Egyptian Journal of Biological Pest Control

RESEARCH

Open Access

Isolation and evaluation of indigenous endophytic entomopathogenic fungus, *Beauveria bassiana* UHSB-END1 (Hypocreales: Cordycipitaceae), against *Spodoptera litura* Fabricius

G. S. Jamunarani[1*], S. H. Ramanagouda[1*], B. Venkateshalu[1], J. Jayappa[1], Gunnaiah Raghavendra[2], D. L. Rudresh[3], M. S. Kulkarni[4], B. N. Naika Mahantesha[5] and J. B. Gopali[6]

Abstract

Background: Microbial biological control agents are gaining worldwide attention to manage insect pests as an alternative to synthetic insecticides. Entomopathogenic fungi (EPF) meet eco-friendly pest management's demand since mere contact of infective propagules is sufficient to cause disease in insect pests, unlike other entomopathogens. However, epiphytic fungal isolates encounter multiple challenges including direct exposure of conidia to sunlight and UV light, high temperature, and low moisture content that reduce their efficacy at the field level. Therefore, utilization of endophytic EPF is becoming more popular because they get protection from adverse conditions compared to the epiphytic EPF as they reside inside the host tissue. In addition, the endophytic EPF also give protection against crop diseases and promote plant growth, degradation of heavy metals, and tolerance to abiotic stress.

Results: The increased mortality of *Spodoptera litura* (Fab.) (Noctuidae: Lepidoptera) was achieved through endophytic colonization of indigenous *Beauveria bassiana* UHSB-END-1 (OM131742). The bioassay proved the highest mortality 2nd instar larvae of *S. litura* at 40 dpi, both in vivo and *in planta* experiment. Further, larvae fed with fungal colonized leaves of tomato plant ended with abnormal growth and developmental process. The recovery of *B. bassiana* from different plant parts (stem, leaves, and roots) was the highest (100%) in all the methods of colonization at 14, 40, 60, 80 dpi, and it was decreased at 120 dpi (80%). The colonization rate was again increased in the next-generation seeds and seedlings (25 days old). This isolate gets vertically transmitted to their progenies via seeds, and it is the first report in tomato crop.

Conclusion: After ensuring the safeness of this isolate against non-target organisms, it can be one of the constitutes in sustainable cost-effective strategy for management of pests affecting tomato as one of the components in integrated pests management. Inoculation of endophytic EPF into seed/seedling reduces environmental impacts and also easy, economical, and sustainable approach for pest management in horticulture crops which are often consumed as raw. Although field studies are required to support the present finding, this appears to be an interesting tool that should be considered for pest biocontrol.

*Correspondence: jamunaprabhas7@gmail.com; goudaento@gmail.com

[1] Department of Entomology, College of Horticulture, University of Horticultural Sciences, Bagalkot, Karnataka 587104, India
Full list of author information is available at the end of the article

Keywords: Entomopathogenic fungi, *Beauveria bassiana*, Endophyte, Tomato, Bioassay, *Spodoptera litura*, Polymerase chain reaction

Background

Tomato, *Solanum lycopersicum* L. (Solanaceae: Solanales), is one of the world's major fresh and processed fruits and is the second most important vegetable crop after the potato worldwide. China, the European Union (EU), India, the USA, and Turkey are the major producers of tomatoes accounting for 70% of global production in 2020. The intensive cultivation of tomatoes either in the open field or under protection (greenhouses) and climate change had led to the emergence of multiple pests and diseases, causing significant yield loss annually. The tobacco leaf-eating caterpillar, *Spodoptera litura* Fabricius (Noctuidae: Lepidoptera), is the top most pest causing a substantial yield loss in tomato production (Mahapatra et al. 2018).

Management of pests with repeated sprays of synthetic chemical insecticides is the most common management practice, followed by farmers. However, insect pests develop resistance against insecticides rapidly. Therefore, the use of insecticides is inefficient in managing insect pests in the long run and also increases the cost of production. Synthetic chemicals are also harmful to non-target organisms and the ecosystem (Sharma et al. 2020). Currently, there is a huge demand for efficient, environmentally safe, and sustainable methods of insect pest management.

Interestingly, microbial biological control agents (mBCAs) are gaining worldwide attention to managing insect pests as an alternative to synthetic insecticides. Entomopathogenic fungi (EPF) meet eco-friendly pest management's demand since mere contact of infective propagules is sufficient to cause disease in insect pests, unlike other entomopathogens such as bacteria, viruses, and nematodes. In addition, they have advantages of ease of isolation and mass production, easy handling of formulations, and field application (Litwin et al. 2020). However, epiphytic fungal isolates for pest management encounter multiple challenges that reduce their efficacy at the field level. The hurdles include direct exposure of propagules (conidia) to sunlight and UV light, higher temperature, and lower moisture content in the environment (Vega 2018). Therefore, the biological management of pests using traditional isolates of fungi is not popular at the field level and attracts less interest among the farming community.

The utilization of endophytic EPF is becoming more popular for pest management in recent days that overcome the limitations of epiphytic EPF (Agbessenou et al. 2020). The endophytic EPF gets protection from adverse environmental conditions compared to the epiphytic EPF as they reside inside the host tissue. In addition, the endophytic EPF also give protection against crop diseases (Mantzoukas and Eliopoulos 2020) and promote plant growth and development through symbiotic association with the host plant for the entire crop period (Kusari et al. 2012). Endophytic EPF favor plant growth by increased nutrient uptake (Yuan et al. 2018), degradation of heavy metals in the rhizosphere (Jaber 2018), and tolerance to abiotic stress (Jaber and Enkerli 2016). The colonization of endophytic fungi in the host plant triggers the production of defensive bioactive compounds of antimicrobial and insecticidal properties. Such compounds get accumulated as a reservoir of preformed defense compounds (Lin et al. 2016). Consequently, plants can conserve the energy that would have been used in the production of defensive compounds against biotic stresses during the initial stages of the infection (Shrivastava et al. 2015). As the growing period of plants advances, the incidence of insect pests and diseases can be suppressed by the combined effect of bioactive compounds produced from both endophytic fungi and host plants (Gualandi et al. 2014).

Endophytic EPF, *Beauveria bassiana* (Bals.-Criv.) Vuill. (Hypocreales: Cordycipitaceae) is one of the hardy EPF, well adapted to the diverse cultivated crops, and protects against insect pests without showing any external symptoms on the plant. Its colonization has been detected in different parts of the plants such as root, stem, and leaf in various field crops and horticultural crops (Reay et al. 2010). The colonization of endophytic *B. bassiana* has been reported in tomato, potato, cucumber, melon, maize, cotton, rice and demonstrated to protect against multiple insect pests and diseases (Vega 2018). In tomatoes, the efficacy of the endophytic *B. bassiana* as a biocontrol agent against insect pests varies with inoculation methods and location-specific isolates (Sinno et al. 2020).

Therefore, an indigenous isolate of endophytic fungus *B. bassiana* was isolated from the Ghataprabha region, a biodiversity hotspot in the Krishna river basin of Western Ghats. Its identity, colonization, and recovery in different tissues of tomatoes were confirmed through molecular techniques. The bio-efficacy of the newly isolated *B. bassiana* for the management of *S. litura* was tested under in vivo (tested under laboratory conditions) and also their efficacy study under in vitro (open conditions) at different crop growth period.

Methods

Isolation and characterization of *Beauveria bassiana*

Isolation of endophytic fungus

For isolation of EPF, leaves, stem, and roots were collected from the tobacco leaf-eating caterpillar infested tomato plants cultivated at Ghataprabha, Gokak taluk, Belagavi district, Karnataka, India (16.23950 N, 74.7580 E). A total of 50 plants were collected randomly covering five fields in the said taluk for tissue sampling. Collected tissues were surface sterilized with 2% sodium hypochlorite for 2 min, treated with 70% alcohol for 3 min, and later thoroughly washed thrice with sterile water. The last rinsed water was plated on PDA medium and incubated to ensure the surface sterilization, and no colonies of fungus were grown on the plate. Sterilized tissues were blotted on sterile filter paper under a laminar flow hood for 45 min to drain the residual water. Tissues were transferred to the Petri plates containing Potato Dextrose Agar (PDA) medium. The Petri plates were incubated at 25 ± 2 °C in a BOD incubator for seven days. The pure culture of the endophytic fungi was isolated by transferring the hyphal tip from 7-day-grown colony to the PDA slants and cultured at 25 ± 2 °C.

Morphological identification

Morphological identification of the endophytic fungi isolated from tomato plant was carried out by observing the colony features (color and texture) of the fungal isolate grown for seven days on a PDA medium. The microscopic characteristics of the fungus were studied based on conidia, conidiophores, and arrangement of spores under a compound microscope (Euromex iScope, Holland).

Molecular identification

The total genomic DNA of the fungal isolate was extracted from the mycelium of the 7-day-old cultures grown on Potato Dextrose Broth (PDB). The mycelia were collected by filtering out the culture through Whatman's filter paper No.1 (125 mm). Mycelium was frozen in liquid nitrogen and ground into fine powder in TissueLyser II (Qiagen, Venlo, Netherlands). The fungal genomic DNA was extracted by CTAB protocol with slight modifications. PCR amplified the fragment of Inter Transcribed Spacer (ITS) region with fungal specific ITS primers; ITS 4-forward primer (5′ TCCGTAGGTGAACCTGCG G 3'), ITS 4-reverse primer (5′ TCCTCCGCTTATTGA TATGC 3′). The amplified PCR product was purified and sequenced on ABI PRISM 3730xL Genetic Analyzer (Thermo Fisher Scientific Company, USA). Quality check was performed on the sequence chromatograms using Codon Code Aligner Version 1.2. The ITS sequences of newly isolated endophytic fungi were submitted to the GenBank, and an accession number was obtained. Fasta sequences were BLAST searched against the non-redundant nucleotide database of NCBI (http://blast.ncbi.nlm.nih.gov/ accessed on 5 January 2022). Multiple sequence alignment of ITS sequence of the newly isolated strain of *B. bassiana* and its homologous sequences was constructed using the MUSCLE program. The phylogenetic tree was built following Neighborhood Joining (NJ) method with 1000 bootstrap values using MEGA X software. v11 (Kumar et al. 2018).

Plant material

Tomato seeds of Arka Rakshak were procured from ICAR-Indian Institute of Horticultural Research, Hesaraghatta, Bengaluru. Seeds were surface disinfected with 2% sodium hypochlorite for two minutes, followed by 70% alcohol for three minutes, and later consecutively washed thrice with autoclaved ultrapure water. The disinfected seeds were dried on sterile filter paper under a laminar airflow hood for 30 min and used for the study. The efficacy of surface sterilization was tested by incubating surface-sterilized seeds onto a PDA medium. Microbial growth was not observed after one week of incubation.

Insect cultures

The wild population of test insects, *S. litura*, was collected from vegetable fields of the College of Horticulture, Bagalkot, India (16.16910 N latitude, 75.66150E longitude). The culture of the insect was reared on tomato leaves under laboratory conditions of 28 ± 1 °C with natural photoperiodic conditions. The second instar larvae were collected from the insect culture as and when required for the *in vivo* and *in vitro* study (Sowmya et al. 2017).

Conidial preparation

The pre-cleaned rice grains were soaked in an equal ratio of distilled water (200 g: 200 ml) in polythene bags added with dry yeast (2 g) and 0.2 g of chloramphenicol antibiotic to avoid bacterial contamination. The mixture was autoclaved (121 °C, 15 lbs pressure) for 30 min and allowed to cool and later inoculated with mother fungal culture (*B. bassiana* UHSB-END1) under a laminar airflow hood. The inoculated bags were incubated at 25 ± 2 °C for 15 days under dark conditions. After 15 days, the fully grown fungus on rice grains was powdered and added with talc powder (1:1) as a talc-based formulation. The spore load of 1×10^8 cfu/g was maintained in the formulation.

Experimental design

Raising of seedlings

The treated and untreated seeds were sown in pro-trays filled with sterilized potting media (coco peat) placed in a growth chamber (Alice Biotech Pvt. Ltd.). The germinated seedlings were grown for 25 days in pro-trays and then transferred into the pots for imposing different treatments filled with autoclaved soil and compost (1:1).

Treatment design

A completely randomized design of six treatments was laid out. Each treatment was replicated thrice with ten plants per replication. Different treatments used to colonize endophytic *B. bassiana* UHSB-END1 in the tomato plant were detailed below.

Seed treatment

The tomato seeds were soaked overnight (12 h.) in freshly prepared fungal conidial suspensions (5 g/l). The soaked seeds were dried for 30 min under a laminar airflow hood and later sown in pro-tray. After 25 days, they were transplanted to the pots for colonization study (Jaber and Enkerli 2016).

Seedling root dip treatment

Roots of 25-day-old seedlings raised in portray were dipped in conidial suspension (5 g/l) for about two hours and then immediately transplanted to pots containing sterilized media (Saragih et al. 2019).

Soil drenching

After two days of transplanting tomato seedlings, the pots were drenched with 500 ml of conidial suspension (10 g/l) (Greenfield et al. 2016).

Foliar Spray

After two days of transplanting tomato seedlings to the pots, the conidial suspension (4 g/l) was sprayed to the entire plant (Greenfield et al. 2016).

Colonization of *B. bassiana* UHSB-END1 in tomato plant

Per cent colonization

The endophytic colonization and persistence of *B. bassiana* UHSB-END1 in the tomato plants were recorded after 14, 40, 60, 80, and 120 days of post-inoculation (dpi). Tomato plant parts (leaves, stem, and root) from different treatments were collected by destructive sampling. Collected plant parts were surface sterilized using the protocol as mentioned in the isolation of fungi from plant parts. The six bits of surface-sterilized stem, leaf, and root of tomato plant parts were transferred to the Petri plates containing Potato Dextrose Agar (PDA) and incubated at 25 ± 2 °C for seven days. After seven days of incubation, the colonization percentage was calculated as detailed below (Petrini and Fisher 1986).

$$\text{Colonization (\%)} = \text{PF/TP} \times 100$$

where PF is the number of pieces exhibiting fungal growth and TP is the total number of pieces plated.

Molecular confirmation of endophytic colonization of *B. bassiana* UHSB-END1 in the tomato plant

Extraction of plant DNA and quantification

The genomic DNA of *B. bassiana* UHSB-END1-colonized and non-colonized tomato plants was extracted after 14, 40, 60, 80, and 120 dpi and next-generation seeds and seedlings of 25 days old using CTAB protocol (Zhang et al. 2010) with slight modification. The isolated DNA was quantified using the nano-drop (Thermo Fisher Nano-drop TM 2000/2000c). The appropriate required quantity of genomic DNA to run PCR was diluted with sterile nuclease-free water.

Specific PCR protocol

All PCRs included a positive control (DNA of mother culture of *B. bassiana*) and negative control (PCR mixture except template). The PCR mixture contained 6 µl of master mix, 0.5 µl ITS 4-forward primer, 0.5 µl ITS 4-reverse primer, 1 µl of template DNA, and total volume made up 20 µl with de-ionized nuclease-free water. The PCR parameters were: initial denaturation of 95 °C for 9 min, denaturation of 94 °C for 1 min, annealing of 52 °C for 1 min, an extension of 72 °C for 1 min, following 35 cycles, and final extension of 3 min period at 72 °C. The reaction mixture was held at 4 °C until the tube was removed from the machine. Amplification products were separated by electrophoresis in 2% agarose gels in 1X TAE buffer for 60 min at 90 V and visualized under gel documentation unit (AlphaImager EC).

Assay of *B. bassiana* UHSB-END1-colonized leaves of tomato against *S. litura*

In-vivo study

The effect of endophytic *B. bassiana* UHSB-END1 was assessed using the standard method, followed by Qayyum et al. (2015). After 40 dpi, the leaves of tomato from different treatments were sampled, and twenty 2nd instar larvae of *S. litura* were allowed to feed on sampled leaves of each treatment. Other 20-larvae of second instar *S. litura* were allowed to feed on non-colonized tomato leaves, which served as control. The food was replaced every day to ensure the freshness of samples. The observation on the morality of *S. litura* was recorded at 1, 3, 5, 7, 10 and 15 days after feeding. The experiment was repeated thrice and maintained at room temperature

28 ± 1 °C and RH of 70–75% and continued up to the end of the insect lifecycle.

In-planta study

Similarly, the pot culture experiment was conducted to evaluate the efficacy of endophytic *B. bassiana* UHSB-END1 against *S. litura*. About 20 larvae of second instar *S. litura* were released on five endophytic *B. bassiana* colonized 40-day-old tomato plants in each treatment. The larvae were confined for their feeding by covering larvae released tomato branch with pin holed polythene bags. The live larvae were carefully transferred to a fresh branch of the same tomato plant daily to ensure fresh food until the completion of the larval stage. The non-colonized tomato plant was also released with 20-s instar larvae of *S. litura* for feeding, which served as control. The observation on the morality of *S. litura* was recorded at 1, 3, 5, 7, 10, and 15 days after release. The experiment was repeated three times. The per cent mortality, pupation, and adult emergence of *S. litura* were calculated.

Confirmation of vertical transmission of *B. bassiana* UHSB-END1

The seed-to-seed transmission of endophytic *B. bassiana* UHSB-END1 was confirmed by comparing the sequences of ITS region of both mother and re-isolated fungal culture from the seedlings of next generation. The genomic DNA was isolated using CTAB method, and ITS region was amplified using PCR protocol as mentioned in Sect. 2.7.1 & 2.7.2. The similarities of ITS sequences of both mother and re-isolated fungus culture were performed using Clustal omega (https://www.ebi.ac.uk/Tools/msa/clustalol(1.81)).

Statistical analysis

The data on cumulative per cent mortality were subjected to arcsine transformation. The information on mortality of larvae was subjected to two-way ANOVA, a completely randomized design. The data on larval mortality in both *in vivo* and *in vitro* were assessed by f-test with a significance of difference at $p < 0.001$ in IBM Statistical Package for the Social Sciences (SPSS v.22). The data on sub-lethal effects (impact on growth and development of larvae) were subjected to one-way ANOVA, a completely randomized design. The mean data on larval mortality and sub-lethal outcomes were compared at 1 and 5% CD using Duncan's multiple range test (DMRT) (Gomez and Gomez 1984).

Results

Fungal identification

Morphological identification

The fungal isolate collected from the tomato plant was identified as EPF, *B. bassiana*. The fungal colonies were white mold, dry powdery, and brownish on the reverse side on PDA medium. Conidia were hyaline, single celled, globose, and smooth walled (Fig. 1). It was identified finally by Agarkar Research Institute, National Fungal Culture Collection of India (NFCCI), Pune, Maharashtra, India. The pure culture of the indigenous isolate was also submitted (Accession no. NAIMCC-SF-0064) at the Indian Council of Agricultural Research (ICAR)—National Bureau of Agriculturally Important Microorganisms (NBAIM), Maunath Bhanjan, Uttara Pradesh, India.

Molecular identification

The sequence of *B. bassiana* UHSB-END1 was deposited in NCBI GenBank with accession number OM131742. Blast analysis of generated fungal sequence showed 99% similarity with other isolates of *B. bassiana* (MK049987; LN886699; MH754663; KT183366; MN710408; MN428792; OK331343; MT635020; MN428795; MG763749). A phylogenetic tree of *B. bassiana* UHSB-END1 at species level was generated using Neighborhood Joining (NJ) method. The phylogenetic analysis showed that the isolate (OM131742) of the present study has a close affinity to *B. bassiana* with strong bootstrap support (100%) (Fig. 2).

Recovery of endophytic *B. bassiana* UHSB-END1 from a tomato plant

Per cent colonization

The 100% colonization in leaves, stem, and root of a plant at 14, 40, 60, and 80 dpi were recorded in all the methods of inoculation of endophytic *B. bassiana* UHSB-END1. It was decreased to less than 80% after 120 dpi (peak reproductive stage of tomato crop) across different methods of inoculation. The rate of colonization of fungi was again increased to 100% when recovered from next generation seeds and seedlings of 25 days old (seed treatment method of inoculation). This indigenous isolate was well colonized in all plant parts during the vegetative stage (Fig. 3).

Molecular confirmation

Endophytic colonization of *B. bassiana* UHSB-END1 was confirmed by molecular characterization after 14, 40, 60, 80, and 120 dpi and next-generation seeds and seedlings. The PCR-amplified product band of approximately 650 base pairs (bp) specific to the ITS region of tomato was observed in all the treatments (seed treatment, seedling

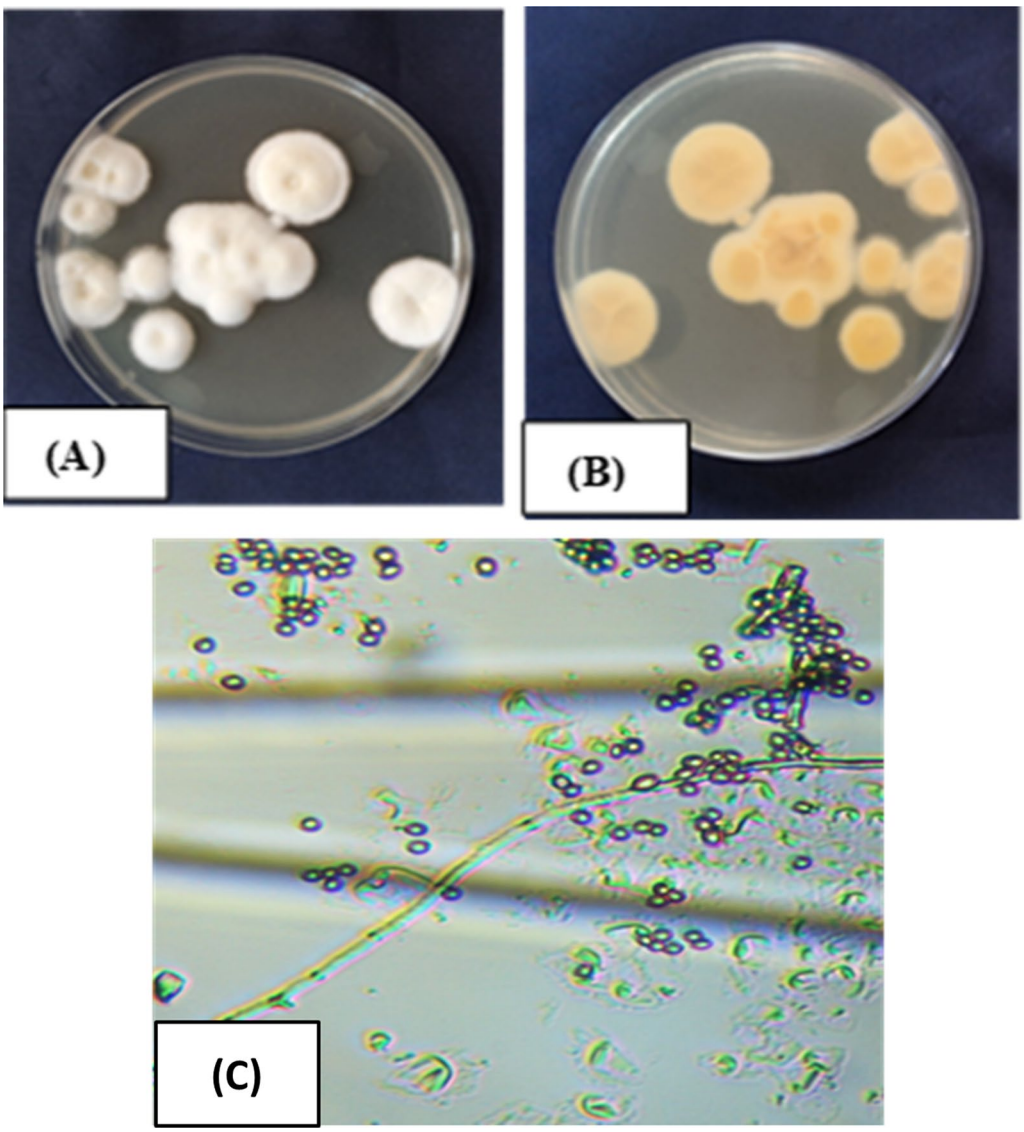

Fig. 1 Morphological characters of *Beauveria bassiana* **A**, **B** *B. bassiana* cultured on PDA medium at 25 ± 2 °C for 7 days **A** the front side **B** the reverse side **C** Conidia were hyaline single celled, globose, and smooth walled

root dip, soil drenching, foliar spray, combination) and control. In addition, all the treated plants with *B. bassiana* UHSB-END1 by different methods showed another amplified band around 566 bp specific to the ITS region of fungal isolate, hence confirming endophytic colonization of *B. bassiana* UHSB-END1 in tomato plant (Fig. 4).

Assay of *B. bassiana* UHSB-END1-colonized leaves of tomato against *S. litura*

In-Vivo study

Mortality of *S. litura* larvae was nil after feeding endophytic *B. bassiana* isolate UHSB-END1-colonized tomato leaves across different methods of colonization after one day of feeding. All the colonization methods recorded more than 50% mortality of larvae after seven days of feeding. The pooled mean mortality of larvae was significantly different between the colonization methods ($F_{5,73} = 118.93$; $p < 0.0001$), between the days after feeding (DAF) ($F_{5,73} = 313.56$; $p < 0.00001$), and also between the interactions of colonization methods and DAF ($F_{24,73} = 5.22$; $p < 0.01$). Statistically, significantly high pooled mortality of larvae was recorded in the treatment T5 (71.33%), followed by T4 (64.33%). The pooled mean mortality of larvae was significantly varied across DAF and mortality initiated at three DAF (30%) and continued up 15 DAF (73.61%). Similarly, the mean mortality

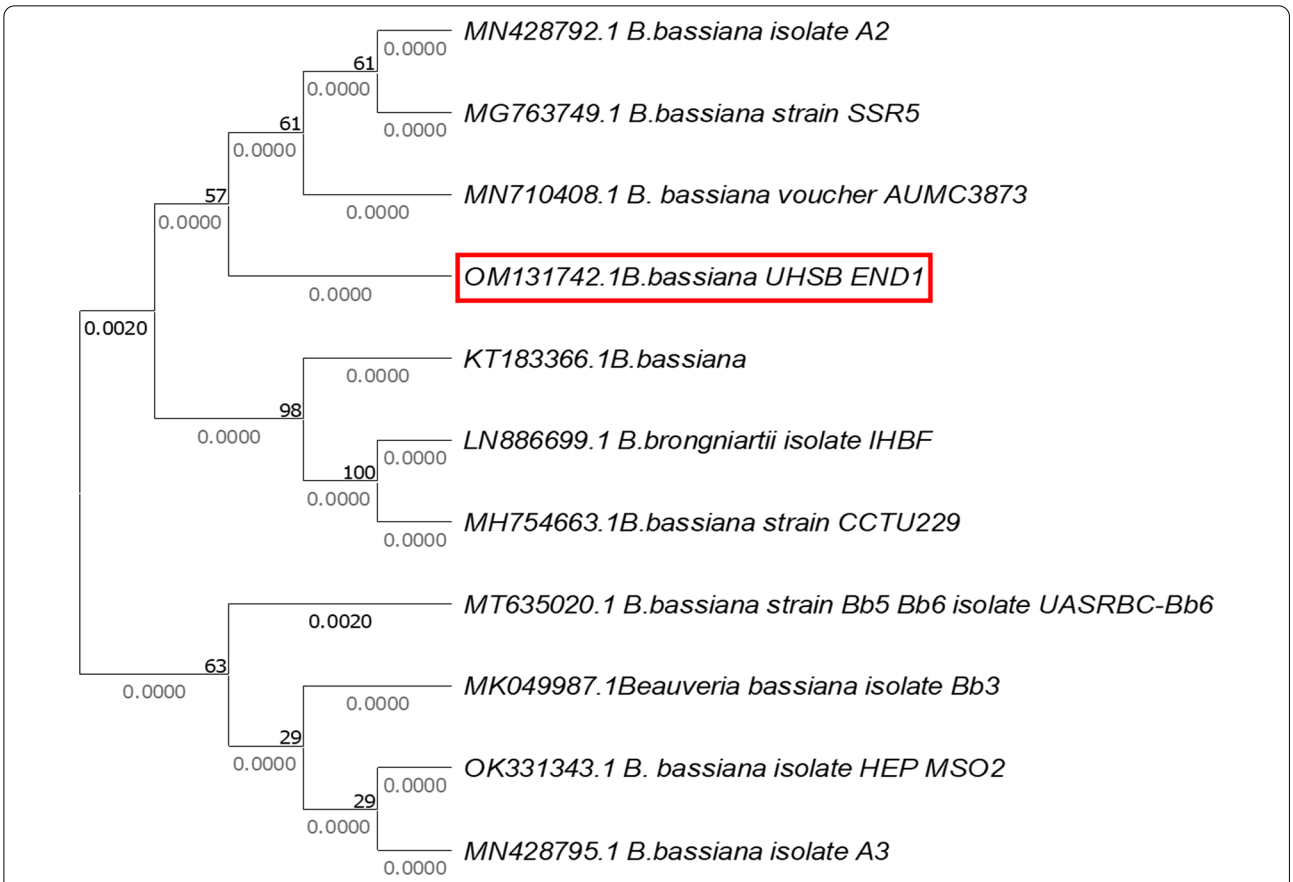

Fig. 2 Phylogenetic tree based on Neighborhood Joining (NJ) analysis. The sequence OM131741 is the sequence generated in the present study of *Beauveria bassiana* UHSB-END1, and it is branched with retrieved sequences of *B. bassiana*

of larvae was also varied across interactions of colonization methods and DAF. The significant highest mortality across different treatments was seen after 15 days of feeding the leaves (Table 1).

The effect of *B. bassiana* on growth and development of *S. litura* larvae by feeding colonized tomato leaves showed significantly lowest pupation (10%) in foliar spray and combination methods. There was a significant difference among all the treatments ($F_{5,12}=6456.134$, $p<0.00001$). The larvae which didn't enter into pupation were malformed; maximum (15%) was recorded in the soil drenching method of colonization of *B. bassiana,* followed by seed treatment and seedling root dip method ($F_{5,12}=1166.717$, $p<0.00001$). The adult emergence of *S. litura* was nil after feeding tomato leaves colonized through combination of methods ($F_{5,12}=3309.164$, $p<0.00001$) showing it was the most virulent to *S. litura* larvae (Table 2).

In planta study

The pot culture experiment showed immediate action of *B. bassiana* colonized tomato plant on *S. litura* larvae as evidenced by 10–25% mortality of larvae after one day of feeding. No mortality of larvae was noticed in the control plant across different days after release of larvae, and they were completed normal life cycle. The pooled mean mortality of larvae was found to significantly different between the colonization methods ($F_{5,73}=3633$; $p<0.00001$), between the days after release (DAR) ($F_{5,73}=2319$; $p<0.0001$), and also between the interactions of colonization methods and DAR ($F_{24,73}=132$; $p<0.0001$). Seed treatment (T1) recorded statistically significantly higher pooled mortality (68.33%) of larvae and followed by other treatments. The pooled mean mortality of larvae was significantly varied across DAR and mortality initiated at one DAR (11.67%) and continued up 15 DAR (77.50%). Similarly, the mean mortality of larvae was also varied across interactions of colonization methods and DAR. All the colonization methods recorded more than 50% mortality of larvae after seven days of release on the plant. After 15 days of release, the colonized leaves showed maximum virulence on larvae in all the treatments. (Table 3).

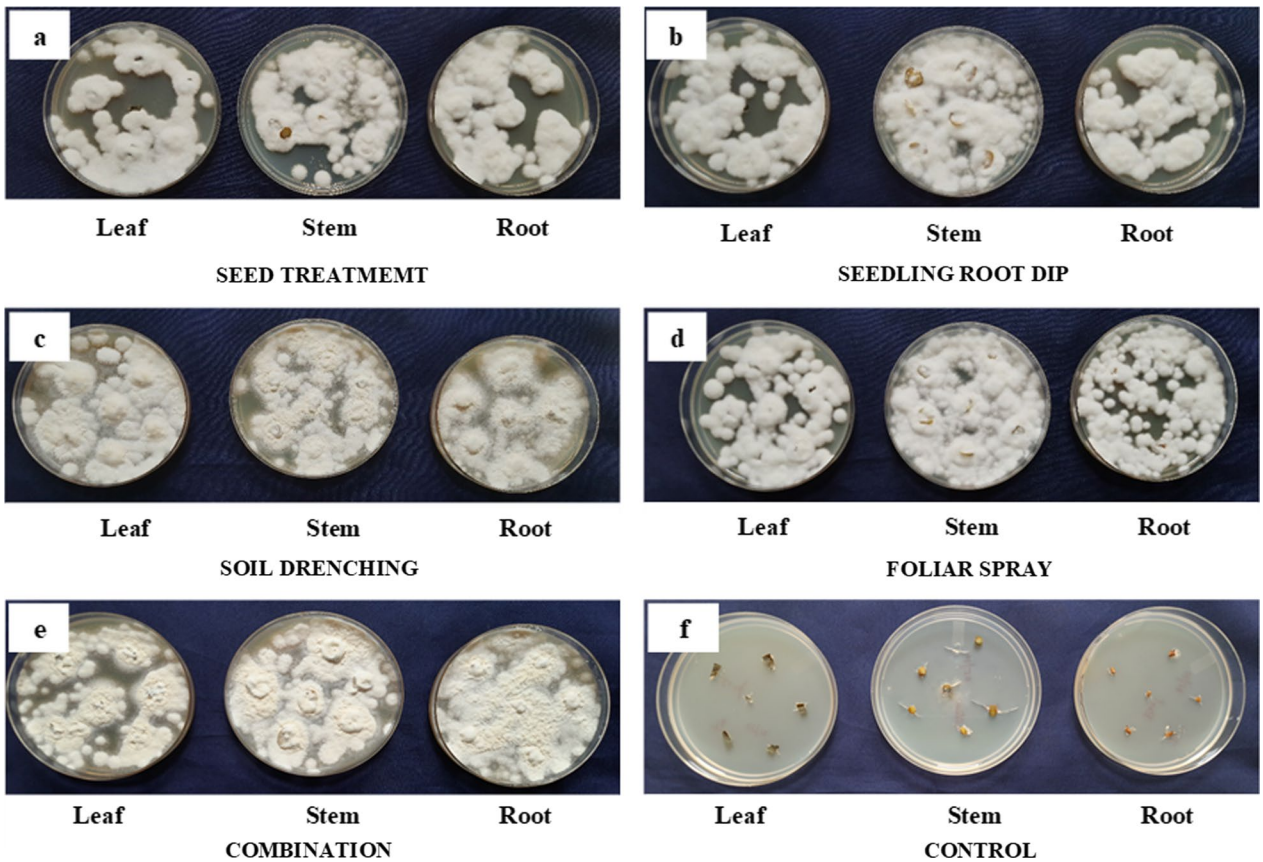

Fig. 3 Recovery of indigenous endophytic, *Beauveria bassiana* UHSB-END1 from tissues (leaf, stem and root) of tomato showing colony growth at different treatments: seed treatment (**a**), seedling root dip (**b**), soil drenching (**c**), foliar spray (**d**), combination of seed treatment, seedling root dip and foliar spray **e** at 40 dpi as compared to control (**f**)

Significant differences between all the treatments on pupation of *S. litura* was documented ($F_{5,12} = 19{,}954.247$, $p < 0.00001$). The pupation was highly reduced in treatments as compared to control. Similarly, the highest malformed pupae were noticed the treatments wherein, feeding of non-colonized tomato plant by *S. litura* larvae showed 5% malformed pupa ($F_{5,12} = 357.667$, $p < 0.00001$). The adult emergence was not noticed in the foliar spray, seedling root dip and combination of methods ($F_{5,12} = 4582.072$, $p < 0.00001$) (Table 4).

Confirmation of vertical transmission of *B. bassiana* UHSB-END1

The sequences of the mother culture of *B. bassiana* isolate UHSB-END1 (566 bp) and re-isolated fungus from next-generation seedlings were 100% identical. Therefore, the re-isolated fungal culture from seedlings was *B. bassiana* isolate UHSB-END1. This research finding reveals that nongrass fungal endophyte, *B. bassiana* UHSB-END1, can be vertically transmitted to next generation via seeds. As per the literature evidence, it is shown that vertical transmission of endophytes was proved in grass species. However, a vertical transmission was demonstrated for the first time in non-grass species in the present study.

Discussion

Identification of endophytic indigenous isolates of *B. bassiana* is necessary since colonization and efficacy of *B. bassiana* depend on isolates collected from different flora and fauna across the geographical regions (Sinno et al. 2020). Therefore, most virulent indigenous isolate of endophytic EPF, *B. bassiana* UHS-END1 from leaf tissues of tomato plant, was isolated and identified. This indigenous isolate was efficient in colonizing different tissues (leaf, stem, and root) of tomato plant in different methods of treatment. The rate of colonization

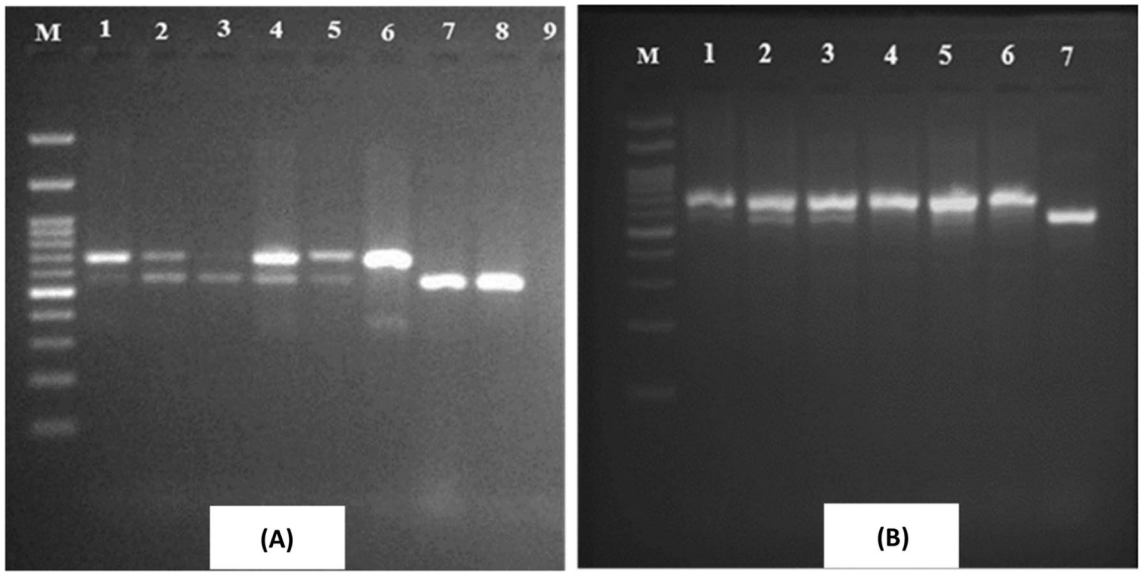

Fig. 4 Confirmation of endophytic colonization of *Beauveria bassiana* UHSB-END1 through PCR using endophyte-specific ITS primers. **a** M-ladder, Lanes 1–14, 2–40, 3–60, 4–80, 5–120 dpi, 6-Control, 7—mother culture (original fungal strain), 8—reisolated fungus, 9—negative control (without DNA template); **b** M-ladder, Lanes 1—fresh seeds, 2–10-day dried seeds, 3–25-day-old seedlings, 4—control (fresh seeds),5—control (10-day dried seeds), 6—control (25-day-old seedlings), 7—mother culture (original fungal strain)

Table 1 In vivo feeding assay of endophytically colonized *Beauveria bassiana* UHSB-END1 tomato leaves against *Spodoptera litura* larvae

	F-Test	S. Em (±)	CD	F Calculated	F Probable
Treatment	118.93; < 0.0001	1.35	3.83	157.699	1.633
Days	82.72; < 0.0001	1.24	3.50		
Treatment x Days	5.21;p < 0.01	3.03	8.57		

S. Em standard error of mean; *CD* critical difference

Table 2 Effect of endophytically colonized *Beauveria bassiana* UHSB-END1 in tomato plant on growth and development of *Spodoptera litura* under in vivo condition

Observations	S. Em (±)	F Calculated	F Probable	CD at 1%	CD at 5%
Pupation (%)	0.27	6456.134	3.850	1.73	1.23
Malformed pupa (%)	0.23	1166.717	1.094	1.01	0.72
Adult emergence (%)	0.40	3309.164	2.126	1.151.23	0.82

S. Em standard error of mean; *CD* critical difference

Table 3 *In-Planta* feeding assay of endophytically colonized *Beauveria bassiana* UHSB-END1 in tomato plant against *Spodoptera litura* larvae

	F-Test	S. Em (±)	CD	F Calculated	F Probable
Treatment	106.46; < 0.0001	1.19	3.34	6858.44	2.685
Days	313.56; < 0.00001	1.19	3.34		
Treatment x Days	5.22;p < 0.01	3.03	8.57		

S. Em standard error of mean; *CD* Critical difference

Table 4 Effect of endophytically colonized *Beauveria bassiana* UHSB-END1 in tomato plant on growth and development of *Spodoptera litura* under *In-Planta* condition

Observations	S. Em (±)	F Calculated	F Probable	CD at 1%	CD at 5%
Pupation (%)	0.22	19,954.247	4.430	0.97	0.69
Malformed pupa (%)	0.31	357.667	1.278	1.34	0.95
Adult emergence (%)	0.44	4582.072	3.012	1.90	1.35

S. Em standard error of mean; *CD* Critical difference

of *B. bassiana* UHSB-END1 in different plant parts was equal across different treatments. It is evidenced by 100% recovery of fungus from root, stem, and leaf in all the methods of colonization so this isolate is having potential to recommend any methods for colonization of *B. bassiana* in tomato. This adds possible movement of fungus not only imbibition through seed but also

through stomatal opening and root uptake. This is an additional benefit over earlier isolates reported wherein they required specific method (seed treatment) of colonization in tomato plant (Sanchez-Rodriguez et al. 2018). The active symbiotic association exists between endophytic fungi and tomato plant only at active plant growth period. This relationship may be hampered as the tomato plant reaches to reproductive stage or end of their lifecycle (Jia et al. 2016). This may be due to poor environmental conditions for growth of fungal endophyte inside the host. In turn, endophytes develop hidden tactics to disseminate to next generation via reproductive propagules of the plant (seeds). The grand growth period of tomato plant (vegetative phase) favors the growth and colonization of fungal endophyte transmitted from maternal seeds. Therefore, per cent colonization of this isolate in tomato fruit and their impact on *S. litura* larvae is novel approach to study. *Beauveria bassiana* UHSB-END1 colonized 100% during active growth period of plant (14, 40, 60 and 80 dpi) in different tissue (leaf, stem, and root) of tomato. The complete colonization of various tissues of the tomato plant is having more advantages in getting protection against insect pests (Wei et al. 2020). The partial or no colonization (Silva et al. 2020) and very poor colonization (Allegrucci et al. 2017) of *B. bassiana* in different tissues of tomato plants may reduce defensive forces against insect pests. The persistence of symbiotic relationship its trade-off between endophytic fungi and host plant at different phenological phases of plant from seedling to harvest needs to be studied for their colonization to avoid pest attack at various stages of the crop. The isolates exhibiting vertical transmission (seed to seed) behavior with symbiotic association and between the host plant and fungi throughout the cropping season gain more importance than other non-symbiotic and non-colonized isolates in pest management (Quesada-Moraga et al. 2014). Therefore, vertical transmission of this fungal isolate via seeds from one generation to another and their physic-chemical interactions needs to be understood for better exploration of the phenomenon.

Vertical transmission of endophytes is proved in most of the grass species (Gagic et al. 2018). The grass endophytes reproduce sexually by ascospores. Anamorphic stages (asexual forms) grow in the seeds of the infected host plant and eventually get transmitted to next generation of plants (Liu et al. 2017). Vertically transmitted endophytes are moved directly from the parents to their progenies (Saikkonen et al. 2002). The endophytic species belonging to the genus *Neotyphodium* are mostly vertically transmitted through seeds to next generation (Hartley and Gange 2009). When transmission is through the maternal seeds, they are mentioned as seed-transmitted endophytes (Schardl et al. 2013). It is also proved that the grass endophytes get horizontally transmitted between host and pass on to next generation (Wiewiora et al. 2015). Unlike in grass endophytes, no much work has been done to study vertical transmission in entomopathogenic nongrass endophytes. However, there is a proof of natural occurrence of endophytic *B. bassiana* get vertically transmitted in Monterey Pine, *Pinus radiate* (Pinaceae: Pinaceae) and positively benefits the plant by protecting them from above and below ground insects (Lefort et al. 2016). In addition, artificial inoculation of *B. bassiana* strain 04/01-Tip to opium poppy (*Papaver somniferum* L.) through seeds endophytically colonized the plant and successfully got transferred from mother plants to their progenies proving the vertical transmission (Quesada-Moraga et al. 2014). Similarly, the present research findings resulted a new insight of non-grass endophytes transmitted vertically to next generation through seeds. For the first time in non-grass crop *i.e.*, tomato, it is demonstrated that *B. bassiana* isolate UHSB-END1 gets vertically transmitted to their progenies via seeds. This insightful result is helpful in understanding vertical transmission in tomato plants at different vegetative phases, and further transmission to the next generation makes them to be called as seed transmitted endophytes.

Utilization of endophytic EPF, *B. bassiana* UHSB-END1 can pave the way as alternative for chemical insecticides in IPM. The bioassay results both in vivo and in vitro showed biological potential of *B. bassiana* UHSB-END1 in bringing the mortality of *S. litura* larvae and impact on their normal life cycle (Fig. 5a, b and Fig. 6a, b). Very least (1%) mycosis was observed on *S. litura* larvae fed with *B. bassiana* colonized tomato plant in both in vivo and in vitro study. The mortality of larvae may be attributed combined toxic effect of bioactive secondary metabolites (insecticidal and antimicrobial) produced from both endophytic fungi and host plant rather than fungal infestation alone on the larvae (Vega 2018). Further study on chemical compounds responsible for the mortality of larvae following feeding of endophytic colonized plants is very much essential. This isolate of *B. bassiana* can be utilized as one of the IPM components of *S. litura* after proving their efficacy under field conditions. Fungal isolate alone may not yield good result under field conditions, because it has to compete for moisture, nutrient, colonization in host plant with other endophytes of fungi or bacteria. Therefore, studying of this fungal endophyte under field condition by incorporating other non-chemical approaches such as use of botanical oils and soap, pheromone traps, trap crops, adding organic manure to the soil in IPM of tomato is most encouraging to produce pesticide free tomato.

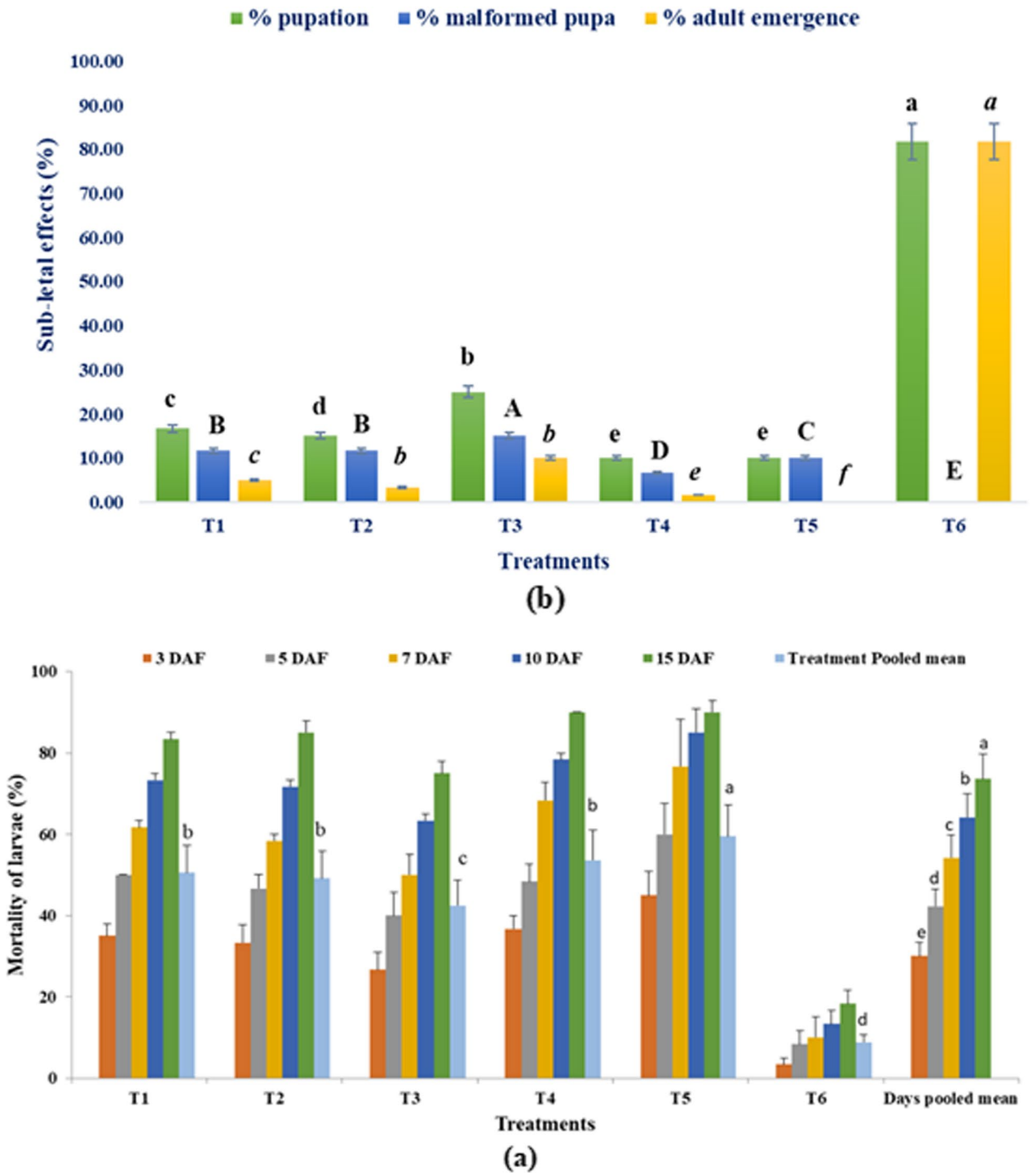

Fig. 5 Efficacy of endophytically colonized tomato plants by *Beauveria bassiana* UHSB-END1 on *Spodoptera litura* after 40 dpi **a** Larval mortality at different Days After Feeding (DAF) by 2nd instar larvae. Data are mean of three tests ($p^{\wedge}0.0001$, f test) **b** Sub-lethal effects in *in vivo* assay. Mortality data are subjected to one-way ANOVA, and means are compared by DMRT (T1—seed treatment, T2—seedling root dip, T3—soil drenching, T4—foliar spray, T5—combination, T6—control)

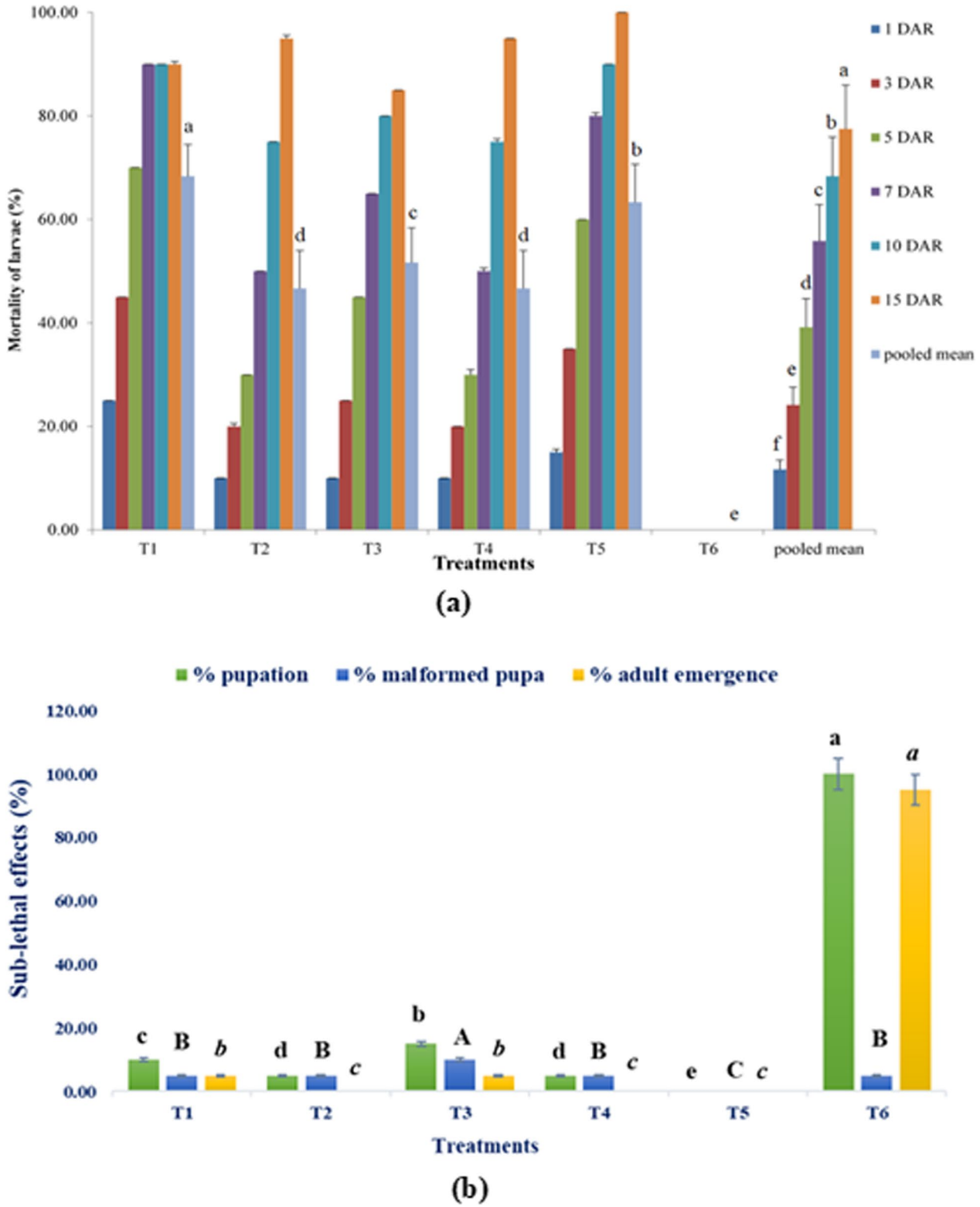

Fig. 6 Efficacy of endophytically colonized tomato plants by *Beauveria bassiana* isolate UHSB-END1 on *Spodoptera litura* after 40 dpi **a** Larval mortality at different Days After Release (DAR) of 2nd *S. litura* larvae. Data were mean ± SE of three tests ($p^{<}0.0001$, f test) **b** Sub-lethal effects in *in vitro* assay. Mortality data are subjected to one-way ANOVA, and means are compared by DMRT. (T1—seed treatment, T2—seedling root dip, T3—soil drenching, T4—foliar spray, T5—combination, T6—control)

Recently, endophytic nature of many EPF has been explored and used extensively as pest management strategies in different cultivated crops (Barra-Bucarei et al. 2020). Likewise, Silva et al. (2020) screened three *B. bassiana* isolates (LEF140, LPP139 and LEF141) against South American tomato pinworm, *Tuta absoluta* (Gelechiidae: Lepidoptera). All three *B. bassiana* isolates were virulent to *T. absoluta*, with approximately 90% mortality over 10 days, where 30- to 40-day-old tomato plants were exposed to fungal suspensions. The spray application of endophytic myco insecticide *B. bassiana* on the tomato plants caused 66.7–76.6% mortality of *S. littoralis* (Resquín-Romero et al. 2016).

Interestingly, in addition to the larval mortality, the effects on growth and development of *S. litura* larvae on colonized plants were also recorded. Noticeably, increased pupal malformation in the colonized plant and the adult emergence were drastically reduced across different treatment and it was nil in combination of methods used for colonization. There are similar results reported by Qayyum et al. 2015 that endophytically colonized tomato plants by three isolates of *B. bassiana* (WG-14, 19, 40) inversely affected the pupation and adult emergence of *Helicoverpa armigera* (Noctuidae: Lepidoptera). In the same line, Akutse et al. (2013) also studied endophytically colonized plants, *Vicia faba* (Fabaceae: Fabales), and *Phaseolus vulgaris* (Fabaceae: Fabales) by Hypocrea were most prominent in reducing number of pupae and adult longevity of pea leafminer, *Liriomyza huidobrensis* (Agromyzidae: Diptera). Adult emergence was significantly reduced in Hypocrea (21.4%) and *Beauveria* (38.0%) treatments. Similarly, Akello and Sikora (2012) evaluated systemic influence of endophytic *B. bassiana* (seed treatment) on *Acyrthosiphon pisum* (Aphididae: Hemiptera) and *Aphis fabae* (Aphididae: Hemiptera). The colonized plants effected offspring fitness, development, and fecundity of both the species of aphids.

The present *B. bassiana* UHSB-END1 was isolated from leaf of tomato plant and colonized tomato plant under controlled condition. Later colonized plants were tested for their efficacy both in vivo and in vitro conditions against *S. litura* larvae. However, there are further confirmation of its colonization in tomato fruit and subsequent accumulation of any toxic metabolites. If there is production of toxic compounds in tomato fruit following colonization, that may be sufficient to bring the mortality of *S. litura* larvae or not. The produced toxins in fruit may affect human health or not by direct consumption fruit as salad. The colonization of this fungal isolate, whether it protects the vegetative stage of the crop only or needs separate management approach at reproductive stage of the crop, needs to be studied. It is also necessary to ensure safety of the isolate to other non-target organisms like predators, parasitoids, and pollinators before incorporating as one of the tools in IPM of *S. litura* in tomato. To conclude, this study is the first report of 100% colonization by indigenous EPF, *B. bassiana* UHS-END1 isolate in tomato plant. This indigenous isolate was identified based on its morphology and molecular studies. In addition, the study demonstrated the virulence of endophytically colonized tomato plant on *S. litura* mortality and effect on normal growth and development of insect. After ensuring the safeness of this isolate against non-target organisms and human beings, it can be one of the constitutes in sustainable cost-effective strategy for management of pests affecting tomato as one of the components in integrated pests management (IPM). Inoculation of endophytic EPF into seed/ seedling reduces environmental impacts and also easy, economical, and sustainable approach for pest management in horticulture crops which are often consumed as raw. Later, it can be expanded for the management of insect pests on other commercially cultivated vegetable crops. Although field studies are required to support the present finding, this appears to be an interesting tool that should be considered for pest biocontrol.

Conclusion

The use of endophytic EPF as a biocontrol agent is popularizing in recent days for the insect pest management in agro-ecosystem. However, identification of indigenous (region-specific) isolates gains the most important since they vary in pathogenicity and virulence against insect pest from one location to other. Therefore, this isolate was identified locally and shown good results in bringing the desirable mortality of *S. litura* larvae and impact on normal growth and development of larvae. Introducing the endophytic EPF into seed level will reduced the environmental pollution, and it is easy, economical, and sustainable approach for pest management. After confirming the safety to non-target organism and field studies, this isolate can be used commercially for the management of lepidopteran complex as one of the components in IPM of tomato.

Abbreviations

EPF: Entomopathogenic fungi; mBCA: Microbial biological control agents; PDA: Potato dextrose agar; PDB: Potato dextrose broth; IPM: Integrated pest management; UHSB-END1: Isolate name (University of Horticultural Sciences-Endophyte 1); DAF: Days after feeding; DAR: Days after release.

Acknowledgements

All authors sincerely acknowledge the University of Horticultural Sciences Bagalkot for extending facilities to carry out this research at the Central Instrumentation Facilities Laboratory and Infrastructure. We would like to express our sincere gratitude to Pioneer Data Solutions, Bengaluru, for data analysis.

Author contributions
JGS and RSH were major contributor in writing the manuscript, and they are the owner of the idea. All authors read and approved the final manuscript. JGS did all experiments, JJ and GJB were analyzed and interpreted the data, RDL and MSK were responsible for the identification of the fungus, and RG and MBNN were responsible for the molecular identification of the fungus. All authors read and approved the final manuscript.

Funding
Not applicable.

Availability of data and materials
Applicable.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Entomology, College of Horticulture, University of Horticultural Sciences, Bagalkot, Karnataka 587104, India. [2]Department of Biotechnology and Crop Improvement, College of Horticulture, University of Horticultural Sciences, Bagalkot, Karnataka 587104, India. [3]Department of Agricultural Microbiology, College of Horticulture, University of Horticultural Sciences, Bagalkot, Karnataka 587104, India. [4]University of Horticultural Sciences, Bagalkot, Karnataka 587104, India. [5]Department of Biotechnology and Crop Improvement, Kittur Rani Channamma College of Horticulture, University of Horticultural Sciences, Arabhavi, Bagalkot 591218, Karnataka, India. [6]Regional Horticulture Research and Extension Center, Kumbapur (Dharwad), University of Horticultural Sciences, Bagalkot, Karnataka 587104, India.

Received: 16 June 2022 Accepted: 13 October 2022
Published online: 23 October 2022

References

Agbessenou A, Akutse KS, Yusuf AA, Ekesi S, Subramanian S, Khamis FM (2020) Endophytic fungi protect tomato and nightshade plants against *Tuta absoluta* (Lepidoptera: Gelechiidae) through a hidden friendship and cryptic battle. Sci Rep 10:1–13

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

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

Allegrucci N, Velazquez MS, Russo ML, Perez ME, Scorsetti AC (2017) Endophytic colonization of tomato by the entomopathogenic fungus *Beauveria bassiana*: the use of different inoculation techniques and their effects on the tomato leafminer *Tuta absoluta* (Lepidoptera: Gelechiidae). J Plant Prot Res 57:331–337

Barra-Bucarei L, Gonzalez MG, Iglesias AF, Aguayo GS, Penalosa MG, Vera PV (2020) *Beauveria bassiana* multifunction as an endophyte: growth promotion and biologic control of *Trialeurodes vaporariorum*, (Westwood) (Hemiptera: Aleyrodidae) in tomato. Insects 11:591

Gagic M, Faville MJ, Zhang W, Forester NT, Rolston MP, Johnson RD, Ganesh S, Koolaard JP, Easton H, Hudson D, Johnson LJ (2018) Seed transmission of *Epichloë* endophytes in *Lolium perenne* is heavily influenced by host genetics. Front Plant Sci 9:1580

Gomez KA, Gomez AA (1984) Statistical procedures for agricultural research. Wiley, New York, p 680

Greenfield M, Gomez-Jimenez MI, Ortiz V, Vega FE, Kramer M, Parsa S (2016) *Beauveria bassiana* and *Metarhizium anisopliae* endophytically colonize cassava roots following soil drench inoculation Biol. Control 95:40–48

Gualandi RJ Jr, Auge RM, Kopsell DA, Ownley BH, Chen F, Toler HD, Dee MM, Gwinn KD (2014) Fungal mutualists enhance growth and phytochemical content of *Echinacea purpurea*. Symbiosis 63:111–121

Hartley SE, Gange AC (2009) Impacts of plant symbiotic fungi on insect herbivores: mutualism in a multitrophic context. Ann Rev Entomol 54:323–342

Jaber LR (2018) Seed inoculation with endophytic fungal entomopathogens promotes plant growth and reduces crown and root rot (CRR) caused by *Fusarium culmorum* in wheat. Planta 248:1525–1535

Jaber LR, Enkerli J (2016) Effect of seed treatment duration on growth and colonization of Vicia faba by endophytic *Beauveria bassiana* and *Metarhizium brunneum*. Biol Control 103:187–195

Jia M, Chen L, Xin H, Zheng C, Rahman K, Han TA (2016) Friendly relationship between endophytic fungi and medicinal Plants: a systematic review. Front Microbiol 7:1–14

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35:1547–1549

Kusari S, Hertweck C, Spiteller M (2012) Chemical ecology of endophytic fungi: origins of secondary metabolites. Chem Biol 19(7):792–798

Lefort MC, McKinnon A, Nelson TL, Glare T (2016) Natural occurrence of the entomopathogenic fungi *Beauveria bassiana* as a vertically transmitted endophyte of *Pinus radiata* and its effect on above-and below-ground insect pests. PeerJ PrePrints 4:6321

Lin Y, Hussain M, Avery PB, Qasim M, Fang D, Wang L (2016) Volatiles from plants induced by multiple aphid attacks promote conidial performance of *Lecanicillium lecanii*. PLoS ONE 11:e0151844

Litwin A, Nowak M, Rozalska S (2020) Entomopathogenic fungi: unconventional applications. Rev Environ Sci Biotechnol 19:23–42

Liu J, Nagabhyru P, Schardl CL (2017) *Epichloë festucae* endophytic growth in florets, seeds, and seedlings of perennial ryegrass (*Lolium perenne*). Mycologia 109:691–700

Mahapatra SS, Sahoo BK, Kariyanna B (2018) Seasonal Incidence of lepidopteran pests (*Helicoverpa armigera* Hub. and *Spodoptera litura* Fab.) in tomato. Int J Curr Microbiol App Sci 7:867–871

Mantzoukas S, Eliopoulos PA (2020) Endophytic entomopathogenic fungi: a valuable biological control tool against plant pests. Appl Sci 10:360

Petrini O, Fisher PJ (1986) Fungal endophytes in *Salicornia perennis*. Trans Br Mycol Soc 87:647–651

Qayyum MA, Wakil W, Arif MJ, Sahi ST, Dunlap CA (2015) Infection of *Helicoverpa armigera* by endophytic *Beauveria bassiana* colonizing tomato plants. Biol Control 90:200–207

Quesada-Moraga E, Lopez-Diaz C, Landa BB (2014) The hidden habit of the entomopathogenic fungus *Beauveria bassiana*: first demonstration of vertical plant transmission. PLoS ONE 9:e89278

Reay SD, Brownbridge M, Gicquel B, Cummings NJ, Nelson TL (2010) Isolation and characterization of endophytic *Beauveria* spp. (Ascomycota: Hypocreales) from *Pinus radiata* in New Zealand forests. Biol Control 54:52–60

Saikkonen K, Gyllenberg M, Ion D (2002) The persistence of fungal endophytes in structured grass metapopulations. Proc R Soc London Ser B Biol Sci 269:1397–1403

Sánchez-Rodriguez AR, Raya-Diaz S, Zamarreno AM, Garcia-Mina JM, Del Campillo MC, Quesada-Moraga E (2018) An endophytic *Beauveria bassiana* strain increases spike production in bread and durum wheat plants and effectively controls cotton leaf worm (*Spodoptera littoralis*) larvae. Biol Control 116:90–102

Saragih M, Trizelia T, Nurbailis N, Yusniwati Y (2019) Endophytic colonization and plant growth promoting effect by Entomopathogenic fungus, *Beauveria bassiana* to Red Chili (*Capsicum annuum* L.) with Different Inoculation Methods. In: IOP Conference series: earth environmental science 305:012070

Schardl CL, Young CA, Pan J, Florea S, Takach JE, Panaccione DG, Farman ML, Webb JS, Jaromczyk J, Charlton ND, Nagabhyru P (2013) Currencies of mutualisms: sources of alkaloid genes in vertically transmitted epichloae. Toxins 5:1064–1088

Sharma A, Srivastava A, Shukla AK, Srivastava K, Srivastava AK, Saxena AK (2020) Entomopathogenic fungi: a potential source for biological control of insect pests. In: Solanki MK, Kashyap PL, Kumari B (eds) Phytobiomes: current insights and future vistas. Springer, Singapore, pp 225–250

Shrivastava G, Ownley BH, Auge RM, Toler H, Dee M, Vu A, Kollner TG, Chen F (2015) Colonization by *arbuscular mycorrhizal* and endophytic fungi enhanced terpene production in tomato plants and their defense against an herbivorous insect. Symbiosis 65:65–74

Silva ACL, Silva GA, Abib PHN, Carolino AT, Samuels RI (2020) Endophytic colonization of tomato plants by the entomopathogenic fungus *Beauveria bassiana* for controlling the South American tomato pinworm, *Tuta absoluta*. CABI Agricul Biosci 1:1–9

Sinno M, Ranesi M, Gioia L, d'Errico G, Woo SL (2020) Endophytic fungi of tomato and their potential applications for crop improvement. Agriculture 10:587

Sowmya G, Ramanagouda SH, Jayappa J (2017) Lethal and sub lethal effects of indigenous isolates of *Metarhizium anisopliae* (Metchnikoff) Sorokin against *Spodoptera litura* (Fabricius). J Entomol Zool Stud 5:1154–1160

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

Wei QY, Li YY, Xu C, Wu YX, Zhang YR, Liu H (2020) Endophytic colonization by *Beauveria bassiana* increases the resistance of tomatoes against *Bemisia tabaci*. Arthropod Plant Interact 14:289–300

Wiewiora B, Zurek G, Panka D (2015) Is the vertical transmission of *Neotyphodium lolii* in perennial ryegrass the only possible way to the spread of endophytes? PLoS ONE 10:e0117231

Yuan ZS, Liu F, Xie BG, Zhang GF (2018) The growth-promoting effects of endophytic bacteria on *Phyllostachys edulis*. Arch Microbiol 200:921–927

Zhang YJ, Zhang S, Liu XZ, Wen HA, Wang MA (2010) Simple method of genomic DNA extraction suitable for analysis of bulk fungal strains. Lett Appl Microbiol 51:114–118

Publisher's Note