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RESEARCH

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Colonization and endophytic effect of *Beauveria bassiana* (Bals.-Criv.) Vuill. UHSB-END1 against *Myzus persicae* (Sulzer) and *Plutella xylostella* (L.) in cabbage

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Abstract

Background Fungal microbial biocontrol agent playing an important role in sustainable pest management with low cost. Conidial contact with cuticle is sufficient to cause disease in insects by entomopathogenic fungi (EPF) compared to bacteria and viruses which required ingestion of contaminated food. The field level application of fungi resulted inconsistent in their efficacy against insects as they withstand in the agroecosystem with adverse environmental conditions. To overcome this bottleneck, endophytic EPF, *Beauveria bassiana* (Bals.-Criv.) Vuill. (Cordycipitaceae: Hypocreales), is well-studied for the management of insects of cultivated crops and got promising results. The colonization of endophytic isolates varied from one host plant to another and virulence against insects too. Hence, the study was undertaken to know the colonization of indigenous *B. bassiana* UHSB-END1 isolate in cabbage plant and its efficacy against major insects of cabbage.

Results The indigenous isolate of *B. bassiana* UHSB-END1 was able to colonize cabbage in all the methods of inoculations (seed treatment, seedling root dip, soil drenching, foliar spray and combination treatment) at 30, 45 and 60 days of post-inoculation (dpi). However, the colonization of the fungus inside the cabbage was restricted to tissues of inoculation, and movement from the site of inoculation to other parts of the plant was poor. In the present study, colonization was the highest at 30 dpi in all methods of colonization with restricted colonization to the site of exposure of fungus. Further, in vivo and *in planta* assay confirmed the effectiveness of *B. bassiana* UHSB-END1 colonized cabbage against *Myzus persicae* (Sulzer) and *Plutella xylostella* (Linnaeus). Under in vivo conditions the maximum mortality of *M. persicae* and *P. xylostella* was recorded at 30 dpi. It was decreased slightly at 45 dpi, and the lowest morality was recorded at 60 dpi. *In planta* experiment also proved better efficacy against both the test insects. Wherein mortality of *M. persicae* ranged from 22 to 36% five days after release (DAR), it was increased to 48–68% at 10 DAR and reached highest mortality rate at 15DAR (72–96%). Similarly, mortality of *P. xylostella* ranged from 14 to 24% after five DAR, and it was 27–44% at 10 DAR, and maximum mortality rate of larvae were recorded at 15 DAR (64–96%).

Conclusion The present study reported 100% colonization of cabbage plant by indigenous *B. bassiana* UHSB-END1 at 30 dpi in combination of treatment and showed a maximum efficacy in managing the population of major insects of cabbage. Introducing this indigenous isolate will pave a ray of hope in managing the both key insects (*P. xylostella*

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and *M. persicae*) in cabbage agroecosystem without disturbing the environment and further helps in production of cabbage with minimum pesticide residue for consumers.

Keywords Endophyte, *Beauveria bassiana*, *Plutella xylostella*, *Myzus persicae*, Cabbage, Potential

Background

Cabbage, (*Brassica oleracea* L. *convar. capitata* (L.) Alef. (Brassicaceae), is an important vegetable crop grown throughout the world. India is the second largest producer of cabbage; still there is a scope for improvement in the production and productivity through efficient pest management. Insect pests are the major bottleneck for significant yield loss, which ultimately lower production and productivity. The diamondback moth, *Plutella xylostella* (Linnaeus) (Plutellidae: Lepidoptera), is the most destructive pest on cruciferous vegetables causing 90% marketable yield loss (Gashawbeza 2006). The first line of defence for the management of insect by farmers is synthetic insecticides. The continuous exposure of insecticides in agroecosystems leads to development of resistance and resurgence in target insect, environmental pollution and serious human health problem.

Among the microbial biocontrol agents, the fungi are playing important role in integrated pest management (Khan et al. 2012). The microbial biocontrol agents such as bacteria and viruses required ingestion of microbes along with contaminated food to cause disease in insects. However, fungi need contact of insect cuticle to cause diseases (Bilgo et al. 2018). The scientific reports highlight the effectiveness of fungal microbes on insect management under laboratory conditions continuously; however, they are not proving inconsistent results in the field. Therefore, their use in pest management by the farmers taking back steps, and hence, they are not popularizing among the farming community. These microbes have a challenge to perform under varied climatic conditions such as direct exposure to ultraviolet (UV) light, high temperature and low relative humidity and moisture (Vega 2018). Hence, studying the various ecological role in relation to use of fungal biocontrol agent is utmost important for the day. Endophytic nature of many entomopathogenic fungi (EPF) has been explored and used extensively as pest management strategy. They can inhabit any parts of the plant (seeds, leaves, branches, stem and roots) with mutual benefit causing no apparent harm to the plants. Among the fungal biocontrol agent, *Beauveria bassiana* (Bals.-Criv.) Vuill. (Cordycipitaceae: Hypocreales) is ubiquitous in the most of cultivated crops and acting as a best plant protecting agent against biotic and abiotic factors (Mantzoukas and Eliopoulos 2020). In addition to protection from pest and adverse climatic conditions, it has associated benefits with host like supply of nutrients, improving soil health, stimulating healthy growth of plant and improved quality of produce. The indigenously collected and identified fungal biocontrol agent, *B. bassiana* UHSB-END1 from tomato, proved its existence among the tomato growers of Karnataka state in India (Jamunarani et al. 2022). Isolated and identified fungal endophytes from one host plant had differential reaction in their colonization and their efficacy against insects in another host plant. Hence, it is important to know the colonization of endophytic fungal biocontrol agent in respective host plant and its efficacy against insects before commercial use at field level case by case. Therefore, an investigation was undertaken to study the colonization and effectiveness of *B. bassiana* UHSB-END1 against key insects of cabbage such as *Myzus persicae* (Sulzer) (Aphididae: Hemiptera) and *P. xylostella*. This study may fast-track to expand the usage of *B. bassiana* UHSB-END1 in cabbage to get rid of key insects and maintaining pesticide residue below maximum residue level (MRL).

Methods

Plant material

The cabbage variety Saint (Seminis®, Monsanto Holdings Private Ltd,) was used in the study. Sodium hypochlorite (2%) were used to remove the surface contaminants of cabbage seeds for two minutes, followed by alcohol (70%) treatment for three minutes and later washed the seeds thrice with autoclaved distilled water to remove left over sodium hypochlorite and alcohol. Sterile filter papers were used to dry the disinfected seeds under laminar air flow hood for 30 min. Surface sterilized seeds were randomly chosen and transferred to PDA medium for incubation to confirm the efficacy of sterilization process. No microbial growth was recorded after one week of incubation.

Fungal isolate

The fungal isolate used was *B. bassiana* UHSB-END1, obtained from entomopathogenic fungal culture collection of "Biological Control Laboratory, Department of Entomology, College of Horticulture, Bagalkot Karnataka (India). Selection of this fungal isolate was based on its laboratory, pot and field efficacy against lepidopteran and sucking insects in tomato (Jamunarani 2022). The species was identified using both molecular (GenBank accession number OM131742) and morphological data (described

by Agarkar Research Institute, National Fungal Culture Collection of India (NFCCI), Pune, Maharashtra, India).

Production of conidia

Cleaned rice grains and distilled water were taken in equal ratio (200 g: 200 ml) in a polythene bags with baker dry yeast (2 g) and chloramphenicol (0.2 g). The mixture was autoclaved (121 °C, 15 lbs pressure) for 30 min and after cooling inoculated with pure fungal culture of *B. bassiana* UHSB-END1 under laminar air flow hood. The inoculated bags were incubated at 25 ± 2 °C for 15 days under dark condition. After 15 days, the fully grown fungus on rice grains was powdered and added with talc powder (1:1). The spore load was determined by using haemocytometer and maintained at the rate of 1×10^8 colony forming units (cfu)/g in the final product.

Experimental details

Raising of cabbage seedlings and plants

The growth chamber (Alice Biotech Pvt. Ltd.) used for raising seedlings and conducting experiment on colonization and efficacy study. Treated and untreated seeds of cabbage were sown in pro-trays filled with sterilized coco peat. The trays were kept for 20 days to get the seedlings. Later seedlings were transferred into the pots filled with autoclaved soil and compost (1:1) for imposing different treatments.

Treatment design

Six treatments with three replications were laid out in completely randomized design, each replication with five plants. The different treatments used to colonize *B. bassiana* UHSB-END1 in the cabbage was detailed below. All the experiments conducted were repeated twice.

Seed treatment

Surface-sterilized cabbage seeds were soaked in fungal solution (5 g/l) for 12 h. The soaked seeds were air-dried on a sterile paper for 20 min. The treated seeds were sown in pro-trays filled with sterilized potting media (coco peat) and placed in growth chamber. The germinated seedlings were grown for 20 days in pro-trays and then transferred into the pots (Muvea et al. 2014).

Seedling root dip treatment

The roots of 20 days old cabbage seedlings were dipped in fungal solution (5 g/l) for 30 min and then transplanted to pots containing sterilized media (Saragih et al. 2019).

Soil drenching

After 10 days of planting, the soil was drenched with fungal culture (10 g/l) (Greenfield et al. 2016).

Foliar spray

Spraying of *B. bassiana* UHSB-END1 @ 5 g/l was done at 15 days after planting on entire plant (Greenfield et al. 2016).

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

Colonization (%)

A destructive sampling was made at 30, 45 and 60 days after post-inoculation (dpi) of fungus to study colonization in leaf, stem and root of cabbage plant. The different plant parts were collected and surface disinfected with sodium hypochlorite (2%) for 2 min then 70% alcohol for 3 min and washed thrice with autoclaved distilled water. The six bits of plant parts were transferred on to PDA plates and incubated (25 ± 2 °C) for seven days. After three days of incubation, the plates were observed for positive fungal growth from plant tissues. The colonization was scored by counting the number of tissues with inoculated fungal growth, and colonization percentage was calculated (Jamunarani et al. 2022).

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

where PF—Number of pieces exhibiting fungal growth and TP—Total number of pieces plated.

Efficacy of *B. bassiana* UHSB-END1 against *M. persicae* and *P. xylostella*

Rearing of M. persicae

Wild population of the test insect, *M. persicae,* were collected from vegetable fields at College of Horticulture, Bagalkot. The culture of the test insect was maintained on cowpea seedlings in pots under net house. Plants of 15–20 days old were used to culture the aphids until all the experiments were completed (Gokak et al. 2017).

Rearing of P. xylostella

P. xylostella was reared on mustard seedlings raised in plastic cups (8 × 4 cm ht × dia) by adopting the method described by Liu and Sun (1984) with suitable modifications under laboratory. The cups were filled with well-soaked vermiculite to a depth of 1.5 cm as a potting medium. Then bold seeded mustard seeds were sown uniformly over the vermiculite medium and watered. Four-day-old seedlings were introduced in to a wooden cage containing freshly emerged *P. xylostella* adults for oviposition. Next morning seedlings were removed from the cage and kept for egg hatching. The larvae were picked up with the help of a soft camel hair brush and then transferred to fresh seedlings as and when required and continued till the larva reached pupation. On completion of the larval period, fully grown larvae were allowed to pupate on the seedlings and paper folds. Then, pupae were collected with the help of bent forceps and

transfer to a Petri dish and kept in a cage for moth emergence. Then, the emerged moths were provided with three- to four-day-old mustard seedlings for oviposition and the cotton swabs with 10% honey solution for moth feeding. After 24 h, mustard seedlings with eggs were taken out and kept in rearing trays and similar procedure continued.

In vivo study

Efficacy of *B. bassiana* UHSB-END1 was tested at different growth period of cabbage (30, 45 and 60 days after inoculation). The fungal colonized and non-colonized cabbage leaves were brought to the laboratory from growth chamber and washed thoroughly under running tap water and later dried and made 9 cm dia. leaf discs and were placed on petri dish (150 mm dia.) containing agar medium to maintain freshness of disc for 24 h. About 20 neonate *M. persicae* nymphs were released inside the Petri dish for feeding cabbage disc. Another group of 20 M*. persicae* were released on the control leaf sample. Each treatment was replicated thrice with a 20 M. *persicae* per replication. The leaf discs were replaced every 24 h to ensure the freshness of samples. Mortality of *M. persicae* was recorded by using stereo zoom microscope (Olympus) at 2, 4, 6 and 10 days after feeding. The moribund aphids were counted as dead. Similar methodology was adopted for testing efficacy of *B. bassiana* UHSB-END1 against *P. xylostella* (Qayyum et al. 2015). The experiment was conducted at room temperature of 27.0 ± 1.0 °C and the relative humidity of 70.0 ± 5.0%.

In planta study

At 30-day post-inoculation of fungus, a pot experiment was conducted to evaluate the efficacy of *B. bassiana* UHSB-END1 colonized cabbage against *M. persicae and P. xylostella.* Nymphs of *M. persicae* and second instar larvae of *P. xylostella* ($n = 20$) were released on *B. bassiana* colonized cabbage separately for each insect. The insects were confined for their feeding by covering insect released cabbage leaf with pin holed polythene bags. As control, a similar test was established with non-colonized cabbage plants. Mortality of insects were recorded at five, ten and 15 days after release. The experiment was repeated twice.

Statistical analysis

The data on per cent colonization was analysed using Pearson Chi-square test. The information on mortality of insects were pre-tested for normality by using Kolmogorov–Smirnov test, a completely randomized design. The data on mortality of insects in *in vivo* and *in planta* assessed by one-way ANOVA (Tukey HSD) in IBM Statistical Package for Social Sciences (SPSS v.16).

Results

Endophytic colonization

At 30 DPI

In leaf, the colonization by fungus in cabbage was 100% in all the methods of treatments except soil drenching (50.00). Similarly, in stem, all treatments recorded 100% colonization except soil drenching (16.66%) and seedling treatment (33.33%). The colonization in root was 100% in all the treatments except foliar spray recorded no colonization of fungus.

At 45 DPI

Colonization of fungus was 100% in leaf tissue in foliar spray and combination treatment and other treatments recorded less than 67%. The least colonization recorded in soil drenching treatment (33.33%). In stem, colonization was less than 67% in all the treatment, wherein the highest colonization was recorded in foliar spray (66.66%) and combination treatment (66.66%) followed by seed treatment (50%), and the least was in drenching (33.33%) and seedling root treatment (33.33%). Similarly, in root, the highest colonization recorded in soil drenching (66.66%) and combination treatment (66.66%) followed by seed (50%) and seedling root treatment (50%). The least case was recorded in foliar spray (16.66%).

At 60 DPI

The colonization was decreased in all the treatments after 60 dpi compared to 30 and 45 dpi. The maximum leaf colonization was recorded in combination treatment (66.66%) followed by foliar spray (50%). The rest of the treatment recorded 33.33% colonization of fungus. Stem colonization of fungus was the highest in combination (33.33%) and foliar spray (33.33%) treatment, while all other treatment recorded 16.66% colonization. The highest colonization of fungus in root was recorded in seedling root treatment (66.66%), followed by combination treatment (50%), seed and seedling root treatments (33.33%), and the least was recorded in foliar spray (16.66%). No colonization of fungus was recorded in control treatment (Table 1, Figs. 1 and 2).

Table 1 Recovery of endophytic *B. bassiana* UHSB-END1 from cabbage

Cabbage plants	Chi-square value	df	Asymp. Sig
Leaves	8.50	8	0.386
Stem	15.90	10	0.103
Root	17.50	10	0.64

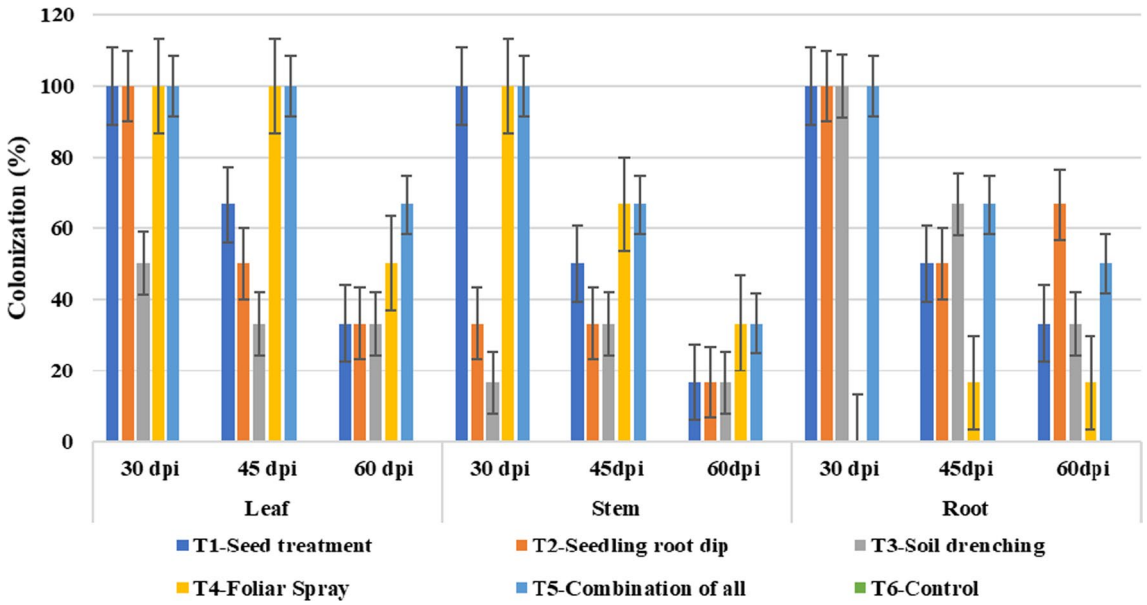

Fig. 1 Recovery of endophytic *B. bassiana* UHSB-END1 from leaves, stem and root of cabbage

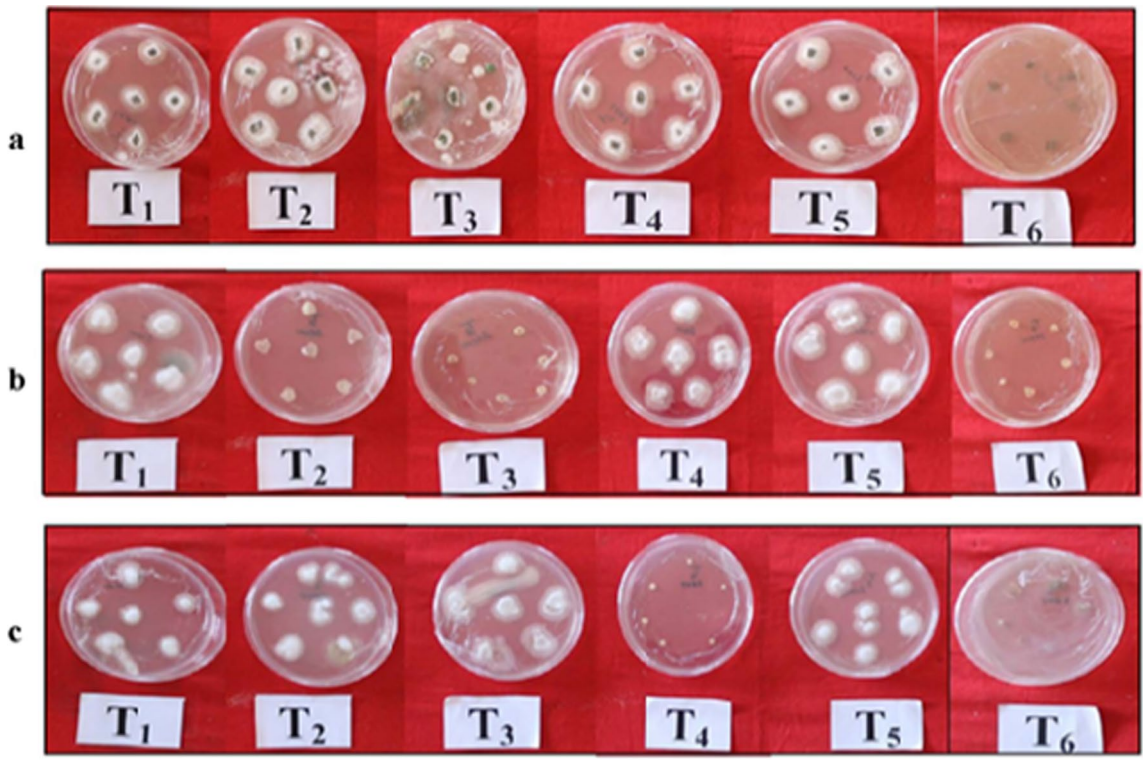

Fig. 2 Recovery of endophytic *B. bassiana* UHSB-END1 from leaves (**a**), stem (**b**) and root (**c**) of cabbage at 30 dpi. Seed treatment (T_1), Seedling root dip (T_2), Soil drenching (T_3), Foliar Spray (T_4), Combination (T_5) and Untreated control (T_6)

Pathogenicity of leaves colonized by *B. bassiana* against *M. persicae* and *P. xylostella*

In vivo study

Effectiveness of *B. bassiana* UHSB-END1 against *P. xylostella* proved significantly different across treatments. The combination treatment recorded the significant highest (88.33%) mortality of *P. xylostella,* which was on par with foliar spray (83.33%) and seed treatment (78.33%). The treatment drenching recorded the least (58.33%) mortality of larvae, and it was on par with seedling root treatment (63.33%) at 30 dpi ($F_{5,17}=127.10$; $p<0.05$). Similar trend was noticed at 45 ($F_{5,17}=231.914$; $p<0.05$) and 60 dpi ($F_{5,17}=42.150$; $p<0.05$) with a mortality ranged from 46.67–83.33 to 31.67–56.67%, respectively, across the different treatments (Table 2 and Fig. 3). The mortality of *M. persicae* was significantly the highest (91.67%) in combination treatment, and it was on par with foliar spray (86.67%) followed by seed treatment (78.33%), seedling (66.67%) and drenching (61.67%) at 30 dpi ($F_{5,17}=290.167$; $p<0.05$). Similar trend was recorded at 45 ($F_{5,17}=209.960$; $p<0.05$) and 60 ($F_{5,17}=85.286$; $p<0.05$) dpi; however, the mortality rate of *M. persicae* was decreased compared to 30 dpi (Table 2 and Fig. 4). The minimum natural mortality of *M. persicae* and *P. xylostella* was recorded in control.

In planta

Effect of *B. bassiana* against *P. xylostella* was confirmed in pot experiment. The combination treatment recorded the significant highest (96%) mortality rate of *P. xylostella,* followed by foliar spray, seed treatment, seedling root dip and drenching (88, 84, 70 and 64%, respectively) after 15 days of release ($F_{5,18}=1.42$; $p<0.05$) (Table 3 and Fig. 5). Similarly, the combination treatment recorded the significantly highest (96%) mortality of *M. persicae* followed by foliar spray (88%), seed treatment (84%), seedling root dip (76%) and drenching (72%) after 15 days of release ($F_{5,18}=4.02$; $p<0.05$) (Table 3 and Fig. 6). About

Table 2 Efficacy of endophytic *B. bassiana* UHSB-END1 against cabbage insects under laboratory conditions

Days post inoculation	Kolmogorov–Smirnov[a]					
	P. xylostella			*M. persicae*		
	Statistic	df	Sig	Statistic	df	Sig
Mortality@30	0.209	18	0.036	0.239	18	0.008
Mortality@45	0.163	18	0.200*	0.188	18	0.092
Mortality@60	0.150	18	0.200*	0.115	18	0.200*

[a] Lilliefors Significance Correction

*Significance difference

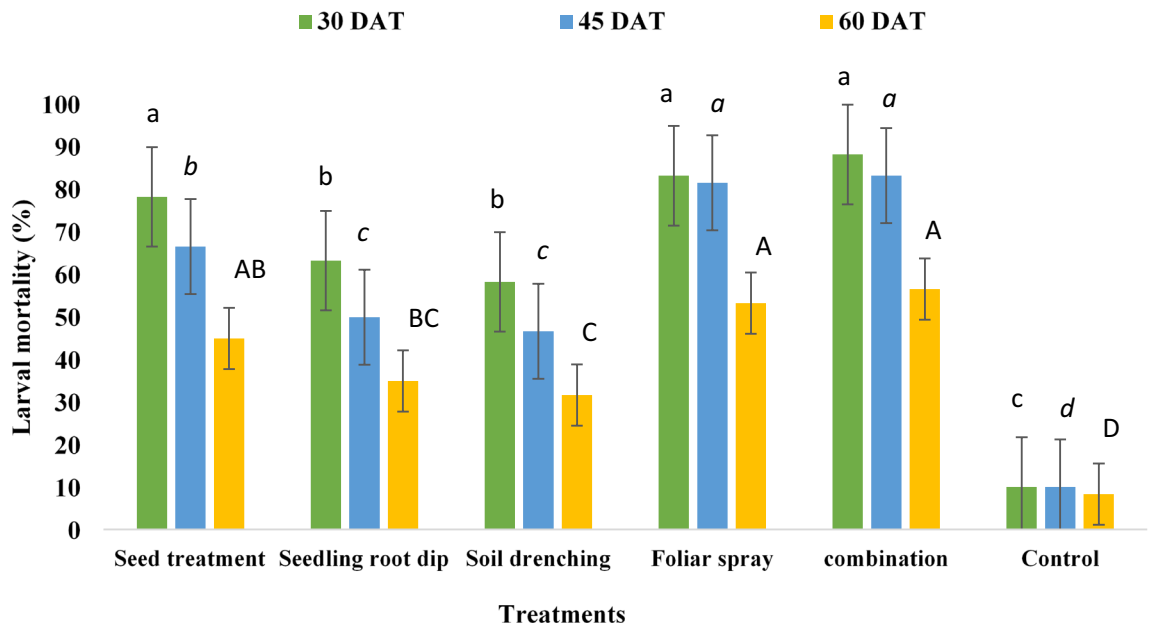

Fig. 3 Efficacy of endophytic *B. bassiana* UHSB-END1 colonized cabbage plant against *P. xylostella* under laboratory condition

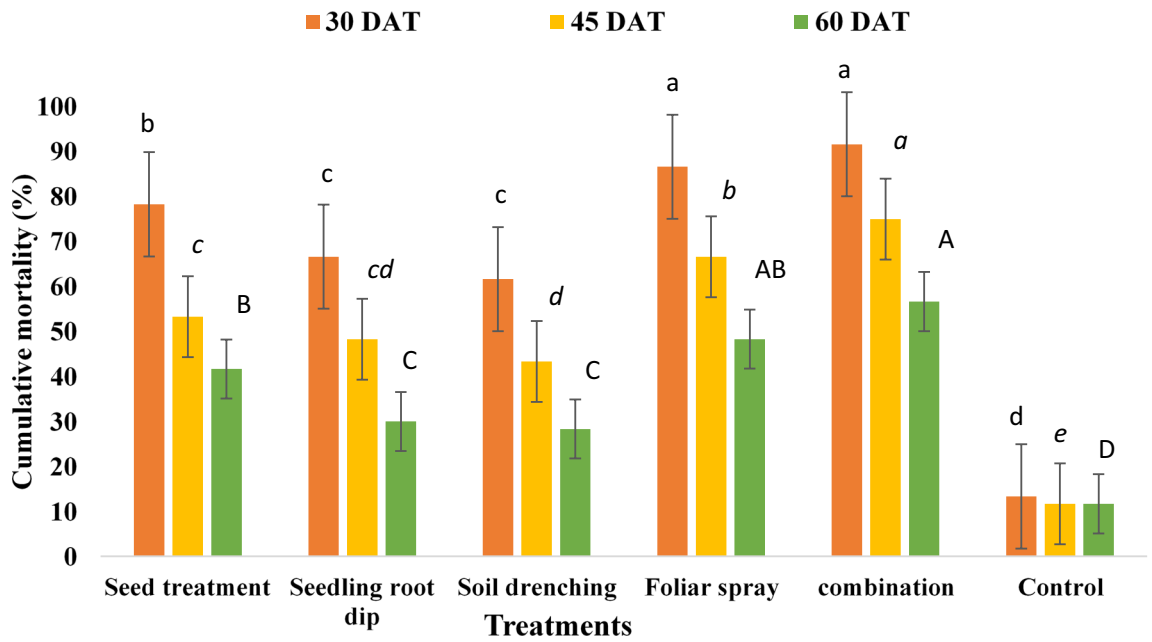

Fig. 4 Efficacy of endophytic *B. bassiana* UHSB-END1 colonized cabbage against *M. persicae* under laboratory condition

Table 3 Efficacy of endophytic *B. bassiana* UHSB-END1 against cabbage insects under pot experiment

Days after release	Kolmogorov–Smirnov[a]					
	P. xylostella			*M. persicae*		
	Statistic	df	Sig	Statistic	df	Sig
5 DAR	0.204	18	0.047	0.248	6	0.200*
10 DAR	0.225	18	0.017	0.345	6	0.025
15 DAR	0.274	18	0.001	0.357	6	0.016

[a] Lilliefors Significance Correction, *DAR*–Days after release

*Significance difference

maximum of 4% mortality of *M. persicae* and *P. xylostella* was recorded in control treatment.

Discussion

Colonization of *B. bassiana* was confirmed in cabbage based on recovery of fungus at 30, 45 and 60 dpi in all the methods of colonization (seed treatment, seedling root dip, soil drenching, foliar spray, combination treatment). This was an additional benefit over other isolates, which were required specific method of colonization (Sanchez-Rodriguez et al. 2018). The present studied isolate was able to invade leaves, stem and roots of cabbage with varied per cent colonization across different treatments (Fig. 2). The *B. bassiana* UHSB-END1 strain isolated from tomato plant, the colonization and recovery of this isolate, was higher (100%) in different tissues of tomato (Jamunarani et al. 2022) than their colonization in cabbage plant during the present study. Generally, colonization and recovery of the endophytic fungi was more at early crop growth period, and it was decreased at flag end of the crop cycle. In support, persistence of fungus in maize was decreased gradually as the crop progressed (Renuka et al. 2016). The 100% colonization of *B. bassiana* in the foliage of the cucumber plants up to 28 days after treatment and then colonization was decreased thereafter as reported by Homayoonzadeh et al. (2022). Similarly, Jamunarani et al. (2022) reported that the recovery of *B. bassiana* UHSB-END1 from different plant parts (stem, leaves and roots) was the highest (100%) in all the methods of colonization at 14, 40, 60 and 80 dpi, and it was decreased at 120 dpi (80%) in tomato. The present study confirmed that the activity of *B. bassiana* in cabbage was high during vegetative stage and decreased at the latest stage of the crop as evidenced by recovery study. All the colonization methods recorded recovery of the *B. bassiana* UHSB-END1 in

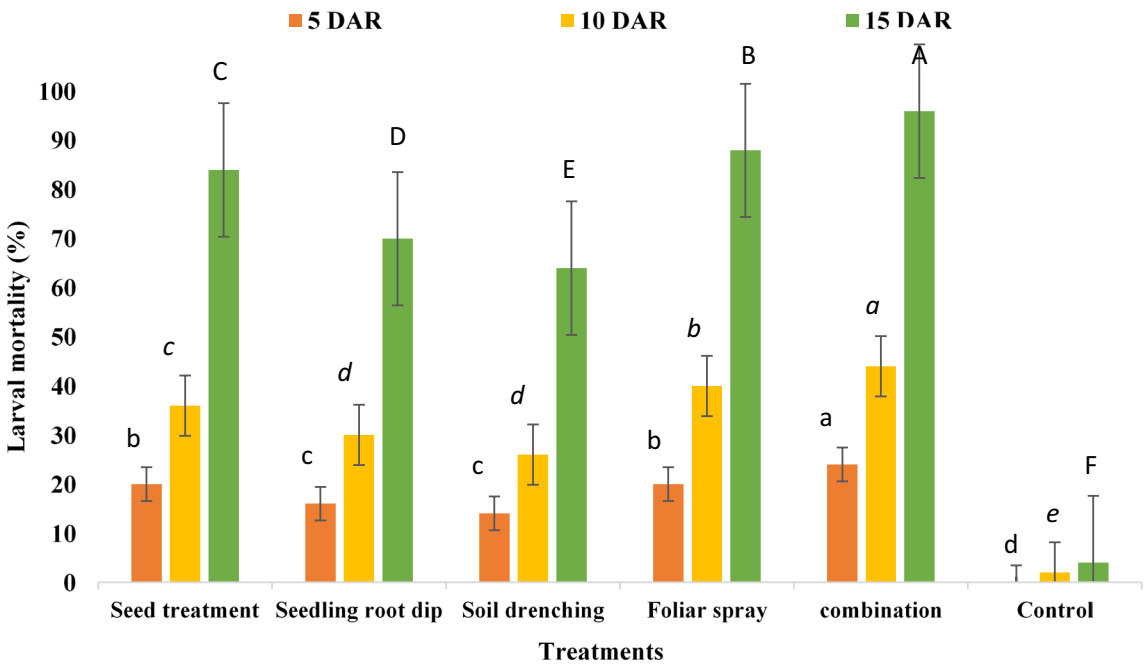

Fig. 5 Efficacy of endophytic *B. bassiana* UHSB-END1 against *P. xylostella* under pot experiment at 30 days of post-inoculation

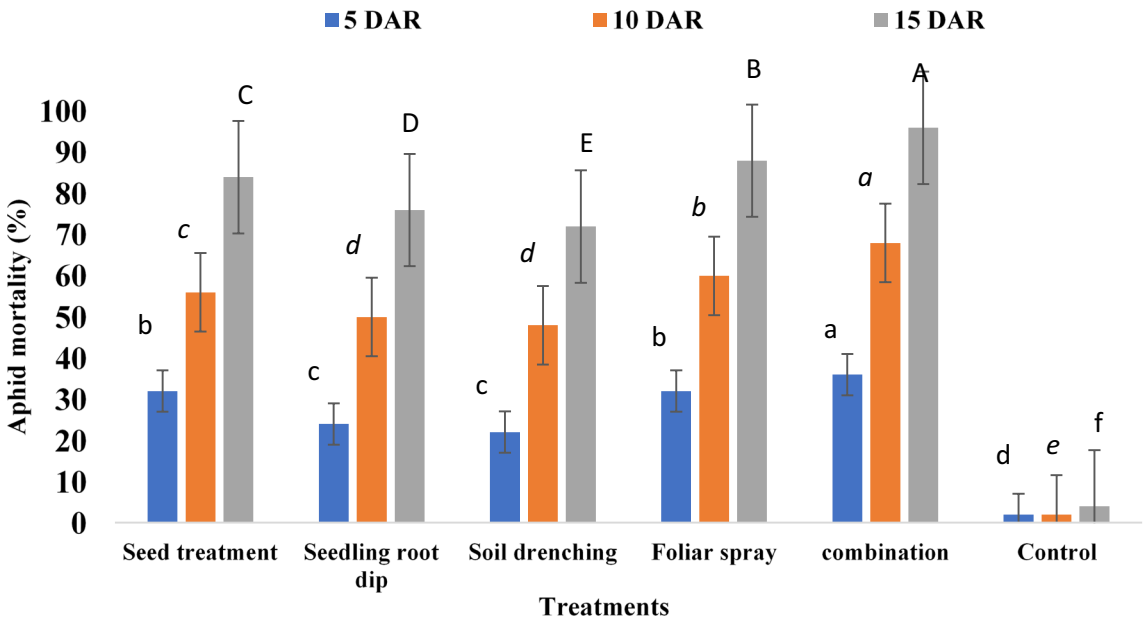

Fig. 6 Efficacy of endophytic *B. bassiana* UHSB-END1 against *M. persicae* under pot culture experiment at 30 days of post-inoculation

cabbage but the movement of fungi from site of inoculation to another part of the plant was very slow as recorded during the study. It was evidenced by foliar application recorded maximum colonization of fungus in leaf tissue than the stem and the least colonization in root tissue of cabbage. Similarly, seedling root and soil drenching recorded higher root colonization than stem and leaf tissues. However, the combination and seed treatment showed good movement and recovery of the fungi from all the tissues of the cabbage (leaf, stem and root). Hence, this isolate can be recommended in combination included seed treatment, seedling root treatment

before transplanting, drenching after ten days of transplanting and foliar spray after 20 days after transplanting to get maximum colonization and movement of fungus inside cabbage plant. Therefore, increased colonization, recovery and movement of fungal biocontrol endophytes inside the host plant depends on the isolate origin and its use in respective plant.

Significant mortality rates of *M. persicae* and *P. xylostella* were recorded in *B. bassiana* treated cabbage. The early period of inoculation of fungus (30 dpi) increased mortality of both the insect due to high persistence and recovery of the fungus during the same period. As the post-inoculation period advanced, the mortality of insects was decreased due to poor persistence and recovery of the fungus. Maximum number of both the insects was died after seven days of feeding the colonized leaves at different intervals tested (30, 45 and 60 dpi) without development of mycosis on cadaver. However, lesser than 5% development of mycosis on cadaver of *M. persicae* and 2% on *P. xylostella* after continuously fed with *B. bassiana* colonized cabbage leaf for 15 days was observed (Figs. 7 and 8). Similar observation was made by Jamunarani et al. (2022), wherein the least (1%) mycosis was observed on *Spodoptera litura* Fabricius larvae fed with *B. bassiana* colonized tomato plant both in vivo and in vitro study. In contradictory to these results, the maximum of 70% development of mycosis was reported by Pelizza et al. (2012) on *Dichroplus maculipennis* (Orthoptera: Acrididae) fed with *B. bassiana* colonized maize plant for 15 days. The death of insects may be due to consumption of food with more secondary metabolites (insecticidal bioactive compounds) which were produced by cabbage following colonization of fungus. Vega (2018) opined that fungal infestation alone may not bring the mortality of insects rather it was a combined effect of bioactive compounds of fungus and host plant. The exact mechanism involved in mortality of insect is not clear and needs to be studied further. Leaf dip bioassay in the suspensions of two endophytic fungi, *B. bassiana* and *Lecanicillium lecanii* R. Zare & W. Gams (Cordycipitaceae: Hypocreales), critically reduced the aphid number and recorded around 45 to 97.5% mortality of aphids due to *B. bassiana* and 24–82% due to *L. lecanii* (Gurulingappa et al. 2011). Colonization in sweet pepper plants by *Metarhizium brunneum* Petch (Clavicipitaceae: Hypocreales) and *B. bassiana* was greatly imparted the life cycle of aphids in the way of reduced growth and development, egg laying, birth rate, delayed reproduction and time (Jaber and Araj 2018). In the cabbage crop, Bathina and Bonam (2020) studied colonization and efficacy against *P. xylostella* through seed treatment, root inoculation and foliar application at different dpi. They

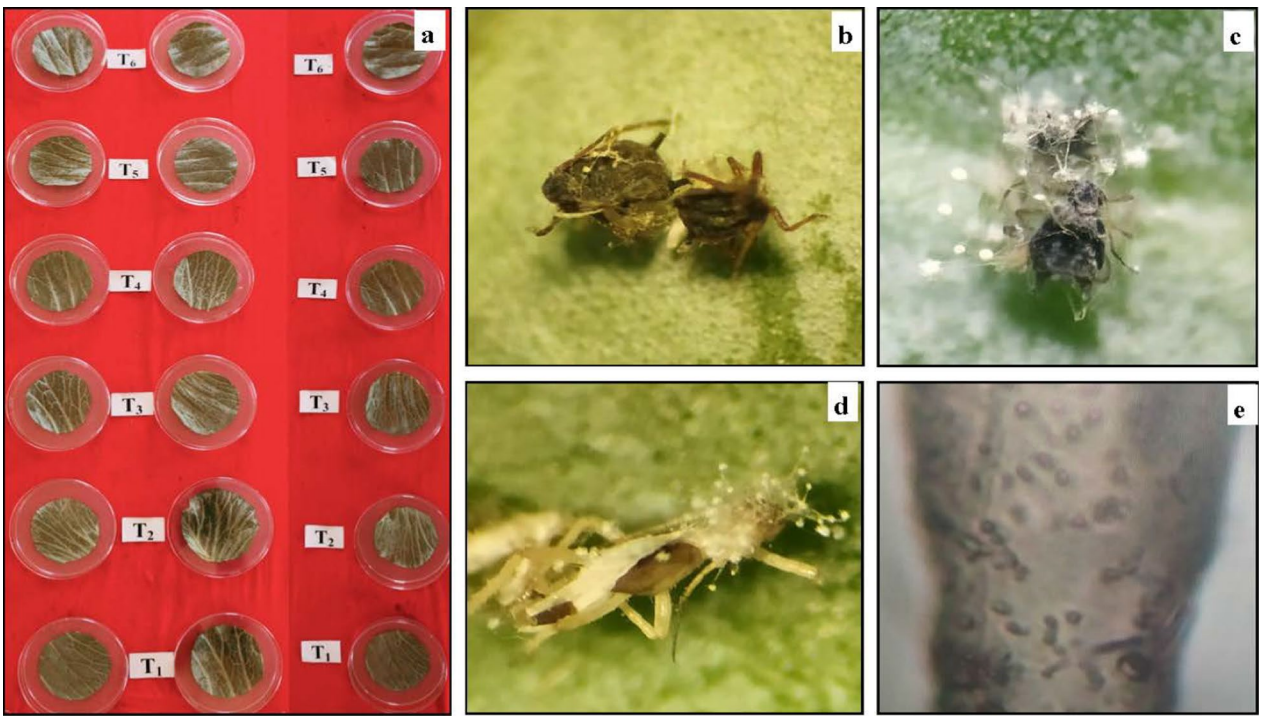

Fig. 7 Efficacy of endophytic *B. bassiana* UHSB-END1 against *M. persicae*. Experimental set up at 30, 45 and 60 dpi under laboratory **a** Dead aphid (**b**), Development of mycosis on dead aphid (**c**), Development of mycosis on exuviae of aphid (**d**) Development of spores inside the leg of aphid (**e**)

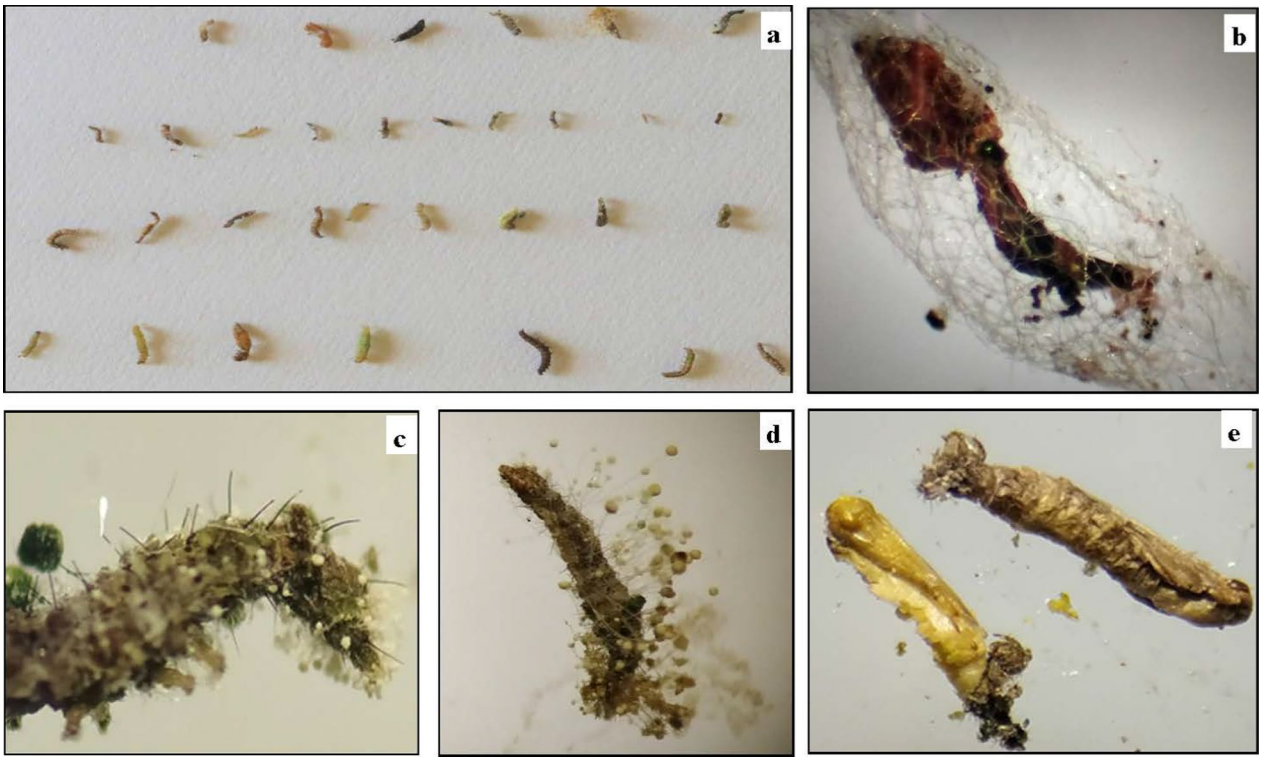

Fig. 8 Efficacy of endophytic *B. bassiana* UHSB-END1 against *P. xylostella*. Dead larvae due to fungus (**a**), Malformed pupae (**b** and **e**), Development of mycosis on dead larvae (**c** and **d**)

reported the mortality of second instar larvae of *P. xylostella* at 15–30 dpi and no mortality after 45 and 60 dpi in all the inoculation methods. Among three application methods, foliar application was recorded the highest mortality of 70–80% at 15 days after treatment (DAT) and 12–58% at 30 DAT on second instar larvae of *P. xylostella*. However, in the present study, even after 45 and 60 dpi, *B. bassiana* UHSB-END1 was able to bring the mortality of *M. persicae* and *P. xylostella*. The foliar spray treatment recorded the highest mortality of both the insects in the present investigation. The highest colonization and efficacy against tested insect were obtained by seed treatment and foliar application methods, respectively. Hence, this isolate can be recommended as seed treatment and repeated foliar application to protect the cabbage crop from both the insects (*M. persicae* and *P. xylostella*). To conclude, *B. bassiana* UHSB-END1 isolate is colonizing cabbage plant even though it was originally isolated from tomato plant. The recovery of the fungus was more at early stage of the crop, and it was decreased at head formation. This coincide with higher mortality of both the tested insects at early crop growth period compared to later stage. After ensuring the safety of *B. bassiana* UHSB-END1 colonized cabbage feeding directly as raw salad, it can be recommended as one of the important bio-input for the insect management in cabbage. Further, continuous field studies at different seasons are required to get the consistence results under open environmental conditions. This is an interesting alternative tool for the insect management in cabbage crop. This non-chemical approach of plant protection will reduce the pesticide usage in cabbage crop resulted in consumption of chemical-free cabbage by the consumers.

Conclusion

The indigenous *B. bassiana* UHSB-END1 proved enough to colonize cabbage plant and showed good mortality of *P. xylostella* and *M. persicae* under both laboratory and pot culture experiment. Field studies are still required for final recommendation of this isolate as one of the safer methods of pest management in cabbage ecosystem. It gave protection against insects at vegetative stage by single concentration of application (up to head formation). Later stage of crop may be protected by repeated applications of fungus or other non-chemical methods which need further studies. This provides scientific platform for other researchers to study on various ecological interactions of this fungus with cabbage.

Abbreviations

EPF	Entomopathogenic fungi
PDA	Potato dextrose agar
UHSB-END1	Isolate name (University of Horticultural Sciences-Endophyte 1)
dpi	Days after post inoculation
UV	Ultraviolet
DAR	Days after release
fMBA	Fungal microbial biocontrol agents
NFCCI	National fungal culture collection of India
cfu	Colony forming unit
CD	Critical difference
ETL	Economic threshold level

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Author contributions
YT 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. YT did all experiments, and VB was analysed and interpreted the data. JGS and RSH helped in conducting laboratory and pot experiment. HHP and AM helped in raising cabbage in pots and conducting the experiment in controlled growth chamber.

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