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Endophytic Potential of Indigenous *Beauveria bassiana* against *Spodoptera litura* Fabricius in Cabbage

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ABSTRACT

The need of today's world is enhanced production and quality of the crops in an eco-friendly manner. In the absence of complete host plant resistance in crops against insect pests, biological control forms a more sustainable and integral part of management ecosystem. Microbial biological control agents, especially entomopathogenic fungi (EPF), offer eco-friendly pest management solutions. Unlike other pathogens, EPF can infect pests upon contact with their propagules. However, environmental factors like temperature, UV light and pollutants often reduce the effectiveness of epiphytic EPF in the field. Endophytic EPF, residing within plant tissues, are gaining popularity for their resilience under adverse conditions. Hence, the research was conducted to observe the endophytic effect of native *Beauveria bassiana* UHSB-END1 against *Spodoptera litura* in cabbage. The bioassay investigations pertaining to both *in-vivo* and *in-planta* revealed that *S. litura* mortality peaked at 30 days after inoculation. Furthermore, larvae that consumed cabbage leaves colonized by fungi showed disrupted growth and abnormal developmental patterns, indicating a negative impact on their physiological development. By introducing this native biological product to control insect pests in cabbage habitats, it is possible to produce with minimal pesticide residue for commercial consumption while maintaining environmental integrity.

Keywords: *Beauveria bassiana,* Biological control, Cabbage, Endophyte, *Spodoptera litura.*

INTRODUCTION

Cabbage (*Brassica oleracea* var. *capitata* (L.) Alef.) is one of the most common cole crops grown worldwide. Despite being the world's second-largest producer of cabbage, India may still do more in terms of productivity, production and insect pest control. Insect pest complexes that infest cabbage from germination to harvesting are the main obstacles to its cultivation (Yogananda *et al,* 2023). Among the various insect pests, the tobacco caterpillar, *Spodoptera litura* F. (Noctuidae: Lepidoptera) stands out as one of the most destructive, causing substantial and recurring yield losses in cabbage cultivation each year. Commonly referred to as the tropical armyworm, *S. litura* is a highly polyphagous and economically significant pest that feeds on a diverse array of crops, including vegetables, fruits, tea, ornamental flowers, green manures and several weed species. Its major host plants include tobacco, chickpea, cotton, cabbage, soybean, lucerne and beet. The larvae of *S. litura* are particularly damaging, with the potential to cause yield losses ranging from 26% to as high as 100% under severe infestation posing a serious threat to agricultural productivity (Tuan *et al*, 2017). Farmers find it challenging to control this polyphagous pest; therefore, they rely solely on synthetic insecticides. Excessive and random pesticide use is known to have a significant negative impact on the environment and society, as well as lead to higher production costs (Aktar *et al,* 2009).

Hence, it is high time to find out an alternate option to reduce the pest population with minimum pesticide residue for consumers and leaves the environment unaffected. One of the major approaches in the IPM is the use of biocontrol agents like fungi, bacteria, viruses and nematodes. Due of their superior ability to transmit disease to insects, by direct penetration of the cuticle unlike bacteria or viruses, fungi are the most important insect pest management agents (Khan *et al,* 2012). Among the Entomopathogenic fungi (EPF), *Beauveria bassiana* is

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well adapted and giving protection against various insect pests that are causing economical injury to the crops (Ownley *et al,* 2008). Although, *B. bassiana* has been widely utilized as an effective biocontrol agent for managing insect pest populations. However, its efficiency in field applications is often compromised due to the direct exposure of its propagules to harmful environmental factors such as ultraviolet (UV) radiation, fluctuating temperatures, and inconsistent humidity levels (Omukoko and Turoop, 2017). These challenges significantly reduce its persistence and performance. To address this limitation, the endophytic establishment of *B. bassiana* within plant tissues has emerged as a promising and sustainable strategy. This approach not only protects the fungus from adverse environmental conditions but also enhances its systemic activity within the plant, offering long-term protection against the notorious pests (Litwin *et al,* 2020). Successful colonization of *B. bassiana* has been documented in various crops, including maize, tomato, cabbage, cucumber, melon, potato, cotton and rice, where it has shown the potential to defend against a broad spectrum of insect pests and plant diseases, thereby contributing to improved crop health and yield. (Vega, 2018). With these evidences about endophytic *B. bassiana,* our main objective is to evaluate the efficacy of native isolate of the fungus, *B. bassiana* UHSB-END1 against *S. litura* at different days post inoculation (dpi) in cabbage.

MATERIALS AND METHODS

The laboratory and pot experiments were conducted in the year 2022 at Department of Entomology, College of Horticulture, Bagalkot, Karnataka, India (16.16910 N latitude, 75.66150 E longitude). The cabbage variety 'Saint' was utilized in the study. The cabbage seeds were surface sterilized using 2% sodium hypochlorite for about 2 minutes, thereafter by a 70% alcohol treatment for 3 minutes. Subsequently, the seeds were rinsed three times with autoclaved sterile water to eliminate any remaining traces of sodium hypochlorite and alcohol. The disinfected seeds were then air dried for 30 minutes under a Laminar Airflow Hood (LAF) using sterile filter papers. To verify that the surface sterilization technique was effective, a batch of randomly selected surface sterilized seeds was plated on PDA media. After incubation for a week, no microbial development was observed. Pro-trays loaded with sterilized growing media (coco peat) were used to sow both treated and untreated seeds and they were placed within a growth chamber. After germination, seedlings were grown up to 25 days in pro-trays in controlled condition and later transferred into the pots containing sterilized soil and compost (1:1) and initiated imposition of treatments accordingly (Jamunarani *et al,* 2022, Yogananda *et al,* 2023).

A wild population of *Spodoptera litura* was initially acquisition from vegetable fields located at the College of Horticulture, Bagalkot, India. The population was subsequently reared in the laboratory on a diet of castor and cabbage leaves, maintained at 28 ± 1 °C under natural light conditions. For experimental purposes, second instar larvae were periodically sourced from this established culture as needed, following the method described by Sowmya *et al* (2017). The experiment was structured using a completely randomized design, consisting of six treatments with three replications each, and each replication included five plants. The different treatments used to colonize endophytic *B. bassiana* in the cabbage are detailed in (Table 1).

The bio-efficacy of *Beauveria bassiana* strain UHSB-END1 was evaluated at various stages of cabbage growth specifically at 30, 45 and 60 days of post-inoculation. For each treatment, 20 second-instar larvae of *Spodoptera litura* were fed with leaves colonized by the fungus, while another group of 20 larvae of the same instar was provided with non-colonized leaves, serving as the control. To maintain leaf freshness, the cabbage samples were replaced every other day. Larval mortality was monitored on the 2^{nd}, 4^{th}, 6^{th}, 10^{th} and 15^{th} days after feeding at a room temperature of 28±1 °C with relative humidity maintained between 70–75% and it continued until the insect life cycle was complete (Jamunarani *et al,* 2022). In the same manner, pot 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 selected and challenge inoculation on cabbage plants at 30 dpi. Insect-released cabbage leaves were covered with pin-holed plastic bags to keep the insects contained while they fed. To assure fresh nourishment till the end of the larval stage the live larvae were carefully moved to a fresh leaf of the same cabbage plant for every fifth day. The observation on the morality of *S. litura* was recorded at five, ten and 15 days after release (DAR) (Yogananda *et al,* 2023).

Insect mortality data from both laboratory and in planta experiments were analysed using one-way

Table 1.Experimental setup to evaluate the effectiveness of *Beauveria bassiana* UHSB-END1 in managing *Spodoptera litura* infestation in cabbage.

Treatment	Method	Dosage (g/l)
T_1	Seed treatment	5
T_2	Seedling root dip treatment	5
T_3	Soil drenching	10
T_4	Foliar Spray	5
T_5	Combination of all treatments	(T_1+T_2+ T_3+T_4)
T_6	Untreated control	-

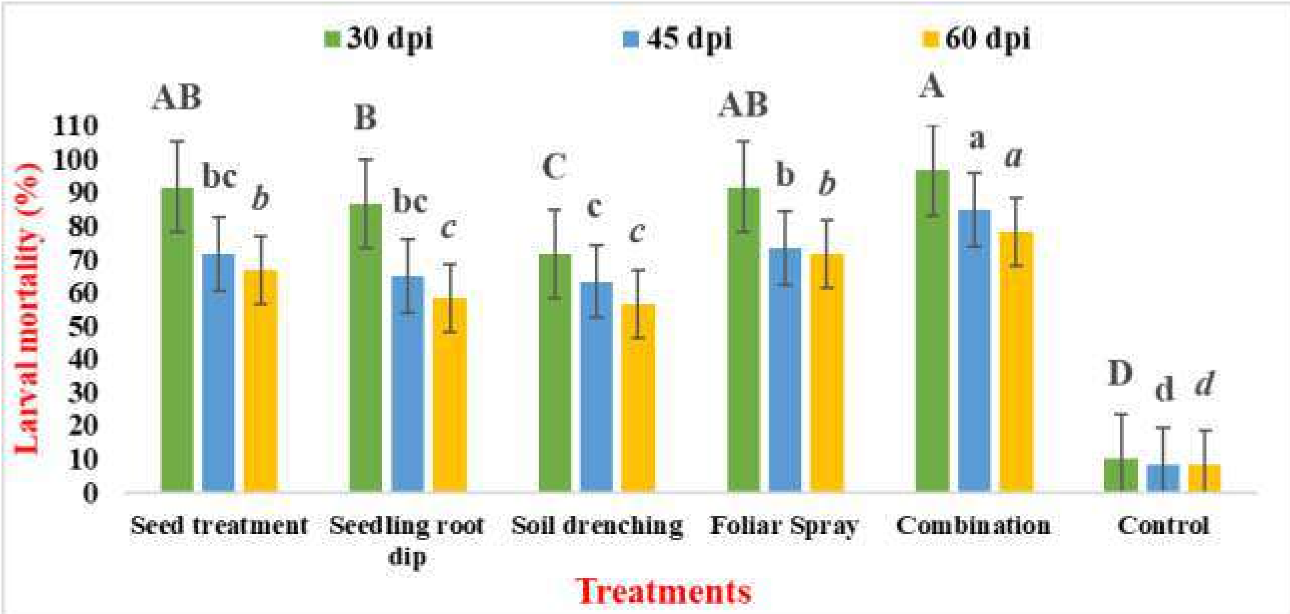

Fig 1. Assessment of the entomopathogenic potential of endophytic *Beauveria bassiana* UHSB-END1 against *Spodoptera litura* at 30, 45 and 60 days of post-inoculation in cabbage

ANOVA. Additionally, data related to sub-lethal effects, such as larval growth and development, were also examined through one-way ANOVA based on a completely randomized design, employing Duncan's Multiple Range Test (DMRT) using IBM SPSS software (version 16).

RESULTS AND DISCUSSION

The pathogenicity of endophytic *Beauveria bassiana* against Spodoptera litura showed significant variation among the different treatments. The combination treatment resulted in the highest mortality rate (96.67%), which was statistically significant, followed by both the foliar spray and seed treatment, each recording a mortality rate of 91.67%. The seedling root dip treatment recorded the mortality of larvae (86.67%) and least mortality was observed in Soil drenching treatment (71.67%) at 30 dpi ($F_{5,17}$=238.182; p<0.05). Similarly, at 45 dpi ($F_{5,17}$=98.200; p<0.05) combination treatment recorded highest mortality (85%), followed by foliar spray (73.33). The mortality in seed treatment (71.67%) was statistically, on par with Seedling root dip treatment (65%) and least mortality was observed in Soil drenching treatment (63.33%). Similar trend was noticed at 60 dpi ($F_{5,17}$=225.600; p<0.05), significantly high mortality of larvae was recorded in the combination treatment (78.33%). The mortality in foliar application (71.67%) was on par with Seed treatment (66.67%). The least mortality was observed in soil drenching (56.67%) which was statistically similar with seedling root treatment (58.33%). The minimum natural mortality of *S. litura* was ranged from 8.33% to 10%, which was recorded in uncolonized control plants (Fig 1).

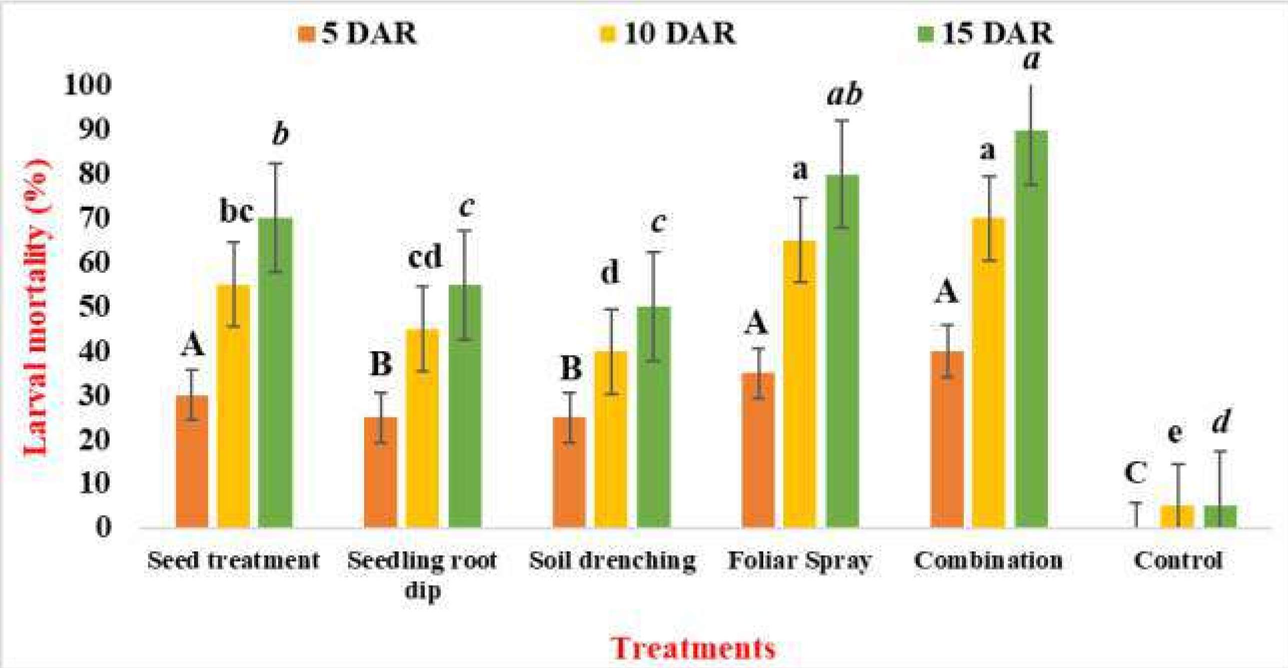

Fig 2. Effectiveness of the endophytic *Beauveria bassiana* UHSB-END1 in cabbage at 30 days after inoculation under *in-planta* conditions against *Spodoptera litura*

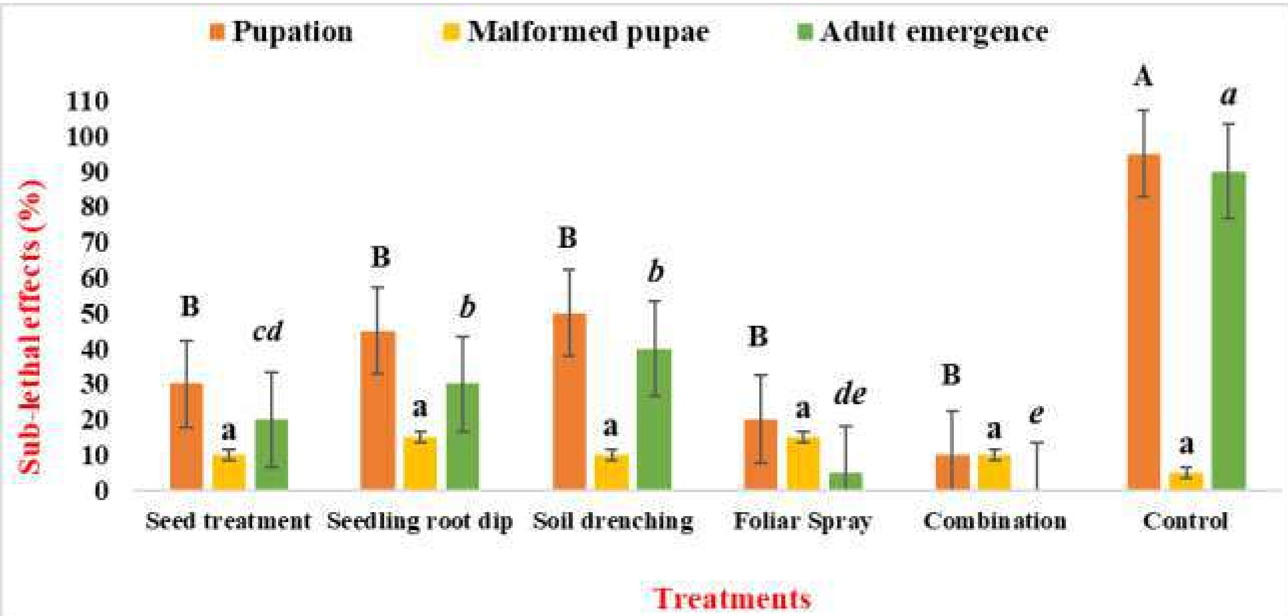

Fig 3. Influence of endophytic *Beauveria bassiana* UHSB-END1 on the growth and development of *Spodoptera litura* under *in planta* in cabbage at 30 days of post inoculation

The pot culture experiment proved impact of established *B. bassiana* on *S. litura* larvae as evidenced by 25-40% mortality of larvae in different treatments at five DAR ($F_{5,17}$=19.971; p<0.05). Similarly, by the end of ten DAR larval mortality was noticed in the range of 40-70% ($F_{5,17}$=49.200; p<0.05). After 15 DAR the combination treatment recorded the significant highest (90%) cumulative mortality of *S. litura*, followed by foliar spray (80%). Seed treatment recorded cumulative mortality of 70%. The mortality in seedling root dip method (55%) and soil drenching (50%) were on par with each other ($F_{5,17}$=46.629; p<0.05). The maximum of 5% mortality was noticed in untreated control even after 15 DAR (Fig 2). Significant

Fig. 4. The effectiveness of endophytic *Beauveria bassiana* UHSB-END1 on *Spodoptera litura* was evaluated at 30, 45, and 60 days of post inoculation under laboratory conditions (a), dead larvae and malformed pupae (b), progression of mycosis on malformed pupae (c) and malformed adult (d)

variations were seen in the effects of each treatment on *S. litura* pupation ($F_{5,17}$=6.400; p<0.05). In comparison to the control, pupation was significantly decreased in all the treatments. Likewise, the highest malformed pupae were recorded in the treatments when compared to control ($F_{5,17}$=0.850; p<0.05). The adult emergence was almost nil in the combination treatment and only 5% in foliar application ($F_{5,17}$=28.378; p<0.05). About 90% healthy adults were emerged in the untreated control treatment (Fig 3).

S. litura is a polyphagous pest that feeds voraciously and has posed a serious threat to various horticultural and agricultural crops around the world (Ahmad *et al,* 2007). In order to reduce the farmers' total reliance on pesticides, Biological control of insect pests has recently received a lot of attention. Entomopathogenic fungi are cheaper and environmentally safe for pest management than pesticides, and they are compatible with IPM. The endophytic colonisation of native *B. bassiana* UHSB-END1 led to an elevation in *S. litura* mortality. The laboratory bioassay results show that the maximum mortality of *S. litura* during early period of the crop (30 dpi) maybe due to high persistence, colonization and recovery of the fungus. The mortality of *S. litura* decreases as the crop matures at 45 and 60 dpi due to poor persistence and recovery of the fungus. In support to this, Yogananda *et al*. (2023) reported that the highest lethality of *M. persicae* and *P. xylostella* under *in vivo* condition was noted at 30 dpi. The morality started to decline significantly around 45 dpi and it was lowest at 60 dpi. Similarly, the indigenous isolate of endophytic *B. bassiana* UHSB-END1 was efficient in causing the mortality of *S. litura* at *in planta* where, mortality is noticed from three days after feeding on to the colonized leaves. Interestingly, beyond inducing larval mortality, the various treatments also influenced the growth and developmental patterns of *Spodoptera litura* larvae. Noticeably, significant reduction in per cent pupation, increased pupal malformation and adult emergence was drastically reduced across different treatments. Coordinating with results of Jamunarani *et* *al*. (2022) where, endophytic *B. bassiana* UHSB-END1, was efficient in causing the mortality of *S. litura* both *in vivo* and *in planta* experiment. Moreover, *S. litura* larvae that consumed colonized leaves showed irregularities in their growth and development. The mortality of the larvae was largely influenced by the method used to introduce the fungus as an endophyte in the host plants (Wagner & Lewis, 2000; Qayyum *et al,* 2015). It is likely that the pest suppression occurred due to antibiosis or feeding deterrence, triggered by secondary metabolites produced by the endophytic entomopathogenic fungi within plant tissues, which possess both insecticidal and anti-feeding characteristics (Vega *et al,* 2008; Gurulingappa *et al,* 2011).

CONCLUSION

Indiscriminate pesticide uses to manage the insect pests in cabbage ecosystem, increases production costs, reduces yield and poses health risks, as seen in a recent tragic incident where a 14-year-old girl died after consuming pesticide-sprayed cabbage. By introducing this native biological product to control insect pests in cabbage habitats, it is possible to produce with minimal pesticide residue for commercial consumption while maintaining environmental integrity. Further, field level studies are required in this direction. This offers a scientific foundation for future researchers to explore the diverse ecological interactions between this fungus and cabbage.

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