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Potential of the Fungus *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Cordycipitaceae) as an Endophytic Fungus for the Growth of Yardlong Bean (*Vigna sinensis* L.)

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Abstract: The main objective was to determine the effectiveness of this technique in establishing endophytic colonies and its impact on plant vegetative growth. The experiment was conducted in the laboratory and greenhouse using a Completely Randomized Design (CRD) approach. We tested four levels of conidial density: control (no conidia), 1×10^6, 1×10^7, and 1×10^8 conidia per mL, with each replicated five times. Parameters we observed included root and stem colonization success, plant height, and leaf number. Data were analyzed using ANOVA followed by a HSD test at the 5% level. The results showed that *B. bassiana* is able to enter and develop internally within root and stem tissues. Interestingly, the highest colonization occurred at the lowest concentration (1×10^6 conidia per mL), with 83.3% in roots and 66.7% in stems. Although statistically, differences in concentration did not always have a significant effect on plant height and leaf number, numerically, inoculated plants showed better growth than controls, especially at 28 days after planting. From these findings, we conclude that soil application of *B. bassiana* is quite effective in establishing endophytic colonies without disrupting vegetative growth. In other words, this method is worth considering as part of a more environmentally friendly and sustainable pest management strategy.

Keywords: Conidial density; colonization; soil wetting; vegetative growth

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INTRODUCTION

Long bean (*Vigna sinensis* L., synonym *Vigna unguiculata* subsp. *sesquipedalis*) is one of the leading horticultural crops in Indonesia with strategic importance in terms of both economic value and nutritional content. As a source of plant-based protein, vitamins, and minerals, this crop contributes to strengthening food security while also serving as a source of income for farmers (FAO, 2022; Syarifuddin, 2020). Stable market demand further positions long bean as a strategic commodity within vegetable production systems. However, its production potential is frequently constrained by various biotic factors. Crop productivity is often limited by pest attacks, which directly reduce both the quality and quantity of agricultural yield.

A major challenge frequently encountered in long bean cultivation is the high intensity of pest and disease infestations occurring from the early growth stage through to harvest. During the vegetative stage, young plants are highly susceptible to leaf-feeding pests that can reduce photosynthetic capacity and inhibit plant growth. Meanwhile, during the generative stage, pest attacks may disrupt flowering and pod formation, thereby directly affecting crop productivity (Apriliyanto & Ariabawani, 2017). At the global level, *Spodoptera frugiperda* has become one of the most concerning pest species due to its polyphagous nature and its rapid adaptability to diverse environments. This pest is capable of causing substantial economic losses across various cultivated crops, including legumes (Goergen et al., 2016; FAO, 2019).

In pest management practices, farmers generally still rely heavily on the use of synthetic insecticides. Although this approach can provide rapid control effects, excessive use and application beyond recommended dosages can result in various adverse impacts. The negative consequences of synthetic pesticide application include the development of pest resistance to active ingredients, pest resurgence, reduction of natural enemy populations, environmental degradation, and chemical residue contamination in agricultural products (Aktar et al., 2009; Popp et al., 2013). Agus et al. (2024) reported that insecticide application practices among farmers often exceed recommended frequencies and concentrations, thereby accelerating resistance development and disrupting the balance of agricultural ecosystems. These conditions highlight the urgent need to transition toward integrated pest management strategies that prioritize environmental sustainability and long-term agricultural resilience.

One promising alternative approach is the utilization of entomopathogenic fungi as biological control agents. *Beauveria bassiana* is a widely distributed entomopathogenic fungus with broad geographical distribution. Its effectiveness in infecting insects is achieved through direct invasion of the insect cuticle, colonization of the body cavity, and subsequent induction of host mortality. Consequently, this fungus has become an important subject in numerous studies (Zimmermann, 2007; Vega et al., 2009). Beyond its role as a biological control agent, recent studies have also demonstrated that *B. bassiana* can function as an endophytic fungus, colonizing plant tissues internally without causing disease symptoms (Vega, 2018).

As an endophyte, *B. bassiana* has been reported to promote plant growth, enhance nutrient uptake, and induce systemic resistance against pest and pathogen attacks (Jaber & Ownley, 2018; Mantzoukas & Eliopoulos, 2020). Endophytic colonization facilitates a symbiotic interaction that provides dual benefits: direct protection against herbivorous insects and improved plant vigor. Several inoculation methods, such as seed treatment and soil drenching, have been proven effective in establishing internal colonization in plant roots and stems (Akello & Sikora, 2012; Ownley et al., 2010).

Although previous studies have demonstrated the dual role of *B. bassiana* as both an insect pathogen and an endophytic microbe capable of colonizing the tissues of various cultivated plants, its application in long bean cultivation in Indonesia remains far from optimal. Information regarding the effectiveness of soil drenching methods with specific conidial densities in promoting endophytic colonization, influencing vegetative growth, and enhancing plant resistance against *Spodoptera frugiperda* is still limited. Furthermore, studies integrating in vitro approaches, such as conidial viability testing, with in vivo approaches, including colonization capability and plant growth responses, are still needed to establish a more comprehensive and robust scientific foundation.

The novelty of this research lies in the integrated evaluation of *B. bassiana* as both an entomopathogenic fungus and an endophyte in long bean plants through the soil drenching method with varying conidial densities under a Completely Randomized Design (CRD). This approach is expected to optimize root colonization, improve nutrient uptake efficiency, strengthen plant defense systems, and sustainably suppress pest populations. Such a strategy supports the development of selective and environmentally safe pest management systems that contribute to the sustainability of horticultural agriculture.

This study aims to determine the extent to which different concentrations of *B. bassiana* conidia applied through the growing medium influence the vegetative growth (plant height and number of leaves) of long bean plants. In addition, the study seeks to provide technical guidance for the application of this fungus as an environmentally friendly and sustainable alternative for pest control.

METHOD

This study was conducted over a three-month period (June–August 2025) at two different facilities within the Faculty of Agriculture, UPN "Veteran" East Java. The in vitro experiment was carried out at the Plant Health Laboratory, while the in vivo experiment was performed in the faculty's screen house.

Tools and Materials

Various laboratory instruments were prepared to support this research, including Petri dishes, a laminar air flow cabinet, orbital shaker, autoclave, binocular microscope, gas stove, glass slides and cover slips, weighing scale, micropipettes, 250 and 500 mL Erlenmeyer flasks, a 30 cm ruler, hemocytometer, inoculation needle, polybags, measuring cylinders, gas lighter, Bunsen burner, scissors, and plastic wrap.

The materials used in this study consisted of *Beauveria bassiana* isolates, yardlong bean (*Vigna unguiculata*) seeds, planting media (soil, sand, compost, and NPK fertilizer), as well as solutions and culture media including sterile distilled water, 70% alcohol, sugar, spiritus, ADK medium, and EKG medium.

Experimental Design

This research was conducted using two experimental approaches: in vitro and in vivo. The in vitro experiment aimed to verify whether *B. bassiana* could function as an endophyte in yardlong bean plants and to determine whether the fungus could contribute to enhancing plant resistance to pests. The in vivo experiment was designed to evaluate the effect of *B. bassiana* on the growth of yardlong bean plants.

The study employed a Completely Randomized Design (CRD) consisting of four treatments. Each treatment was replicated five times, resulting in a total of 20 experimental units. Each unit consisted of two plants, giving a total of 40 plants observed in the experiment. The four treatments were as follows:

P1: Direct application to planting media (soil drenching) using sterile distilled water (control)
P2: Direct application to planting media (soil drenching) using *B. bassiana* at a density of 1×10^8 conidia/mL distilled water
P3: Direct application to planting media (soil drenching) using *B. bassiana* at a density of 1×10^7 conidia/mL distilled water
P4: Direct application to planting media (soil drenching) using *B. bassiana* at a density of 1×10^6 conidia/mL distilled water

Preparation of ADK and EKG Media

The ADK (Potato Dextrose Agar) medium was prepared by boiling 250 g of thinly sliced potatoes in 1 L of distilled water until boiling. The boiled mixture was then filtered to separate the potato residues. The resulting extract was mixed with 20 g of agar and stirred until homogeneous. The volume was adjusted to 1 L using distilled water and poured into a 500 mL Erlenmeyer flask. Sterilization was performed using an autoclave at 120°C and 1 atm pressure for 30 minutes.

The EKG (Potato Sugar Extract) medium was prepared by boiling 125 g of chopped potatoes in 500 mL of distilled water until boiling. After filtration, the potato extract was mixed with 10 g of sugar until completely dissolved. The final volume was adjusted to 500 mL using distilled water, then transferred into a 500 mL Erlenmeyer flask and sterilized in an autoclave at 120°C and 1 atm pressure for 30 minutes.

Propagation of *Beauveria bassiana* Isolates

The propagation of *B. bassiana* began by culturing conidia on ADK medium using the pour plate method and incubating them for 12 days until colonies developed. The resulting colonies were then transferred to EKG medium and incubated for an additional 8 days.

To determine the conidial concentration, 1 mL of suspension was diluted with 10 mL of sterile distilled water. Subsequently, 0.01 mL of the solution was taken and the number of conidia was counted using a hemocytometer in five medium-sized counting squares. The conidial density was calculated according to the formula described by Ardiyati et al. (2015):

$$\mathrm{c} = \frac{t}{n.0{,}25} \times 10^6$$

Where:
c = Conidial density per mL of solution (conidia/mL)
t = Total number of conidia in the observed counting squares
n = Number of counting squares observed
0.25 = Correction factor

Initiation of *Beauveria bassiana*

The initiation of *B. bassiana* in yardlong bean plants was conducted from 10 to 40 days after planting. Soil drenching was applied once per week using 10 mL of *B. bassiana* conidial suspension with densities of 1×10^8, 1×10^7, and 1×10^6 conidia/mL on the soil surface. This procedure follows the findings of Isrin & Fauzan (2018), which indicate that the most effective application of *Beauveria* is conducted once per week and applied in the afternoon.

The control treatment consisted of soil drenching using only sterile distilled water (without conidia). This group served as a reference to evaluate the effects of different conidial densities of *B. bassiana* on the potential endophytic colonization in yardlong bean plants and their effects on plant growth and resistance.

Evaluation of *Beauveria bassiana* as an Endophyte

After the planting period was completed, the plants were carefully removed from their containers. The roots and stems were washed thoroughly with running water to remove any remaining planting media. From each plant, one 3 cm segment of stem and one 3 cm segment of root were collected as samples. All samples were subsequently surface-sterilized inside a laminar air flow cabinet (LAF). The sterilization process began with immersion in sterile distilled water for 1 minute, followed by immersion in 70% alcohol for 30 seconds, and then rinsing again in sterile distilled water for 1 minute. This procedure was repeated three times consecutively to ensure effective surface sterilization.

After sterilization, the samples were dried on filter paper in a Petri dish. The ends of the root and stem segments were slightly trimmed to minimize the possibility of contamination from tissues that might still be exposed to external microorganisms. The samples were then placed in the center of ADK medium plates. Colony growth was observed daily for 10 days. The initial indicator of successful colonization was the appearance of white mycelium, which gradually turned pale yellow over time, consistent with the characteristics reported by Kumar et al. (2016). To maintain isolate purity, purification of the growing colonies was performed. Further observations were conducted on the 14th day after purification, both macroscopically and microscopically. Macroscopically, colony shape and color on ADK medium were recorded.

Microscopically, the characteristics of conidia, hyphal dimensions, and conidiophore morphology were observed using a microscope.

Data Analysis

The data obtained were analyzed using analysis of variance (ANOVA) to evaluate the effects of treatments. If the calculated F value exceeded the F table value at the 5% significance level (α = 0.05), the null hypothesis was rejected. Subsequently, the Honest Significant Difference (HSD) test was performed to determine the best treatment.

RESULTS AND DISCUSSION

Characteristics of *Beauveria bassiana*

The *B. bassiana* isolate was rejuvenated on ADK medium and incubated for 14 days to observe fungal colony morphology. The results of the observation of *B. bassiana* characteristics are presented in Figure 1.

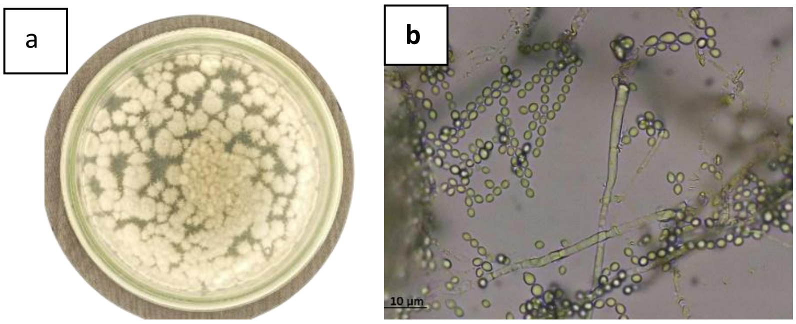

Figure 1. Characteristics of *B. bassiana*: (a) macroscopic appearance of the *B. bassiana* isolate, (b) microscopic appearance of the *B. bassiana* isolate

Based on the observations, the growing *B. bassiana* exhibited typical macroscopic characteristics, namely colonies with a yellowish-white color, a powder-like texture, and an irregular spreading pattern. These findings are consistent with the report of Wahjono and Yuliani (2024), who described colonies as white with a fine powdery texture that may gradually turn yellowish over time, with round conidia ranging in color from white to yellowish and arranged along transparent septate hyphae.

Microscopically, septate hyphae, conidiophores with pointed tips, and round to oval conidia were observed. These characteristics correspond with the description provided by Sopialena (2022), who reported that *B. bassiana* conidiophores grow upright and solitary with pointed tips, while the conidia are spherical, single-celled, and hyaline. Overall, the observed features confirm that the obtained isolate is *Beauveria basiana*, consistent with the morphological characteristics reported in previous studies.

Effect of *Beauveria bassiana* Initiation as an Endophyte on the Growth of Yardlong Bean Plants

Observations of yardlong bean plant height from 7 to 42 days after planting showed that growth responses varied among treatments inoculated with *B. bassiana* as an endophyte. In general, the presence of this fungus produced noticeable effects compared with the control plants. The differences observed among treatments are likely closely related to the active role of endophytic fungi in promoting plant growth, particularly through their ability to produce secondary metabolites that regulate physiological processes during the vegetative growth phase. This assumption is consistent with the findings of Ramdan et al. (2013), who reported that increased plant growth associated with endophytic fungi is often linked to the secondary metabolites they produce. Detailed data regarding the effect of treatments on plant height are presented in Table 1, which indicates that endophytic inoculation with *B. bassiana* significantly influenced the height of yardlong bean plants.

Table 1. Observation results of yardlong bean plant height

Treatment	**Plant Height (cm)**					
	Day-					
	7	**14**	**21**	**28**	**35**	**42**
P1	7,00	15,00 b	25,00 a	40,00 c	60,00 c	80,00 c
P2	9,00	19,00 a	35,00 b	65,00 a	95,00 a	107,70 a
P3	8,00	17,00 ab	30,30 ab	51,00 b	75,00 b	95,00 b
P4	8,00	17,00 ab	30,00 ab	54,30 b	80,00 b	101,00 ab
BNJ 5%	tn	0,45	1,19	2,00	2,24	1,86

Note: Mean values followed by the same letter are not significantly different according to the Honest Significant Difference (HSD) test at α = 0.05.

Based on the data presented in Table 1, variation among treatments began to appear at 14 days after planting (DAP). Treatment P2 was categorized in group “a”, while P1 was categorized in group “b”, whereas P3 and P4 were grouped as “ab”. This condition indicates that treatments P3 and P4 did not differ significantly from either P1 or P2. This phenomenon is likely associated with the early phase of endophytic fungal colonization, during which *B. bassiana* begins to enter plant tissues, although its effect on plant height has not yet become fully stable. Plant height in treatments P1 to P4 generally increased with plant age. However, statistically, no significant differences were detected among all tested treatments during the early observation period.

At 28 DAP, the differences among treatments became more pronounced. Treatment P2 showed the highest average plant height compared with the control treatment and other conidial density treatments, whereas treatment P1 recorded the lowest value. Treatments P3 and P4 showed intermediate plant heights between these two treatments. The increasing differences in plant height at this stage are likely associated with the optimal progression of the vegetative growth phase. During this phase, plants may begin to effectively utilize the presence of *B. bassiana* as an endophytic associate, thereby contributing to increased plant height. This finding supports the report of Rondot & Reineke (2018), who stated that endophytic colonization by *B. bassiana* during the vegetative phase tends to be stable and has the potential to stimulate plant growth.

Overall, plant height data from 7–42 DAP indicate that inoculation with *B. bassiana* did not inhibit the growth of yardlong beans. In fact, several treatments tended to produce taller plants than the control. This suggests that *B. bassiana* can live endophytically without harming its host plant, consistent with the statement of Mantzoukas & Eliopoulos (2020) that endophytic entomopathogenic fungi can be neutral to beneficial for plant growth. Furthermore, Athifa et al. (2018) reported that increasing the density of applied conidia can increase the number of conidia adhering to the host plant, thereby enhancing the potential for successful colonization.

Table 2. Observation results of the number of leaves of yardlong bean plants

Treatment	**Number of Leaves (Leaves)**					
	Day-					
	7	**14**	**21**	**28**	**35**	**42**
P1	3,67 a	8,00 a	15,33 a	27,00b	39,33 a	47,67 b
P2	3,33 a	8,67 a	19,33 a	37,67a	45,33 a	59,00 a

Treatment	Number of Leaves (Leaves)					
	Day-					
	7	14	21	28	35	42
P3	3,33 a	7,00 a	17,00 a	32,67ab	40,67 a	51,67 b
P4	3,33 a	8,67 a	17,66 a	32,00ab	43,00 a	50,67 b
BNJ 5%	tn	tn	tn	1,00	tn	0,92

Note: Mean values followed by the same letter are not significantly different according to the Honest Significant Difference (HSD) test at α = 0.05.

Observations of the leaf number parameter conducted from 7 to 42 days after planting, as presented in Table 2, show that all treatments experienced an increase in leaf number as the plants aged. This indicates that yardlong bean plants maintained normal vegetative growth throughout the observation period, both in the control treatment and in treatments inoculated with *B. bassiana*.

At 7 DAP, the ANOVA results indicated that variations in conidial density did not statistically influence leaf number. All treatments were grouped within the same significance category. This condition is likely due to the early growth stage of the plants, during which leaf formation is still strongly influenced by nutrient reserves within the seeds and the initial adaptation of plants to the growing environment. This situation is reasonable because early leaf development is largely supported by seed nutrient reserves and plant adaptation processes, while endophytic colonization by *B. bassiana* may not yet have reached an optimal level. Jaber & Enkerli (2016) also reported that endophytic effects are usually not evident during the early stages of plant growth.

At 28 DAP, although the number of leaves continued to increase, the differences among treatments were not yet statistically consistent. ANOVA results indicated a treatment response; however, the differences among treatments were not fully consistent statistically. This may be because leaf number is a relatively stable growth parameter, so the observed changes were not yet large enough to produce significant differences. Tomilova et al. (2023) reported that colonization by endophytic fungi does not always immediately increase certain morphological parameters, as plants may allocate energy to maintain physiological balance and internal defense mechanisms.

Differences in leaf number observed at 28, 35, and 42 DAP showed variation among observation times. At 28 DAP, significant differences among treatments were observed, whereas at 35 DAP statistical analysis indicated no significant differences among treatments. Subsequently, at 42 DAP, differences among treatments became significant again. This pattern suggests that the response of leaf number to the treatments does not always appear consistently at each observation time and may be influenced by the dynamics of plant growth during the observation period, without necessarily indicating a direct causal relationship (Wei et al., 2020).

CONCLUSION

The results of this study indicate that differences in the concentration of *Beauveria bassiana* conidia did not produce a statistically significant effect on plant height or the number of leaves of yardlong bean during the observation period from 7 to 42 days after planting. Nevertheless, numerically, plants that received inoculation treatments tended to exhibit better average growth than the control, particularly within the age range of 28 to 42 days after planting (DAP). This finding suggests that although the effects were not always statistically significant, the presence of this fungus appears to provide a positive contribution to plant growth. Interestingly, *B. bassiana* successfully colonized the root and stem tissues of yardlong bean plants endophytically in all inoculation treatments. The highest colonization percentage was observed in the treatment with a concentration of 1×10⁶ conidia per mL (P2), reaching 83.3% in roots and 66.7% in stems. In contrast, no fungal colonization was detected in the tissues of control plants, as expected.

RECOMMENDATION

Based on the results obtained, the authors recommend further research extending to the final generative stage (fruiting) in order to determine the effect of *B. bassiana* application on the fruit quality of yardlong bean plants.

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