M2RT4, *Hypocrea lixii* (Hypo) F3ST1, and *Metarhizium anisopliae* (Met) 20, 30, 69, 78, and GZP after 12 days post-inoculation. Bars indicate mean \pm one standard error at 95 % CI ($P < 0.0001$)

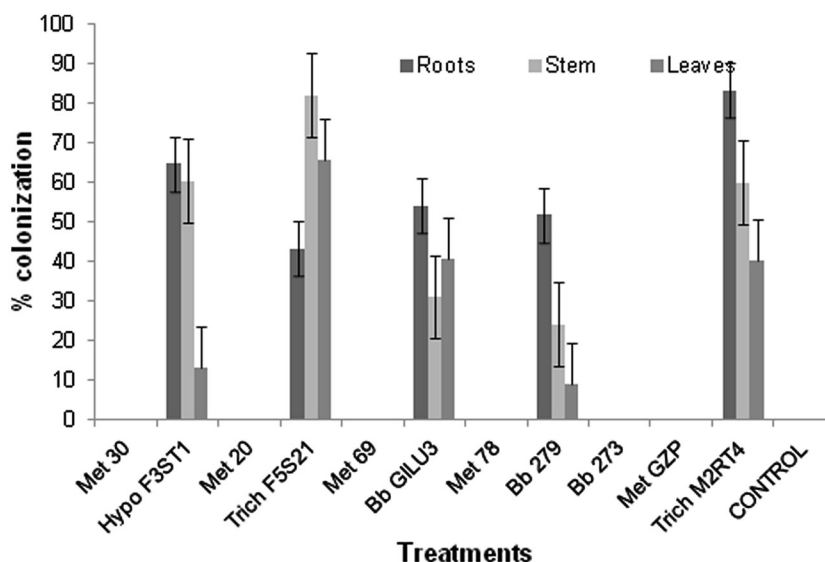


Fig. 2 Effects of exposure to *Phaseolus vulgaris* plants inoculated with different fungal pathogens, *Beauveria bassiana* (Bb) G1LU3, 279 and 273, *Trichoderma atroviride* (Trich) F5S21, *T. asperellum* M2RT4, *Hypocrea lixii* (Hypo) F3ST1, and *Metarhizium anisopliae* (Met) 20, 30, 69, 78, and GZP on oviposition and feeding of adult *Ophiomyia phaseoli* after 72 h. Bars denote means \pm one standard error at 95 % CI ($P < 0.0001$)

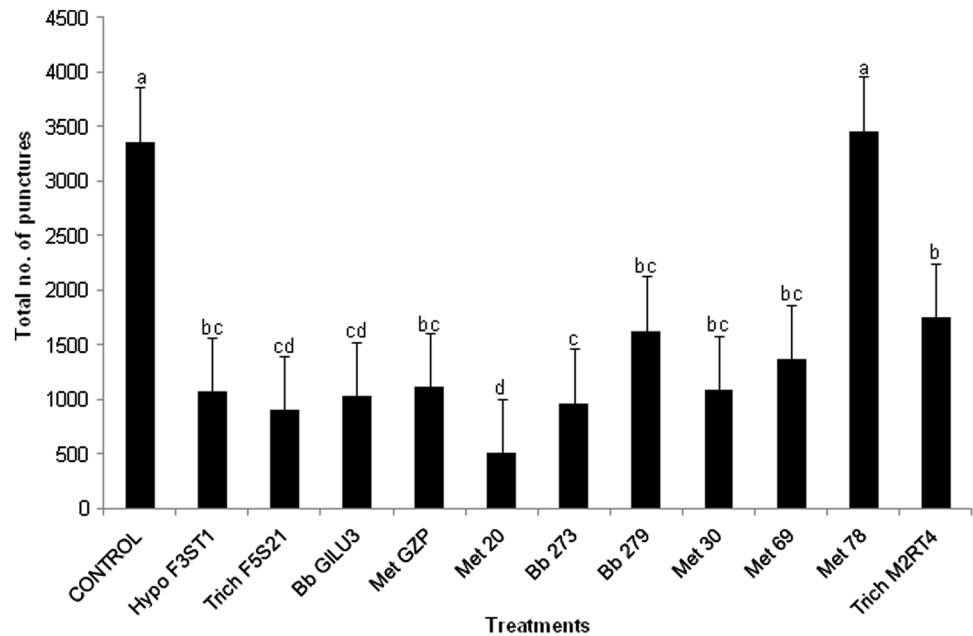
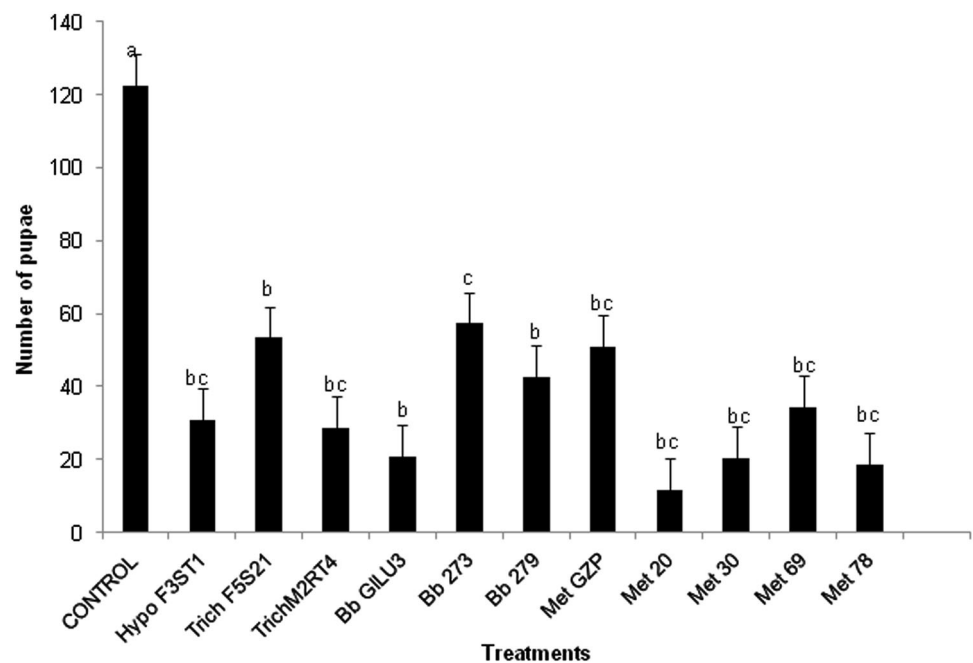


Fig. 3 Effects of exposure to *Phaseolus vulgaris* plants inoculated with different fungal pathogens, *Beauveria bassiana* (Bb) G1LU3, 279 and 273, *Trichoderma atroviride* (Trich) F5S21, *T. asperellum* M2RT4, *Hypocrea lixii* (Hypo) F3ST1, and *Metarhizium anisopliae* (Met) 20, 30, 69, 78, and GZP on pupation of adult *Ophiomyia phaseoli* after 35 days post-inoculation. Bars denote means \pm one standard error at 95 % CI ($P < 0.0001$)



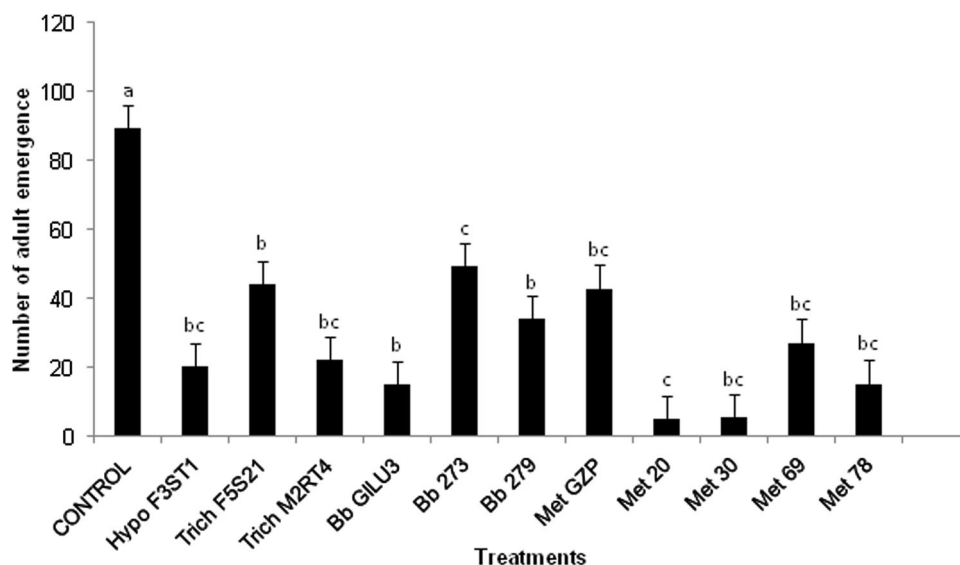
were plated in our study. Furthermore, the inhibitor used in the selective media may also have an effect on *Metarhizium* competing with other microorganisms (García et al. 2011).

Fungus-inoculated bean plants significantly reduced BSM feeding and oviposition, which is in agreement with previous reports (Quesada-Moraga et al. 2006; Akello et al. 2008; Muvea et al. 2014, 2015). In addition, the number of pupae produced by female BSM and their adult emergence were also significantly reduced. Similar results were

reported on another agromyzid, *L. huidobrensis* (Akutse et al. 2013). Significant reduction in adult emergence, longevity, and oviposition period of *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) following exposure of larvae to diet supplemented with ethyl acetate extract of *Alternaria alternata* (Fr.) Keissl. (Ascomycota: Pleosporales) has also been reported (Kaur et al. 2013).

The mechanism by which fungal endophytes interfere could be explained by the production of active metabolites by endophytes that could deter insect feeding (Bing and

Fig. 4 Effects of inoculation of bean plants by fungal pathogens, *Beauveria bassiana* (Bb) G1LU3, 279 and 273, *Trichoderma atroviride* (Trich) F5S21, *T. asperellum* M2RT4, *Hypocrea lixii* (Hypo) F3ST1, and *Metarhizium anisopliae* (Met) 20, 30, 69, 78, and GZP on emergence of adult *Ophiomyia phaseoli* after 35 days post-inoculation. Bars denote means \pm one standard error at 95 % CI ($P < 0.0001$)



Lewis 1991; Vega et al. 2008). Studies by Cherry et al. (2004) on *Sesamia calamistis* (Lepidoptera: Noctuidae) seem to support the feeding deterrence/antibiosis hypothesis since larvae feeding on plants injected with *B. bassiana* were smaller than those in the control plants. Investigating the behavioral responses of *T. tabaci* to endophyte-inoculated onion plants, (Muvea et al. 2015) observed that thrips (adults and nymphs) orientate preferentially to endophyte-free host plants and it was hypothesized that volatiles produced as a result of endophyte–host plant interactions were responsible for thrips non-preference to endophyte-inoculated plant. Jasmonic acid (JA) is known to play essential role in plant defense against herbivore insects (Mcconn et al. 1997). Navarro-Meléndez and Heil (2014) found that endophytic colonization with *Fusarium* sp. (Ascomycota: Hypocreales) or *Cochliobolus lunatus* R.R. Nelson & Haasis, (Ascomycota: Pleosporales) enhanced the number of detectable volatile organic compounds (VOCs) (salicylic acid and JA) emitted from intact leaves of the wild Lima bean, *Phaseolus lunatus* L. (Fabales: Fabaceae).

Fungal isolates, *H. lixii* isolate F3ST1 and *B. bassiana* isolate G1LU3, which were reported earlier to have effect on the number of pupae and emergence of *L. huidobrensis* (Akutse et al. 2013), produced similar effects against BSM. Although the effect of endophyte inoculation on the growth of bean plants was not carried out, it is well documented that fungal endophytes promote plant growth (García et al. 2011), as illustrated by Fig. 5 in our study.

The present study has demonstrated that fungal endophytes can be considered as promising tools for the management of BSM. Fungus-inoculated bean plants significantly reduced BSM feeding and oviposition which in turn affected pupation and adult emergence. Future research should focus on the traceability of the endophytes

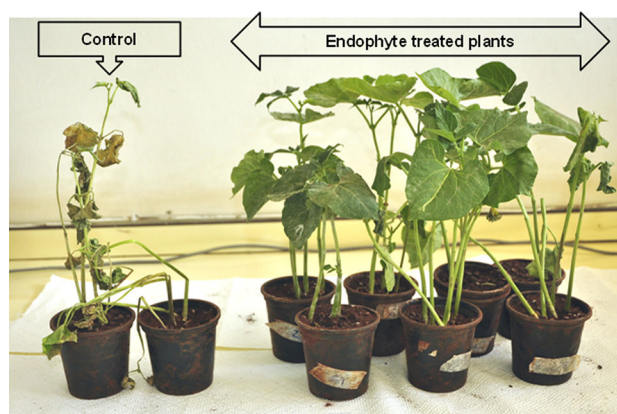


Fig. 5 Leaf damage caused by BSM on untreated bean (*Phaseolus vulgaris*) plants (control: left) and endophyte-inoculated bean plants (right)

within plant tissue and their performance in field conditions.

Author contribution

All authors conceived and designed the research. BMM conducted the experiments. NKM and SE provided the reagents, fungal isolates, analytical tools, and the facilities. BMM, NKM, SE, and NS analyzed the data and wrote the manuscript. All authors read and approved the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

- Abate T (1991) The bean fly *Ophiomyia phaseoli* (Tryon) (Diptera: Agromyzidae) and its parasitoids in Ethiopia. *J Appl Entomol* 111:278–285
- Abate T, Ampofo JKO (1996) Insect pests of beans in Africa: their ecology and management. *Ann Rev Entomol* 41:45–73
- Akello J (2012) Biodiversity of fungal endophytes associated with maize, sorghum and Napier grass and their influence of biopriming on resistance to leaf mining, stem boring and sap sucking insect pests. Ph.D., University of Bonn, Bonn
- Akello J, Dubois T, Coyne D, Kyamanywa S (2008) Effect of endophytic *Beauveria bassiana* on populations of the banana weevil, *Cosmopolites sordidus*, and their damage in tissue-cultured banana plants. *Entomol Exp Appl* 129:157–165
- Akutse KS, Maniania NK, Fiaboe KKM, Van den Berg J, 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 Ecol* 6:293–301
- Ampofo JKO, Massomo SMS (1998) Some cultural strategies for management of bean stem maggots (Diptera: Agromyzidae) on beans in Tanzania. *Afr Crop Sci J* 6:351–356
- Behie S, Jones S, Bidochka M (2014) Plant tissue localization of the endophytic insect pathogenic fungi *Metarhizium* and *Beauveria*. *Fungal Ecol* 13:112–119
- Bing L, Lewis L (1991) Suppression of *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae) by endophytic *Beauveria bassiana* (Balsamo) Vuillemin. *Environ Entomol* 20:1207–1211
- Blair M, Fregene S, Beebe S, Ceballos H (2007) Marker-assisted selection in common beans and cassava. In: Guimarães E, Ruane J, Scherf B, Sonnino A, Dargie J (eds) Marker-assisted selection: current status and future perspectives in crops, livestock, forestry and fish. FAO, Rome, pp 81–115
- Cherry AJ, Banito A, Djegui D, Lomer C (2004) Suppression of the stem-borer *Sesamia calamistis* (Lepidoptera: Noctuidae) in maize following seed dressing, topical application and stem injection with African isolates of *Beauveria bassiana*. *Int J Pest Manag* 50:67–73
- Ekesi S, Maniania NK (2002) *Metarhizium anisopliae*: an effective biological control agent for the management of thrips in Horti- and Floricultural Crops in Africa. In: Upadhyay RK (ed) Advances in microbial control of insect pests. Kluwer Academic/Plenum Publishers, The Netherlands, pp 165–180
- García JE, Posadas JB, Peticari A, Lecuona RE (2011) *Metarhizium anisopliae* (Metschnikoff) Sorokin promotes growth and has endophytic activity in tomato plants. *Adv Biol Res* 5:22–27
- Goettel MS, Inglis GD (1997) Fungi: Hyphomycetes. In: Lacey L (ed) Manual techniques in insect pathology. Academic press, San Diego, pp 213–214
- Greathead DJ (1969) A study in East Africa of beanflies (Diptera: Agromyzidae) affecting *Phaseolus vulgaris* and of their natural enemies with the description of a new species of *Melanagromyza* Hend. *Bull Entomol Res* 59:541–556
- Gurulingappa P, Sword GA, Murdoch G, McGee PA (2010) Colonization of crop plants by fungal entomopathogen and their effects on two insect pests when *in planta*. *Biol Control* 55:34–41
- Istifadah N, McGee PA (2006) Endophytic *Chaetomium globosum* reduces development of tanspot in wheat caused by *Pyrenophora tritici-repentis*. *Aust Plant Pathol* 35:411–418
- Jandricic SE, Filotas M, Sanderson JP, Wraight SP (2014) Pathogenicity of conidia-based preparations of entomopathogenic fungi against the greenhouse pest aphids *Myzus persicae*, *Aphis gossypii*, and *Aulacorthum solani* (Hemiptera: Aphididae). *J Invertebr Pathol* 118:34–46
- Karel AK (1985) A Bibliography of bean flies, *Ophiomyia phaseoli* (Tryon), *O. centrosematis* (de Meij) and *Melanogromyza spencerella* (Greathead) (Diptera: Agromyzidae). Michigan State University East Lansing
- Karel AK, Autrique A (1989) Insects and other pests in Africa. In: Schwartz HF, Pasto-corales MA (eds) Bean production problems in the tropics. CIAT, Cali, pp 455–504
- Kaur HP, Singh B, Kaur A, Kaur S (2013) Antifeedent and toxic activity of endophytic *Alternaria alternata* against tobacco caterpillar *Spodoptera litura*. *J Pest Sci* 86:543–550
- Lopez DC, Sword GA (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*). *Biol Control* 89:53–60
- Mcconn M, Creelman RA, Bell E, Mullet JE, Browse J (1997) Plant Biology Jasmonate is essential for insect defense in *Arabidopsis*. *Proc Natl Acad Sci* 94:5473–5477
- Migiro L, Maniania N, Chabi-Olaye A, Van den Berg J (2010) Pathogenicity of entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* (Hypocreales: Clavicipitaceae) isolates to the adult pea leafminer (Diptera: Agromyzidae) and prospects of an autoinoculation device for infection in the field. *Environ Entomol* 39:468–475
- Muvea AM, Meyhofer R, Subramanian S, Poehling HM, Ekesi S, Maniania NK (2014) Colonization of onions by endophytic fungi and their impacts on the biology of *Thrips tabaci*. *PLoS ONE* 9(9):e108242
- Muvea AM, Meyhofer R, Subramanian S, Poehling HM, Ekesi S, Maniania NK (2015) Behavioral responses of *Thrips tabaci* Lindeman to endophyte-inoculated onion plants. *J Pest Sci* 88:555–562
- Navarro-Meléndez A, Heil M (2014) Symptomless endophytic fungi suppress endogenous levels of salicylic acid and interact with the jasmonate-dependent indirect defense traits of their host, Lima bean (*Phaseolus lunatus*). *J Chem Ecol* 40:816–825
- Nuessly GS, Nagata RT, Skiles ES, Christenson JR, Elliott C (1995) Techniques for differentially staining *Liriomyza trifolii* (Diptera: Agromyzidae) eggs and stipples within *Cos lettuce* leaves. *Fla Entomol* 78:258–264
- Ochilo W, Nyamasyo G (2011) Pest status of bean stem maggot (*Ophiomyia* spp.) and black bean aphid (*Aphis fabae*) in Taita district, Kenya. *J Trop Subtrop Bot Agroecosyst* 13:91–97
- Pachico DH (1993) The demand for bean technology. Cali, Colombia
- Petrini O, Fisher PJ (1987) Fungal endophytes in *Salicornia perennis*. *Trans Soc Br Mycol* 87:647–651
- Pyndji MM, Trutmann P (1992) Managing angular leaf spot on common beans in Africa by supplementing farmer mixtures with resistant varieties. *Plant Dis* 76:1144–1473
- Quesada-Moraga E, Landa BB, Muñoz-Ledesma J, Jiménez-Díaz RM, Santiago Alvarez C (2006) Endophytic colonisation of opium poppy, *Papaver somniferum*, by an entomopathogenic *Beauveria bassiana* strain. *Mycopathologia* 161:323–329
- Sasan RK, Bidochka MJ (2012) The insect-pathogenic fungus *Metarhizium robertsii* (Clavicipitaceae) is also an endophyte that stimulates plant root development. *Am J Bot* 99:101–107

- Schultz B, Guske S, Dammann U, Boyle C (1998) Endophyte-host interactions II. Defining symbiosis of the endophyte-host interaction *Symbiosis* 25:213–227
- Singh SR (1990) Fighting Insect Damage to Legumes http://www.anancy.net/tpl/fulltext.tpl.php?file_id=1088&language=english&acs 15. Accessed 25 Jul 2009
- Songa JM, Ampofo JKO (1998) Ecology of bean stem maggot attacking dry bean (*Phaseolus vulgaris* L.) in semi-arid areas of eastern Kenya. *Int J Pest Manag* 45:35–40
- Spencer KA (1973) *Agromyzidae* (Diptera) of economic importance. Junk, The Hague
- R Development Core Team (2013) A Language and environment for statistical computing. <http://www.r-project.org/>. Accessed 20 Apr 2014
- Tefera T, Vidal S (2009) Effect of inoculation method and plant growth medium on endophytic colonization of sorghum by the entomopathogenic fungus *Beauveria bassiana*. *Biol Control* 54:663–669
- USAID (2010) Staple foods value chain analysis. In Country report Kenya. USAID, Kenya
- Vega FE, Posada F, Aime MC, Pava-Ripoll M, Infante F, Rehner SA (2008) Entomopathogenic fungal endophytes. *Biol Control* 46:72–82
- Vega FE, Goettel MS, Blackwell M, Chandler D, Jackson MA, Keller S, Koike M, Maniania NK, Monzón A, Ownley BH, Pell JK, Rangel DEN, Roy HE (2009) Fungal entomopathogens: news insights of their ecology. *Fungal Ecol* 2:149–159
- Wickramasinghe N, Fernando HE (1962) Investigations on insecticidal seed dressings, soil treatments and foliar sprays for the control of *Melanagromyza phaseoli* (Tryon) in Ceylon. *Bull Entomol Res* 53:223–240
- Wortmann CS, Kirkby RA, Eledu CA, Allen DJ (1999) Atlas of common bean (*Phaseolus vulgaris* L.) production in Africa. CIAT, Cali