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RESEARCH PAPER

Endophytic *Beauveria bassiana* in root, seed, and foliar-treated maize affecting on *Spodoptera frugiperda* larvae and its parasitization eggs

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ABSTRACT

Spodoptera frugiperda is a highly polyphagous pest capable of causing up to 100% yield loss in maize. This study aimed to evaluate the effects of endophytic *Beauveria bassiana* applied through root drench, seed treatment, and foliar spray on the severity of larval feeding damage and egg parasitization by *S. frugiperda.* The *B. bassiana* isolate (JgSPK) used in this study was molecularly identified and deposited in GenBank (acc. no. MZ356494). Results indicated that among the three inoculation methods, seed treatment resulted in the highest endophytic colonization, followed by soil drench and foliar spray. Feeding on leaves colonized by the fungus increased larval mortality and resulted in abnormal pupation. Seed treatment showed the greatest potential in reducing larval feeding severity compared with foliar spray and soil drench applications. However, none of the inoculation methods significantly affected egg parasitization rates. Overall, endophytic *B. bassiana* reduced larval attack severity but did not influence egg parasitization, indicating compatibility with egg parasitoids. Further research is recommended to evaluate potential effects on larval parasitoids.

Keywords: Abnormal pupae, entomopathogenic fungi, fall armyworm, maize, egg parasitization, *Telenomus remus*

INTRODUCTION

The fall armyworm (FAW), *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae), has been established in Indonesia, Lampung since 2015, indicating that its introduction occurred earlier than previously reported (Andrianto et al., 2024). In March 2019, this invasive pest caused severe damage to maize crops in West Sumatra (Sartiami et al., 2020). Since then, FAW has spread throughout various provinces in Indonesia, including Bengkulu (Ginting et al., 2020), Bali (Supartha et al., 2021), and West Java (Russianzi et al., 2021). FAW is a highly polyphagous pest, feeding on 353 plant species from 76 families in the Americas (Montezano et al., 2018). In maize, particularly during the vegetative stage, FAW infestation can result in up to 100% crop loss (Herlinda et al., 2022b), as larval feeding severely damages leaves, growing points, and shoots (Herlinda et al., 2021a). In Indonesia, two FAW strains—corn and rice—have been identified (Herlinda et al., 2022b).

Management of FAW in Indonesia, as well as in Kenya, Brazil, and Puerto Rico, has relied heavily on synthetic insecticides, including carbamates and organophosphates. However, resistance to these insecticides has already been documented (Boaventura et al., 2020). Furthermore, the use of synthetic insecticides can negatively affect natural enemies, including parasitoids (Amaro et al., 2018). Several parasitoids are known to regulate FAW populations, including egg parasitoids (*Trichogramma* spp. (Hymenoptera: Trichogrammatidae), *Telenomus remus* Nixon (Hymenoptera: Scelionidae) and larval parasitoids (*Chelonus annulipes* Wesm. and *Chelonus formosanus* Sonan (Hymenoptera: Braconidae) (Herlinda et al., 2023).

Entomopathogenic fungi such as *Beauveria bassiana* (Balsamo) Vuillemin (Faddilah et al., 2022), *Metarhizium anisopliae* (Metsch.) Sorokin (Lestari et

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al., 2022), and *Penicillium citrinum* Thom (Herlinda et al., 2021b) have been shown to infect and kill FAW larvae. However, topical applications of these fungi can negatively affect beneficial organisms, such as shortening the longevity of the parasitoid *T. remus* (Putri et al., 2024). Additionally, their field effectiveness is limited due to susceptibility to low humidity, ultraviolet exposure (Russo et al., 2020), and difficulty reaching larvae hidden deep inside maize whorls (Herlinda et al., 2022a). To overcome these limitations, the use of entomopathogenic fungi as endophytes has emerged as a promising approach.

Our previous studies demonstrated that *B. bassiana*, *M. anisopliae*, and *P. citrinum* isolates are capable of colonizing maize endophytically and killing FAW larvae (Sari et al., 2022). In laboratory studies, endophytic *B. bassiana* applied via seed treatment resulted in up to 51.33% FAW larval mortality (Sari et al., 2022). Endophytic fungal colonization has also been shown to impair FAW growth and development (Faddilah et al., 2022; Lestari et al., 2022; Sari et al., 2022) and reduce damage severity in maize (Kuzhuppillymyal-Prabhakarankutty et al., 2021). Despite these findings, no study has compared different inoculation methods—such as soil drenching, seed treatment, and foliar spraying—on FAW infestation severity and parasitization levels under field conditions.

Therefore, this study aimed to evaluate the effects of *B. bassiana* applied through root (soil drench), seed, and foliar inoculations on the severity of *S. frugiperda* larval damage and the parasitization rate of FAW eggs in maize.

MATERIALS AND METHODS

Research Site. The field experiment was conducted at the experimental farm of the Department of Plant Protection, Faculty of Agriculture, Universitas Sriwijaya. The study was arranged in a completely randomized block design, consisting of three inoculation methods of *B. bassiana* (soil drench or root inoculation, seed treatment, and foliar spray) and a control (no fungal treatment), with seven replications. Seed treatment was performed prior to planting, while soil drench and foliar spray treatments were applied to 14-day-old maize plants. Each replication contained 128 plants. Leaf tissues were collected at 21 days after planting.

Inoculation of Endophytic *Beauveria bassiana*. The *B. bassiana* JgSPK isolate used in this research was molecularly identified in 2021 and deposited in GenBank (Accession No. MZ356494) (Herlinda et al., 2021b). The isolate originated from maize leaves from Simpang Padang Karet, Pagar Alam, South Sumatra (103°15’30.1788” E, 4°1’28.0308” S). The fungus was cultured on sabouraud dextrose agar (SDA) and incubated for two weeks.

Surface sterilization of seeds was performed using alcohol and sodium hypochlorite (Russo et al., 2020). One kilogram of maize seed was immersed in 1 L of fungal suspension (1×10^{10} conidia mL^{-1}) for 12 hours. The maize variety used was sweet corn (*Zea mays* L. var. *Saccharata* Sturt). Treated seeds were planted in the field.

Soil sterilization followed the method of Donga et al. (2018), where soil was heated in a sealed plastic bag at 95–100 ºC for 5 hours, cooled for 24 hours, and mixed with 25 g of diammonium phosphate. Prior to planting, soil was moistened with tap water for 24 hours.

For soil drench inoculation, 50 mL of fungal suspension (1×10^{10} conidia mL^{-1}) was applied per plant at the soil surface near the roots of 14-day-old maize. For foliar spray, 50 mL of the same suspension was sprayed on leaves at the same plant age, applied between 08:00–09:00 when stomata were open. Control plants received sterile water.

To confirm fungal colonization, 1 cm leaf tips from 21-day-old maize were collected following Sari et al. (2022). Detection was conducted weekly until 63 days after planting by culturing leaf tissues on SDA medium. Colonization percentage was determined by counting the number of leaf pieces showing fungal growth.

Effects of Fungal Colonization on *Spodoptera frugiperda* Larvae and Egg Parasitization. To evaluate the effects of fungal colonization on FAW larvae, colonized and non-colonized maize leaves from the field were fed to laboratory-reared third-instar larvae. The larvae were reared individually. First-instar larvae were fed *Amaranthus hybridus* leaves, while second–sixth instars were fed fresh maize leaves (Lestari et al., 2022). Fresh leaves were provided daily until death or pupation, and abnormal pupae were recorded.

The pupal emergence were transferred to a transparent plastic cage ($50 \times 50 \times 50$ cm^3) housing over 100 pupae, with fresh corn leaves provided for adult egg-laying. This experiment conducted weekly with the larvae were provided with 2×2 cm leaves from the fungal treated and untreated maize at 21, 28, 35, 42, 49, 56, and 63 days of age, the larval diet was replaced with the fresh corn leaves every day. They were monitored until they either died or transformed into pupae. The morphology of abnormal pupae was observed every day.

In the field experiment, a “W” pattern sampling method was used to evaluate leaf damage severity (Prasanna et al., 2018). Thirty plants per treatment were assessed. Damage symptoms included shot holes, tattered leaves, lesions, dead hearts, and pinholes. Severity was visually scored on a scale of 1–5: 1) no damage; 2) 1−10% damage or < 5 mm feeding marks; 3) 11−25% damage with chewing >5 mm, whorl intact; 4) 26−50% damage with chewing >1 cm, moderate whorl damag; 5) > 50% damage, severe whorl damage and stunting (Kuate et al., 2019). Artificial infestation was unnecessary due to high natural FAW pressure.

Parasitization rate of *S. frugiperda* eggs during a maize planting season was observed every week and began at 21-day-old-maize, 28-day-old-maize, 35-day-old-maize, 42-day-old-maize, 49-day-old-maize, 56-day-old-maize, 63-day-old-maize. The eggs of *S. frugiperda* were collected from all plots of treatments using a purposive sampling technique (Mukkun et al., 2021). The selected sample eggs (2-3 days old) were not covered with a protective layer of silk anymore and characterized by a yellowish-white color. The eggs were placed in a test tube (Ø 15 cm, 20 cm in height), and the emerging parasitoid adults were monitored daily.

The hatching of *S. frugiperda* eggs was documented to assess the rate of egg parasitization. The rate of egg parasitization was calculated from number of eggs parasitized divided by the total number of eggs observed. Parasitized egg were identified through direct observation by their black coloration. The morphology of the adult parasitoids was examined to identify the species. The emerging parasitoid adults were put into vial containing removed from the containers and killed by immersion in 70–96% alcohol. The adult parasitoids were identified morphologically (Herlinda et al., 2023).

Data Analysis. The effect of fungal inoculation methods on fungal colonization percentage, larval mortality and pupal deformation, FAW leaf damage severity, and egg parasitization rate were analyzed using analysis of variance (ANOVA), followed by Tukey’s test at a significance level of 0.05.

RESULTS AND DISCUSSION

Colonization of Endophytic *Beauveria bassiana* in Maize Plants. The percentage of maize leaves colonized by endophytic *B. bassiana* throughout the growing season indicated that colonization began at 21-day-old maize (7 days after fungal inoculation). Leaves colonized by endophytic *B. bassiana* showed visible fungal hyphae and mycelium, whereas control leaves did not exhibit such structures (Figure 1). The percentage of leaf colonization differed significantly from the control ($P < 0.05$) (Table 1).

Among the three inoculation methods, seed treatment tended to demonstrate higher colonization ability compared with soil drench and foliar spray. Although fungal colonization remained detectable from 21 to 63 days after planting, the percentage of colonized leaves gradually declined as the maize aged. The seed treatment method was the most effective in establishing systemic fungal colonization. This result is consistent with previous studies reporting that seed inoculation results in successful colonization across all plant parts, although colonization typically decreases over time (Kinyungu et al., 2023). Other studies have reported colonization rates of 50–60% and 72–80% at 14 days post-treatment using seed treatment and foliar spray methods, respectively (Altaf et al., 2023). The relatively low colonization rate observed in the present

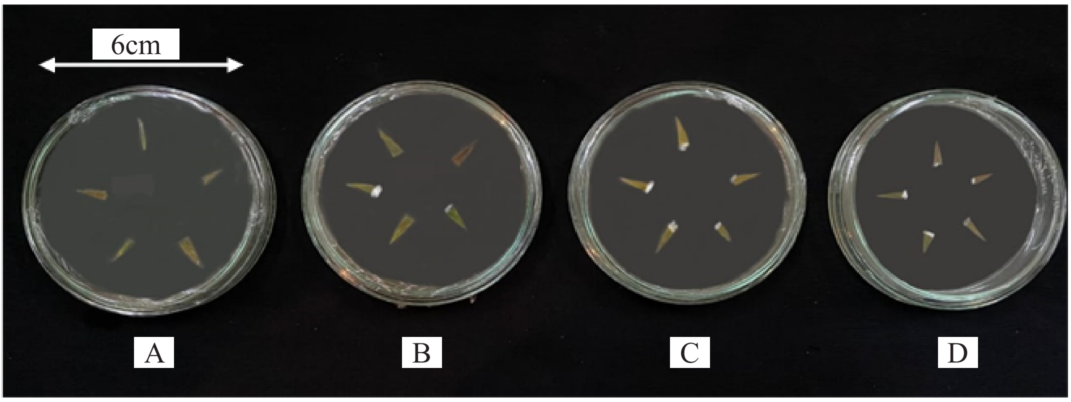

Figure 1. Colony morphology of endophytic *Beauveria bassiana* isolated from different maize tissues. A. Control (non-inoculated maize); B. Root tissue inoculated with *B. bassiana*; C. Seed; D. Leaf tissue.

study may be related to the single inoculation event.

Effects of Fungal Colonization on *Spodoptera frugiperda* Larvae. Abnormal pupae and larval mortality were recorded when *S. frugiperda* larvae fed on leaves from 35 to 56-day-old maize (Table 2). No mortality or abnormal pupae were observed in larvae fed on leaves from 21 and 28-day-old maize. Larvae feeding on leaves from 35 and 42-day-old maize showed significantly higher mortality and abnormal pupation ($P < 0.05$) in seed treatment and soil drench applications than in the foliar spray treatment. Feeding on leaves colonized by the fungus induced larval death and morphological deformities.

Although fungal colonization was detected in leaves of 21 and 28-day-old maize, no larval mortality or abnormal pupation occurred at these stages, likely due to reduced larval consumption of leaf tissue. In contrast, larvae feeding on colonized leaves from 35 to 56-day-old maize resulted in abnormal pupae and a larval mortality rate of 33.33%. However, larvae feeding on leaves from 63-day-old maize did not produce mortality or abnormalities. Dead larvae exhibited typical fungal infection symptoms, including reduced size, shriveling, and mummification, whereas larvae in the control treatment were longer and larger. Abnormal pupae resulting from larvae fed on colonized leaves displayed malformation and shriveling (Figure 2). These findings are consistent with symptoms previously reported in similar studies (Herlinda et al., 2022a; Sari et al., 2022).

In the field, overall larval damage severity did not significantly differ among treatments ($P > 0.05$) (Table 3). However, at 56 days after planting, seed-inoculated maize exhibited significantly lower larval severity ($P < 0.05$) compared to soil drench, foliar

Table 1. Effects of fungal treatments on leaf colonization percentage by endophytic *Beauveria bassiana* across different maize growth stages

Fungal treatment	Maize growth stage (days old)						
	21	28	35	42	49	56	63
	Mean *Beauveria bassiana* colonization (%)						
Control	0 a	0 a	0 a	0 a	0 a	0 a	0 a
Root	2.86 b	2.14 b	6.67 bc	7.62 b	4.76 b	3.81 b	3.81 b
Seed	12.38 c	34.29 c	9.52 c	12.38 b	4.76 b	4.76 b	1.90 b
Foliar	15.24 c	14.29 c	3.81 b	8.57 b	8.57 c	4.76 b	5.71 c
F-value	16.45*	21.79*	3.51*	5.44*	3.19*	3.44*	3.55*
P-value	0.00002	0.000003	0.036	0.007	0.04	0.03	0.03
HSD value	3.76	5.21	4.76	4.96	4.69	3.57	3.75

* = significantly differences, ns = not significantly different, data within same column followed by the same letters were not significantly different at $p < 0.05$ according to HSD test (Tukey's test). The data were transformed using the Arcsin before statistical analysis.

Table 2. Larval mortality and abnormal pupae of *Spodoptera frugiperda* consuming fungal-treated maize leaves in the laboratory

Fungal treatment	Maize growth stage (days old)						
	21	28	35	42	49	56	63
	Larval mortality and abnormal pupae (%)						
Control	0.00	0.00	0.00 b	0.00 b	0.00	0.00	0
Root	0.00	0.00	25.00 ab	25.00 a	25.00	16.67	0
Seed	0.00	0.00	33.33 a	25.00 a	16.67	16.67	0
Foliar	0.00	0.00	16.67 ab	0.00 b	8.33	0.00	0
F-value	-	-	6.01*	3.74*	4.0	0.43^{ns}	-
P-value	-	-	0.03	2×10^{-16}	0.07	0.67	-
HSD value	-	-	30.37	1.35	-	-	-

* = significantly different, ns = not significantly different, data within same column followed by the same letters were not significantly different at $p < 0.05$ according to HSD test (Tukey's test). The data were transformed using the Arcsin before statistical analysis.

spray, and control treatments. Because seed treatment resulted in the highest colonization level (Table 1), it demonstrated the strongest potential to suppress larval damage in the field.

Previous studies have shown that fungal seed treatment can achieve 100% colonization in all maize seedling tissues under hydroponic conditions (Herlinda et al., 2022a; Sari et al., 2022). The present findings align with those studies, although field colonization levels were lower than those achieved under controlled hydroponic conditions. Nevertheless, seed treatment showed superior colonization efficiency compared to the other inoculation methods.

Larval severity exhibited a temporal trend, beginning at 21 days after planting, peaking at 35 days, and gradually decreasing from 49 days onward. Similar trends have been reported previously, where peak infestation occurred at four weeks after planting and declined after eight weeks (Supartha et al., 2021). Seasonal larval population dynamics in maize fields frequently show multiple fluctuations (Dassou et al., 2021). Young maize plants (3–6 weeks old) typically experience the most severe infestation, whereas reproductive-stage maize experiences minimal damage (Herlinda et al., 2022b).

Effects of Fungal Colonization on *Spodoptera frugiperda* Egg Parasitoids. Parasitized *S. frugiperda* eggs appeared uniformly black, whereas non-parasitized eggs were greenish-white (Figure 3). The egg parasitoid species recorded in this study was *Telenomus remus* (Figure 4). The parasitization rate of *S. frugiperda* eggs throughout the growing season did not differ significantly among treatments (P > 0.05) (Table 4). Egg parasitization was first observed at 21 days after planting, gradually increased to peak at 35 days, and subsequently declined until no parasitoids were detected near harvest.

Previous laboratory studies demonstrated that topical application of *B. bassiana* on parasitoid mummies did not reduce parasitization rate or adult emergence (Putri et al., 2024). Similarly, in the present field experiment, endophytic fungal inoculation did not negatively affect egg parasitization. Since the fungus resided internally within plant tissues and did not physically contact the parasitoid eggs or mummies,

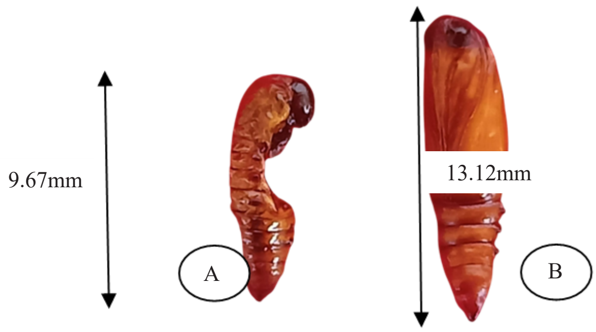

Figure 2. Pupae of *Spodoptera frugiperda*. A. Abnormal, infected *B. bassiana*; B. Normal pupa from the control.

Table 3. Effects of fungal colonization on the severity of damage caused by *Spodoptera frugiperda* larvae during the maize growing season under field conditions

Fungal treatment	Maize growth stage (days old)						
	21	28	35	42	49	56	63
	Mean severity (on a scale of 1 to 5)						
Control	3.87	7.48	8.14	6.68	2.80	2.49ab	3.27
Root	6.21	9.53	8.49	4.87	3.47	3.05a	0.92
Seed	1.06	4.91	5.89	4.54	2.37	1.30b	0.87
Foliar	7.33	7.89	8.34	3.85	1.58	1.95ab	2.23
F-value	2.67ns	2.17ns	0.16ns	0.35ns	2.74ns	3.52*	0.43ns
P-value	0.11	0.16	0.91	0.78	0.11	0.06	1.00
HSD value	-	-	-	-	-	5.34	-

* = significantly different, ns = not significantly different, data within same column followed by the same letters were not significantly different at p < 0.05 according to HSD test (Tukey's test). The data were transformed using the Arcsin before statistical analysis.

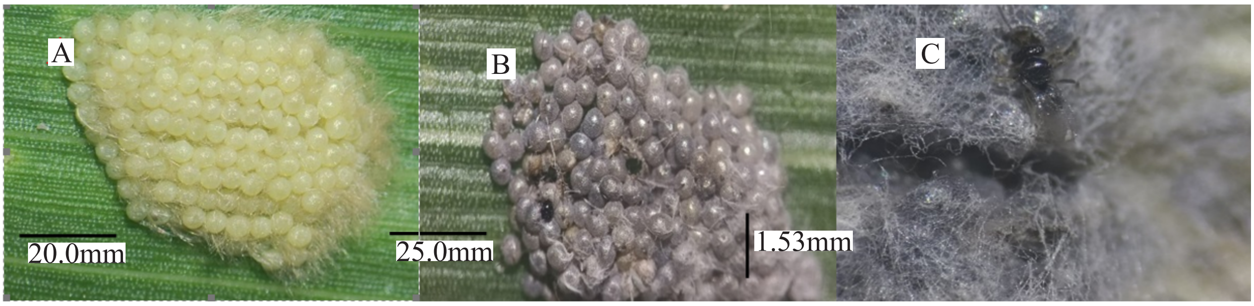

Figure 3. Condition of *Spodoptera frugiperda* eggs. A. Healthy eggs; B. Parasitized eggs; C. Eggs from which both *Spodoptera frugiperda* and *Telenomus remus* emerged.

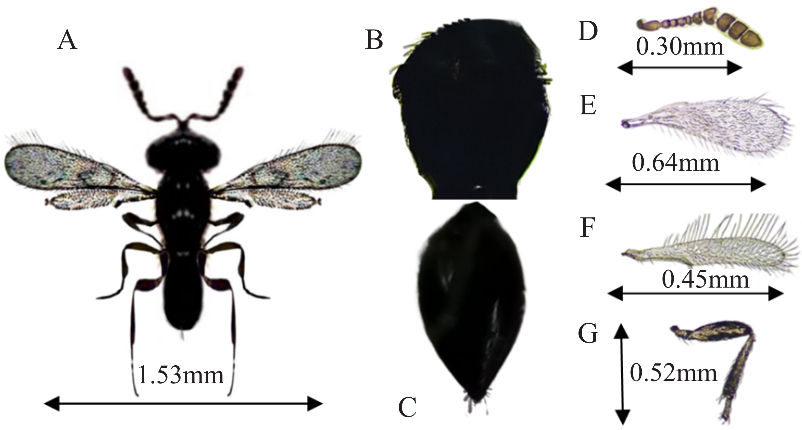

Figure 4. Morphological characteristics of *Telenomus remus*. A. Adult; B. Thorax; C. Abdomen; D. Antena; E. Forewing; F. Hind wing; G. Leg.

Table 4. Effects of fungal colonization on the parasitization rate of *Spodoptera frugiperda* eggs during a maize planting season

Fungal treatment	Maize growth stage (days old)						
	21	28	35	42	49	56	63
	Parasitization rate of *Spodoptera frugiperda* eggs (%)						
Control	0	33.06	28.00	28.42	32.70	18.34	28.56
Root	0	28.57	56.85	33.25	34.88	33.53	14.29
Seed	0	32.54	28.57	24.65	34.05	34.16	28.57
Foliar	3.28	57.14	42.86	11.30	4.13	14.29	28.57
F-value	2.38	0.57	1.91	0.46	0.99	0.55	0.21
P-value	0.10^{ns}	0.64^{ns}	0.16^{ns}	0.71^{ns}	0.42^{ns}	0.66^{ns}	0.89^{ns}
HSD value	-	-	-	-	-	-	-

Ns = not significantly different; data within same column followed by the same letters were not significantly different at $p < 0.05$ according to HSD test (Tukey's test). The data were transformed using the Arcsin before statistical analysis.

no harmful effects were observed.

These findings demonstrate that endophytic *B. bassiana* application in maize fields is compatible with biological control by egg parasitoids such as *T. remus*. Further research is needed to determine whether endophytic fungi may influence larval parasitoids or parasitoid emergence success in later stages of the pest life cycle.

CONCLUSION

The endophytic *B. bassiana* in root, seed, and foliar-treated maize can decrease the severity of *S. frugiperda* larvae under laboratory and field conditions, but does not impact its parasitization eggs. Integrated application of egg parasitoids and fungal seed treatment in cornfields may enhance the control of *S. frugiperda* and support integrated pest management (IPM) within the maize ecosystem. Further research is necessary to determine whether the larval parasitization rate and the emergence of larval parasitoid adults could be influenced by the application of endophytic entomopathogenic fungus.

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AUTHORS' CONTRIBUTIONS

SH performed research concept and design, data interpretation, writing the article, and final approval of article. SS performed re-culturing and applying endophytic *Beauveria bassiana*. TT and SPM performed collection and assembly of data. JMPS prepared and performed data analysis. SS performed interpretation and critical revision of the article. RS identified fungal species molecularly. All the authors read and approved the manuscript.

COMPETING INTEREST

All authors declare that we have no competing interests, such as financial or non-financial interests, professional or personal relationships that are directly or indirectly connected to the work submitted for publication.

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