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Growth of fall armyworm, *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae) fed on young maize colonized with endophytic fungus *Beauveria bassiana* from South Sumatra, Indonesia

DETYA ROSA FADDILAH[1], MARIESKA VERAWATY[1,2], SITI HERLINDA[3,4,♥]

[1]Graduate Program of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya. Jl. Padang Selasa No. 524, Palembang 30139, South Sumatra, Indonesia
[2]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya. Jl. Raya Palembang-Prabumulih Km. 32, Indralaya, Ogan Komering Ulu 30662, South Sumatra, Indonesia
[3]Department of Plant Protection, Faculty of Agriculture, Universitas Sriwijaya. Jl. Raya Palembang-Prabumulih Km. 32, Indralaya, Ogan Komering Ulu 30662, South Sumatra, Indonesia. Tel./fax.: +62-711-580059, ♥email: sitiherlinda@unsri.ac.id
[4]Research Center for Sub-optimal Lands, Universitas Sriwijaya. Jl. Padang Selasa No. 524, Palembang 30139, South Sumatra, Indonesia

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Abstract. *Faddilah DR, Verawaty M, Herlinda S. 2022. Growth of fall armyworm,* Spodoptera frugiperda *J.E. Smith (Lepidoptera: Noctuidae) fed on young maize colonized with endophytic fungus* Beauveria bassiana *from South Sumatra, Indonesia. Biodiversitas 23: 6652-6660.* The aim of present research was to evaluate the seed treated effect of endophytic *Beauveria bassiana* isolated from soil and infected-host cadavers on the growth of *S. frugiperda*. The three isolates (TaBrPGA, LtApPGA, and TaTtLH) of *B. bassiana* identified molecularly were used for bioassay. The research confirmed that isolates of *B. bassiana* isolated from soil and infected-host cadavers of Lepidoptera were endophytic entomopathogenic fungi All isolates of endophytic *B. bassiana* caused negative effects on the growth of *S. frugiperda* larvae. TaTtLH isolate of *B. bassiana* was the most pathogenic isolate (73% of larvae mortality) among the other isolates. *B. bassiana* in seed treatment could retard the growth of *S. frugiperda* larvae, pupae, and adults. The endophytic *B. bassiana* could also kill the pupae and adults of *S. frugiperda*, and decrease the pupae and adult emergence. *Beauveria bassiana* could decrease 86% of adult emergence. *Beauveria bassiana* applied in seed treatment could protect she stored corn seeds and the young maize plant against *S. frugiperda*. So, endophytic *B. bassiana* could be recommended to protect the stored corn seeds.

Keywords: Corn, endophytes, entomopathogen, neonate larvae, seed treatment, *Spodoptera frugiperda*

INTRODUCTION

Fall armyworm (FAW), *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae) is the most destructive insect pest of corn worldwide, and it can migrate all over the world (Montezano et al. 2018) and cause crop losses (De Groote et al. 2020). The FAW came from South America (Otim et al. 2018) and entered Africa in 2016 (Goergen et al. 2016), while in 2017, it was found in Europe (Early et al. 2018). This pest was found in Asia in 2018, specifically for the first time in India (Ganiger et al. 2018) and then crossed over to Indonesia at the beginning of 2019 in West Sumatra (Sartiami et al. 2020). Currently, FAW has spread throughout Indonesia (Maharani et al. 2019). Furthermore, this pest can attack a wide range of plants (polyphagous) and 353 host plant species from 76 plant families are its hosts (Montezano et al. 2018). The FAW induced the percent of infested corn ranging 80% to 100% in Ethiopia and 82.2% to 100% in Kenya (Sisay et al. 2019). It caused corn losses about 1 million tonnes per year in Kenya (De Groote et al. 2020). The losses reached US $ 13 million per year in Africa (Harrison et al. 2019). In Indonesia, FAW severely attacked corn and caused up to 100% damage (Herlinda et al. 2022a; Mukkun et al. 2021). The FAW larvae destroy corn or other crops by eating young leaves, stems, flowers, fruits, and growing points (Montezano et al. 2018; Herlinda et al. 2022a). In the morning, the larvae are always appear for feeding on the surface of maize leaves, but at daylight up to night they begin to hide within the leaves' midribs of maize (Gustianingtyas et al. 2021).

Synthetic insecticides are commonly used to control *S. frugiperda* (Kumela et al. 2018) because insecticides are easy to spray and fast acting. Nevertheless, some synthetic insecticides have negative effects, such as pyrethroid, spinosad, and organophosphorus insecticides (Zhang et al. 2021) and cause problem for human health and environment (Harrison et al. 2019). An alternative eco-friendly approach to control FAW is urgently need. The preferred control option for FAW is biological control by entomopathogenic fungi (EPF) (Herlinda et al. 2020; Herlinda et al. 2022b). Previous study showed that topical application or direct contact of EPF such as *Beauveria bassiana* (Balsamo) Vuillemin killed more than 80% of *S. frugiperda* larvae (Ramanujam et al. 2020). *Metarhizium anisopliae* (Metsch.) Sorok. (Deuteromycotina: Hyphomycetes) caused 75% mortality of *S. frugiperda* larvae (Ramos et al. 2020).

In the field, it is difficult to control FAW larvae by topical spraying of EPF because larvae almost hide all day within the leaves midribs (Herlinda et al. 2021). The EPF that enable to colonize whitin plant tissues referred to as

endophytic fungi are urgently needed to control such hiding larvae (Gustianingtyas et al. 2021). The endophytic fungi are able to suppress the insect pest growth (Russo et al. 2020) and can provide beneficial effects to their host plants by stimulating their growth (Lira et al. 2020). *Beauveria bassiana* isolated from plants from South Sumatra that were inoculated by seed treatment could only result 22.67% of the FAW larva mortality (Herlinda et al. 2021). The other previous experiment showed that endophytic *B. bassiana* sprayed on leaves could suppress *S. frugiperda* growth (Russo et al. 2020). The endophytic fungi have negative effects on the developmental time of *S. frugiperda* (Lestari et al. 2022; Sari et al. 2022). However, there is no information about the seed treated effect of the endophytic fungi isolated from soil and infected-host cadavers on the growth of *S. frugiperda*. Further studies should be performed to confirm that the fungus isolated from soil and infected-host corpses is an endophytic entomopathogen. So, the seed treated effect of the endophytic fungi isolated from soil and infected-host cadavers on the growth of *S. frugiperda* need to be evaluated. The aim to this research was to evaluate the seed treated effect of endophytic *B. bassiana* isolated from soil and infected-host cadavers from South Sumatra (Indonesia) on the growth of *S. frugiperda*.

MATERIALS AND METHODS

Preparation of *Spodoptera frugiperda* culture

The eggs and larvae of *S. frugiperda* were obtained from the Laboratory of Entomology, Department of Plant Protection, Faculty of Agriculture, Universitas Sriwijaya. The FAW cultures were mass-reared in the laboratory few years ago (Herlinda et al. 2020) and *S. frugiperda* was identified molecularly (Herlinda et al. 2022a). The FAW was mass-reared in the laboratory according to the methods of Herlinda et al. (2020) with temperature of 28 ± 1°C, 82 ± 1% RH, 12 L:12 D photoperiod. The larvae were kept individually in order to avoid larval cannibalism in plastic cups (Ø 6.5 cm, height 4.6 cm) filled with the fresh maize leaves (2 cm x 5 cm). The leaves were replaced daily with the fresh new ones until they reached the prepupal stage. The prepupae were transferred to a rectangular plastic or PVC container (50 x 50 x 50 cm^3) whose bottom was filled with sterile soil (5 cm in thickness) used for pupal habitat. Within the PVC container, young maize was also put for the adults laying their eggs. The eggs were collected from maize leaves and put in plastic cup (Ø 6.5 cm, height 4.6 cm) until the larvae hatched. Then, the first instar neonate larvae that hatched within 24 hours were used for bioassays.

The ability of endophytic fungal colonization in young maize plant tissue

The three isolates of *B. bassiana* used for bioassay were collected from the Laboratory of Entomology. *B. bassiana* isolates were identified molecularly (Herlinda et al. 2021). The fungal isolates were grouped into species of *B. bassiana* TaBrPGA isolate (GenBank acc. no. OM791682), *B. bassiana* LtApPGA isolate (GenBank acc. no. OM791685), and *B. bassiana* TaTtLH isolate (GenBank acc. no. OM791683) (Table 1). The fungal isolates were originated from soil in Bangun Rejo, Pagar Alam (4°01'28"S 103°13'58"E), Lepidoptera larval cadavers in Air Perikan, Pagaralam (4°01'45"S 103°14'04"E), and soil in Tanjung Tebat, Lahat (3°59'14"S 103°26'22"E), respectively.

To confirm that all fungal isolates were endophytic, they were assessed to colonize maize plant tissue by inoculating the fungi onto maize seeds. The fungal isolates used were grown on SDA medium (sabouraud dextrose agar) and incubated for 2 weeks. -Forty five maize seeds were first surface sterilized (Russo et al. 2020), and then dipped in 10 ml of fungal suspension with a concentration of 1 x 10^8 conidia ml^{-1} for 24 hours, while untreated seeds (control) were submerged with 10 ml of sterilized water. The treated and control seeds were cultured in hydroponic medium (Novianti et al. 2020). To known that fungal isolates have colonized maize plant tissues, leaf tips (1 cm length) were cut from 7 and 14-day-old plants. Then tips were surface-sterilized by dipped them in 70% ethanol, followed by sodium hypochlorite solution and rinsed twice with sterile distilled water (Russo et al. 2020). The tips were grown onto agar medium (SDA). The colonization percentage was resulted from the number of leaf tissue overgrown with fungus divided by the number of leaf tissue observed x 100. The final rinse water was grown onto the agar medium, if no fungal mycelia were found, indicated that surface-sterilization effectively killed epiphytic microorganisms. So, the fungus found on agar medium was endophytic. The rest maize plant leaves were given to the first instar neonate larvae (hatching within 24 hours) of *S. frugiperda* for bioassays.

Bioassay for evaluating the growth of *Spodoptera frugiperda* fed on maize leaves colonized by *Beauveria bassiana*

The bioassay for evaluating the growth of *S. frugiperda* fed on maize leaves colonized by *Beauveria bassiana* was carried out at the Laboratory of Entomology, Department of Plant Protection, Faculty of Agriculture, Universitas Sriwijaya and mean of temperature and RH during experiment was 28.12°C and 82.75%, respectively.

Table 1. Isolates of endophytic-entomopathogenic fungi from South Sumatra, Indonesia

Location (village, district/city)	Isolate origin	Altitude (m)	Fungal species	Fungal isolates code	GenBank acc. no.	References
Bangun Rejo, Pagar Alam	Soil	789.5	*Beauveria bassiana*	TaBrPGA	OM791682	Ramayanti et al. (2022)
Air Perikan, Pagaralam	Lepidoptera	625.9	*Beauveria bassiana*	LtApPGA	OM791685	Ramayanti et al. (2022)
Tanjung Tebat, Lahat	Soil	377.0	*Beauveria bassiana*	TaTtLH	OM791683	Ramayanti et al. (2022)

The maize leaves used were obtained from maize seedling colonized with endophytic *B. bassiana* via seed treatment. The endophytic colonized leaves were provided to first instar neonate larvae of *S. frugiperda*, while non-treated leaves were given to control larvae. A hundred neonate larvae were provided with 15 treated leaves and untreated leaves as control for 12 hours or until the leaves were eaten. Then, larvae were transferred to a porous plastic cup (Ø 6.5 cm) containing fresh non-treated leaves (2 cm x 5 cm) and individually maintained. The non-treated leaves were replaced daily. The research was completely randomized block design with fungal isolates as treatments and repeated three times.

The variables observed were fungal colonization, larval weight and fecal weight of each instar. The leaf area eaten, length of larval body, mortality of larvae were recorded daily from the first instar up to the last instar. The pupae and adult emergence were also monitored daily and the sex of adult emergence of *S. frugiperda* was recorded daily. The morphology of unhatched eggs, the unhealthy larvae and pupae, and the dead larvae and pupae were observed every day. The behavior of the unhealthy larvae was also monitored day by day.

Data analysis

The differences in larval weight and fecal weight of each instar, the leaf area eaten, length of larval body and the mortality of each stage, the pupae and adult emergence, and the sex ratio of *S. frugiperda* from each treatment were analyzed using analysis of variance (ANOVA). Original data were transformed using Arcsin transformation or Square Root transformation prior to statistical analysis. Tukey's test or Tukey's Honestly Significant Difference (HSD) test or was applied to determine the significant differences among the isolates at p = 0.05. All data were calculated using software of SAS University Edition 2.7 9.4 M5.

RESULTS AND DISCUSSION

Endophytic fungal colonization in young maize tissue

All *B. bassiana* isolates (TaBrPGA, LtApPGA, and TaTtLH) were confirmed as fungal endophytes in maize plant. The mycelia of three fungal isolates were able to colonize within treated maize tissue, whereas mycelia were not found within untreated maize tisssue (control). All *B. bassiana* isolates could colonize the leaves of treated maize plant when the leaves were cultured on the SDA medium. All leaves of treated maize plant were overgrown and covered by fungus (Figure 1), but fungal mycelia were not found on the final flushing water and on leaves of control maize. The percentage of *B. bassiana* colonization within the leaves began to increase from 7 to 14 days after seed immersion treatment occurred. The percentage of fungal colonization among *B. bassiana* isolates (TaBrPGA, LtApPGA, and TaTtLH isolates) (80-100%) were not found significant (Table 2). However, significant difference were found between the *B. bassiana* isolates and control. The result confirmed that *B. bassiana* isolates (TaBrPGA, LtApPGA, and TaTtLH) isolated from soil and infected-host cadavers were an endophytic entomopathogenic fungus.

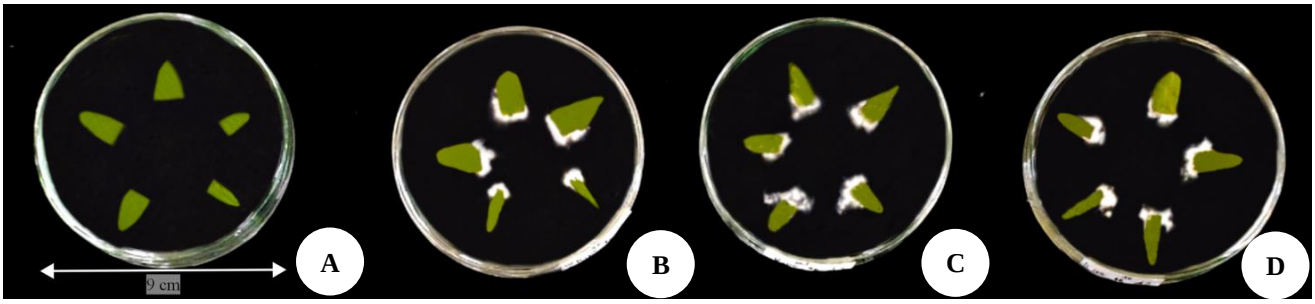

Figure 1. Colony morphology of endophytic fungi from the leaves of maize: Control (A), *Beauveria bassiana* of TaBrPGA isolate (B), LtApPGA (C), and TaTtLH isolates (D)

Table 2. Mean colonization (%) of fungi within leaves treated with endophytic-entomopathogenic *Beauveria bassiana* at 7 and 14 days after inoculation

Isolates	Species	Mean colonization (%)	
		7 days after inoculation	14 days after inoculation
Control	-	0.00d	0.00b
TaBrPGA	*Beauveria bassiana*	46.67c	80.00a
LtApPGA	*Beauveria bassiana*	73.33b	93.33a
TaTtLH	*Beauveria bassiana*	100.00a	100.00a
F-value		127.83*	40.66*
P-value		7.95×10^{-6}	2.21×10^{-4}
HSD value		16.13	31.06

Note: *: significantly different; values within a column followed by the same letters were not significantly different at P < 0.05 according to Tukey's HSD test

Effect of fungal colonization maize on *S. frugiperda* growth

The first instar neonate larvae (hatching within 24 hours) were fed on *B. bassiana* colonized maize leaves, which caused the 2nd, 3rd, 4th, 5th, and 6th larvae significantly reduce their leaf area eaten. However, leaf area eaten by 1st *S. frugiperda* larvae fed on young maize colonized with TaBrPGA, LtApPGA, and TaTtLH isolates was not significantly different from those of control. Leaf area eaten by the 2nd, 3rd, 4th, 5th, and 6th larvae of *S. frugiperda* larvae fed on young maize colonized with the fungal isolates decreased significantly compared to those eaten by untreated or control *S. frugiperda* larvae (P<0.0001) (Table 3). The results showed that all *B. bassiana* isolates reduced *S. frugiperda* larvae's appetite.

Fecal weight of *S. frugiperda* larvae fed on *B. bassiana* colonized maize leaves significantly reduced. However, at the 2nd and 3rd larvae, all isolates of *B. bassiana* did not decreased larvae fecal weight. Fecal weight of 4th, 5th, and 6th larvae was significantly lighter than that of the control (P<0.0001) (Table 4), but fecal weight of those treated larvae was not significantly different among isolates. All isolates of *B. bassiana* have the ability to reduced fecal weight of *S. frugiperda* larvae.

The first instar neonate larvae treated with *B. bassiana* had lower weight. In the 1st and 2nd larvae, all isolates of *B. bassiana* caused significantly lower larvae weight than untreated ones (control), but the effect among isolates was not significantly different. The older the larvae were, the more significant the difference in effect among isolates was found. The lightest larvae weight was found on larvae fed on leaves colonized by TaBrPGA and TaTtLH isolates and was significantly different from those colonized by LtApPGA isolates and control. However, all isolates of *B. bassiana* could significantly decline larvae weight of *S. frugiperda* (P<0.0001) (Table 5). In addition to reducing larvae weight, *B. bassiana* also reduced larvae length of *S. frugiperda*. The length of larvae fed on corn leaves inoculated with the fungus was shorter than control ones that ate non-colonized maize leaves (P<0.0001) (Table 6).

The first instar neonate larvae fed on *B. bassiana* colonized maize leaves could increase the mortality of all instar larvae significantly compared to mortality of larvae fed on non-colonized maize leaves (control). The mortality of larvae treated with TaTtLH isolate of *B. bassiana* was the highest among other isolates. The cumulative mortality at last (6th) larvae could reach 73% found on TaTtLH isolate treatment and the mortality was significantly higher than those of TaBrPGA and LtApPGA isolates (P<0.0001) (Table 7). Based on the cumulative mortality occurred at the last larvae, TaTtLH isolate was the most pathogenic isolates among other isolates of *B. bassiana*.

The first instar neonate larvae fed on *B. bassiana* colonized maize leaves could produce reduction of pupae and adult emergence of *S. frugiperda* significantly compared to those fed on non-colonized maize leaves (control). The lowest percentage of pupae and adult emergence was caused by TaTtLH isolate (P<0.0001) (Table 8). However, all isolates of *B. bassiana* could significantly reduce the percentage of pupae and adult emergence of *S. frugiperda* compared to the control. The neonate larvae fed on *B. bassiana* colonized maize leaves did not significantly influence the sex ratio of *S. frugiperda* adults.

Table 3. Leaf area eaten by *Spodoptera frugiperda* larvae fed on young maize colonized with TaBrPGA, LtApPGA, and TaTtLH isolates of *Beauveria bassiana*

Isolates	Species	Mean of leaf area eaten by larvae ($cm^2 larvae^{-1} day^{-1}$)					
		1st larvae	2nd larvae	3rd larvae	4th larvae	5th larvae	6th larvae
Control	-	3.63	8.48a	11.01a	12.15a	12.76a	9.68a
TaBrPGA	*Beauveria bassiana*	2.73	8.11b	9.54a	10.10b	11.18a	7.64b
LtApPGA	*Beauveria bassiana*	2.64	5.94b	7.59b	10.29b	11.15a	6.71b
TaTtLH	*Beauveria bassiana*	2.54	5.13b	6.85b	10.24b	7.05b	4.35c
F-value		1.62ns	55.72*	43.57*	8.17*	31.97*	60.20*
P-value		0.28	8.98×10^{-5}	1.82×10^{-4}	0.02	4.37×10^{-4}	7.18×10^{-5}
HSD value		-	0.19	0.23	0.24	0.33	0.26

Note: ns: not significantly different; *: significantly different; values within a column followed by the same letters were not significantly different at P < 0.05 according to Tukey's HSD test.

Table 4. Fecal weight of *Spodoptera frugiperda* larvae fed on young maize colonized with TaBrPGA, LtApPGA, and TaTtLH isolates of *Beauveria bassiana*

Isolates	Species	Mean of larva fecal weight ($mg\ larvae^{-1}\ day^{-1}$)					
		1st larvae	2nd larvae	3rd larvae	4th larvae	5th larvae	6th larvae
Control	-	0.17ab	2.39	8.07	18.97a	31.73a	36.04a
TaBrPGA	*Beauveria bassiana*	0.18a	1.05	5.65	16.35ab	25.11b	29.51b
LtApPGA	*Beauveria bassiana*	0.13ab	0.94	5.60	13.93ab	24.98b	27.99b
TaTtLH	*Beauveria bassiana*	0.11b	0.87	4.71	10.87b	20.75b	26.57b
F-value		6.91*	1.85ns	4.04ns	7.54*	15.66*	29.65*
P-value		0.02	0.23	0.06	0.02	3.04×10^{-3}	5.39×10^{-4}
HSD value		0.03	-	-	0.79	0.54	0.33

Note: *: significantly different; values within a column followed by the same letters were not significantly different at P < 0.05 according to Tukey's HSD test.

Table 5. Weight of *Spodoptera frugiperda* larvae fed on young maize colonized with TaBrPGA, LtApPGA, and TaTtLH isolates of *Beauveria bassiana*

Isolates	Species	Mean of larvae weight (mg larvae^{-1})					
		1st larvae	2nd larvae	3rd larvae	4th larvae	5th larvae	6th larvae
Control	-	7.83a	18.34a	38.26a	79.34a	186.30a	262.61a
TaBrPGA	*Beauveria bassiana*	5.12b	11.90b	21.39b	38.78b	58.23c	88.36c
LtApPGA	*Beauveria bassiana*	4.83b	10.50b	17.21bc	31.59bc	68.52b	94.55b
TaTtLH	*Beauveria bassiana*	4.04b	10.04b	13.99c	27.09c	53.11c	83.11c
F-value		17.13*	79.45*	112.42*	174.48*	1470.716*	1470.72*
P-value		2.40×10^{-3}	3.21×10^{-5}	1.16×10^{-5}	3.17×10^{-6}	5.48×10^{-9}	5.48×10^{-9}
HSD value		0.38	0.27	0.49	0.61	0.38	0.38

Note: *: significantly different; values within a column followed by the same letters were not significantly different at $P < 0.05$ according to Tukey's HSD test.

Table 6. Length of *Spodoptera frugiperda* larvae fed on young maize colonized with TaBrPGA, LtApPGA, and TaTtLHisolates of *Beauveria bassiana*

Isolates	Species	Mean of larvae length (mm)					
		1st larvae	2nd larvae	3rd larvae	4th larvae	5th larvae	6th larvae
Control	-	6.43a	14.97a	36.89a	77.97a	184.92a	261.24a
TaBrPGA	*Beauveria bassiana*	4.00b	8.73b	20.01b	38.99b	61.64b	93.18b
LtApPGA	*Beauveria bassiana*	3.77b	7.40b	15.24bc	31.42c	62.95b	86.99c
TaTtLH	*Beauveria bassiana*	2.97b	6.86b	12.46c	28.07c	51.73c	80.63d
F-value		18.02*	68.47*	73.57*	174.29*	564.82*	291.05*
P-value		0.02	4.95×10^{-5}	4.01×10^{-5}	3.18×10^{-6}	96.00×10^{-8}	7.06×10^{-10}
HSD value		0.37	0.32	0.63	0.59	0.61	0.31

Note: *: significantly different; values within a column followed by the same letters were not significantly different at $P < 0.05$ according to Tukey's HSD test.

Table 7. Cumulative mortality of *Spodoptera frugiperda* larvae fed on young maize colonized with TaBrPGA, LtApPGA, and TaTtLH isolates of *Beauveria bassiana*

Isolates	Species	Mean of larvae cumulative mortality (%)					
		1st larvae	2nd larvae	3rd larvae	4th larvae	5th larvae	6th larvae
Control	-	4.33c	6.00d	6.33d	6.33c	6.33d	6.33d
TaBrPGA	*Beauveria bassiana*	17.67ab	22.33c	30.00c	35.33b	39.33c	43.67c
LtApPGA	*Beauveria bassiana*	16.67b	33.67b	41.33b	44.00b	47.67b	52.00b
TaTtLH	*Beauveria bassiana*	24.33a	56.00a	68.67a	70.00a	71.33a	73.00a
F-value		54.66*	131.01*	212.84*	200.52*	390.43*	668.96*
P-value		9.49×10^{-5}	7.39×10^{-6}	1.76×10^{-6}	2.10×10^{-6}	2.89×10^{-7}	5.79×10^{-8}
HSD value		0.27	0.34	0.31	0.33	0.24	0.19

Note: *: significantly different; values within a column followed by the same letters were not significantly different at $P < 0.05$ according to Tukey's HSD test.

Table 8. Mean of percentage of pupae and adult emergence, and adult sex ratio of *Spodoptera frugiperda* fed on young maize treated with TaBrPGA, LtApPGA and TaTtLH isolates of *Beauveria bassiana*

Isolates	Fungal species	Pupae emergence (%)	Adult emergence (%)	Sex ratio of adults
Control	-	93.67a	93.67a	0.75
TaBrPGA	*Beauveria bassiana*	53.00b	49.33b	0.86
LtApPGA	*Beauveria bassiana*	44.33c	39.33c	0.71
TaTtLH	*Beauveria bassiana*	21.67d	14.00d	0.59
F-value		1172.60*	766.09*	3.028ns
P-value		1.08×10^{-8}	3.86×10^{-8}	0.115
HSD value		0.16	0.22	-

Note: ns: not significantly different; *: significantly different; values within a column followed by the same letters were not significantly different at $P < 0.05$ according to Tukey's HSD test.

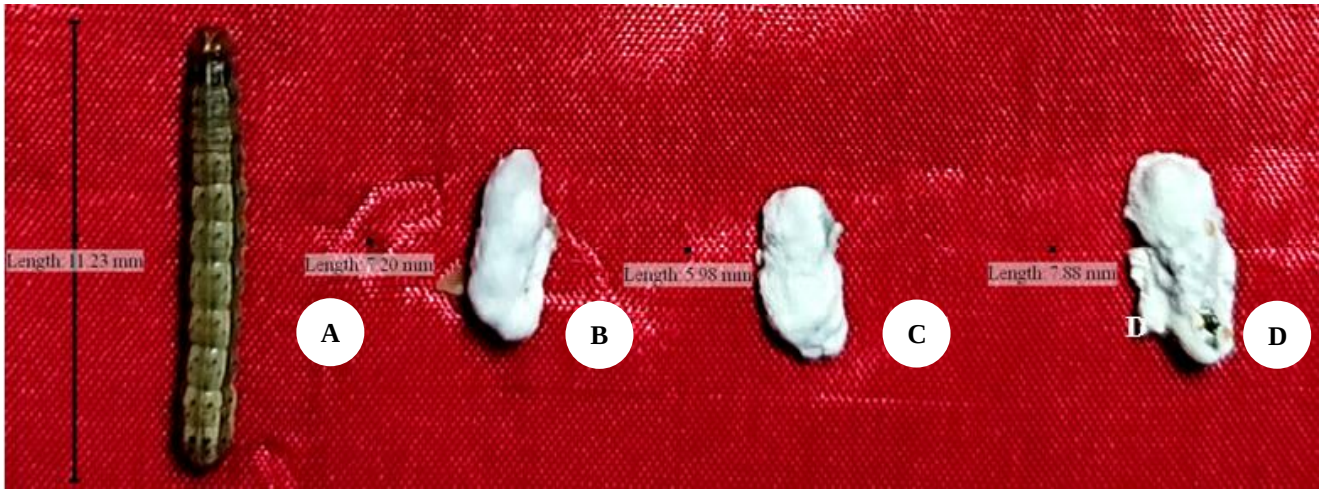

Figure 2. The cadavers from larvae fed on maize leaves uncolonized with fungi or control (A), and cadavers from larvae fed on maize leaves colonized by *Beauveria bassiana* of TaBrPGA isolate (B), LtApPGA (C), and TaTtLH isolates (D)

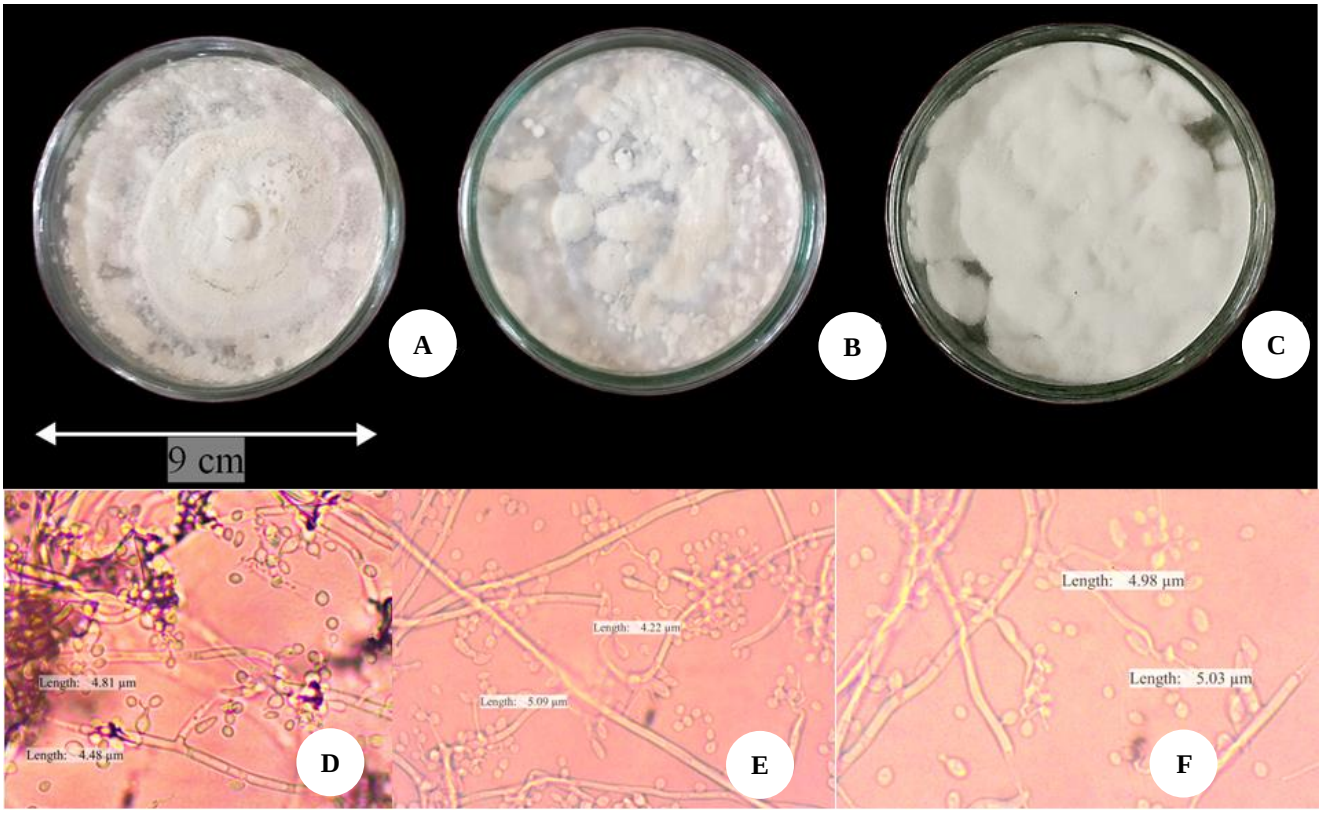

Figure 3. Colony morphology of endophytic fungi on SDA media (*above*) isolated from cadavers, and conidial and hyphal morphology (*below*) of fungi: *Beauveria bassiana* of TaBrPGA isolate (A and D), LtApPGA (B and E), and TaTtLH isolate (C and F)

Symptoms of mycosis occurred on larvae, pupae, and adults of *Spodoptera frugiperda*

The first instar neonate larvae of *S. frugiperda* fed on *B. bassiana* colonized maize leaves showed mycosis on their larvae, pupae, and the adults. Mycosis on infected larvae began with a change in larvae color and behavior. The infected larvae were less active and their appetite decreased as evidenced by the lower leaf area eaten compared to the control (Table 3). Infected and sick larvae had unique symptoms, such as smaller size, and their color became darker than healthy ones. About 4-6 days after feeding on colonized maize leaves, larvae began to die. The cadaver of infected larvae became shrunken, hardened and mummified. When cadaver was grown onto water-agar medium, after being incubated for 4-6 days, the white mycelia of *B. bassiana* began to emerge from the cadaver body. The mycelia completely cover the cadaver body after incubated for more than 10 days (Figure 2). Conidia

isolated from the cadaver were grown on SDA medium, the grown fungus showed the same morphology as the fungal isolate used for corn seed treatment. The colony morphology of each fungal isolate from the cadaver re-isolation had white color (Figure 3). Hyphae of all isolates had hyaline color, and conidia were globose. The result revealed that larvae cadaver produced *B. bassiana* conidia and mycelia. So, the larva was able to cause mycosis.

Some of the infected larvae that survived could became abnormal larvae, and some of them got into pupae and adult stage (Table 8). The abnormal larvae had smaller and shrinkage body with darker color than the healthy ones (Figure 4). The abnormal larvae had harder integument than healthy larvae. The survival infected larvae that reached pupae stage could also produce abnormal pupae. The symptoms of abnormal pupae were similar to abnormal larvae with smaller and shrinken bodies and darker color compared to the healthy pupae (Figure 5). The infected pupae that were survival could produce the abnomal adults. Abnormal adults had smaller and malformed body with folded wings, so that adults were unable to fly (Figure 6).

Discussion

The present study confirmed that endophytic *B. bassiana* isolates (TaBrPGA, LtApPGA, and TaTtLH) isolated from soil and infected-host cadavers of Lepidoptera were the endophytic entomopathogenic fungus. All isolates of *B. bassiana* could colonize maize corn plant tissue. The ability of *B. bassiana* isolates to colonize the young maize leaves via seed treatment ranged 80-100% after 14 days inoculation. The obtained result revealed that *B. bassiana* isolated from soil and infected-host cadavers confirm as a fungal endophyte. *B. bassiana* isolated from *S. frugiperda* larvae was reported as a fungal endophyte (Sari et al. 2022). Endophytic *B. bassiana*, and *M. anisopliae* isolated from maize and red pepper, respectively were able to colonize 100% in maize leaves when inoculated by seed treatment, and at 14 days after fungal seed treatment (Lestari et al. 2022). Endophytic *B. bassiana* and *M. anisopliae* could be inoculated by foliar spray and root dipping to colonize plant leaves, stem, or roots (Russo et al. 2020). The existence of endophytic fungi in plant tissues could be more than 14 days and found within leaves of tomatoes up to 30 days after inoculation (Silva et al. 2020). In the present study, all isolates of *B. bassiana* could colonize the young maize leaf tissue (80-100%). The ability of fungus to colonize young maize could increase the young plant's defense against *S. frugiperda* larvae (Lestari et al. 2022). The young corn plant (vegetative stage) is most susceptible to *S. frugiperda* larvae (Supartha et al. 2021), so colonized young maize is beneficial for early prevention from *S. frugiperda* larvae (Lestari et al. 2022). Furthermore, *S. frugiperda* larvae that always hide within the corn midribs are effectively controlled by using the endophytic fungi (Sari et al. 2022).

The leaf area eaten by 2nd, 3rd, 4th, 5th, and 6 larvae of *S. frugiperda* fed on young maize colonized with the *B. bassiana* isolates decreased significantly. So, all *B. bassiana* isolates reduced *S. frugiperda* larvae's appetite. The fecal weight of *S. frugiperda* larvae treated with all isolates of *B. bassiana* also decreased significantly. The reduction in leaf area eaten by larvae of *S. frugiperda* fed on *B. bassiana* colonized maize leaves resulted in a significant decline in the weight and body length of all instar larvae. So, all isolates of *B. bassiana* caused negative effects on the growth of *S. frugiperda* larvae.

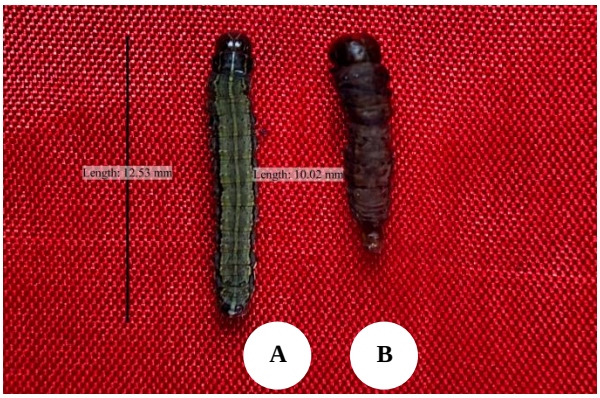

Figure 4. Larval *Spodoptera frugiperda*: healthy larvae (A) and larvae infected by endophytic *Beauveria bassiana* (B)

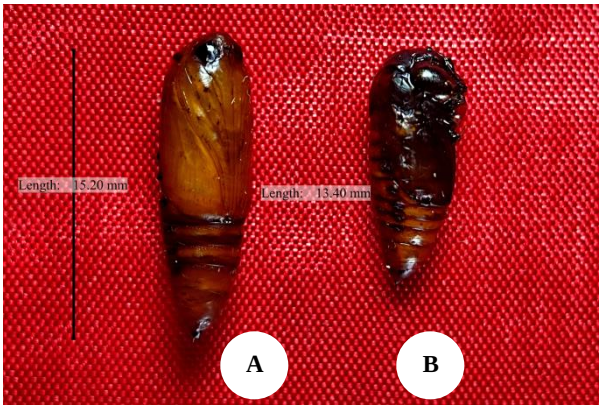

Figure 5. Pupal *Spodoptera frugiperda*: healthy pupae (A) and pupae infected by endophytic *Beauveria bassiana* (B)

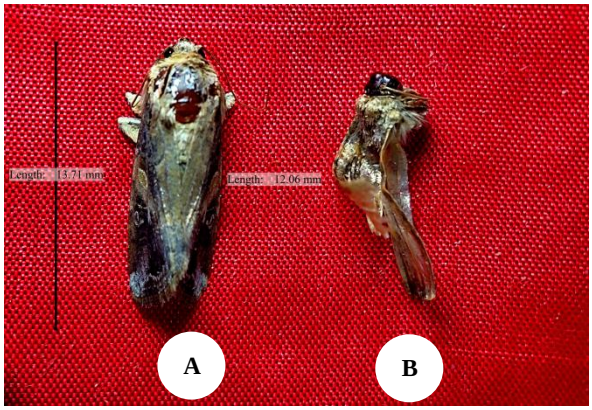

Figure 6. *Spodoptera frugiperda* adults: healthy adult (A) and adult infected by endophytic *Beauveria bassiana* (B)

Furthermore, reduction in leaf area eaten by *S. frugiperda* larvae induced the increase of larvae mortality. All *B. bassiana* isolates significantly increased the mortality of all instar larvae of *S. frugiperda*. The highest mortality of the 6th larvae reached 73% of mortality induced by TaTtLH isolate treatment. The previous study showed that endophytic *B. bassiana* with conidial suspension of 1×10^6 conidia mL^{-1} could kill only 29.33% of the *S. frugiperda* larvae mortality (Gustianingtyas et al. 2021). This present study was successful in increasing the mortality (73%) of *S. frugiperda* larvae by increasing conidial suspension (1×10^8 conidia mL^{-1}) of endophytic *B. bassiana*. The commercial strains of *B. bassiana* Bb-18 at 1×10^8 conidia mL^{-1} could kill 87% of *S. frugiperda* larvae, however fungus was applied using the soil drench method not by seed treatment (Ramos et al. 2020). The seed treatment method is more beneficial for applying endophytic *B. bassiana* is more advantageous because the fungus could protect plants from the time the corn seeds are stored seed.

In addition to killing the larvae of *S. frugiperda*, endophytic *B. bassiana* could kill pupae and adults of *S. frugiperda*, and decrease the pupae and adult emergence. Some infected pupae and adults emerged were abnormal. The obtained data showed that first instar neonate larvae fed on *B. bassiana* colonized maize leaves could induce 14% of adult emergence. So, endophytic *B. bassiana* could decrease 86% of adult emergence. From 14% of adult emergence, some of them had deformed wings or folded wings. The deformed wings or folded wings could make the adults unable to copulate so that their population will decrease on the next generation. The previous research found that endophytic *B. bassiana* could retard the adult emergence more than 50% (Lestari et al. 2022; Sari et al. 2022). The endophytic *B. bassiana* in seed treatment could retard the growth of *S. frugiperda* larvae and adult reproduction and survival (Russo et al. 2020). The adverse effects of endophytic fungi on growth of *S. frugiperda* began with the reduction of leaf area consumed by the larvae of *S. frugiperda. Spodoptera frugiperda* larvae's appetite decrease due to secondary metabolites and toxic protein or toxins produced by the fungal blastospores (Vidal and Jaber 2015). The toxins secreted by blastospores of *B. bassiana* were bassiacridin (Quesada-moraga and Vey 2004) and beauvericin (Safavi 2012). The blastospores in the larvae hemolymph were produced from the mycelia of endophytic fungi within maize tissue that were consumed by the larvae of *S. frugiperda*. The maize colonized with *B. bassiana* could also produce deterrent properties and terpenoid *in planta* (within plant) (Russo et al. 2020). The toxins secreted by blastospores are toxic to larvae (Mancillas-Paredes et al. 2019). The secondary metabolites *in planta* are also toxic and resulted antibiosis and feeding deterrence for larvae and could kill the insects (Jaber and Ownley 2018). If the larvae died, the endophytic fungus began to grow saprophytically by living on dead body (cadaver) of larvae (Vidal and Jaber 2015). The present study found that mycosis occurred on larvae of *S. frugiperda* consuming the *B. bassiana* colonized leaves. No mycosis was found on the larvae consuming uncolonized leaves. The *S. frugiperda* larvae fed on maize leaves colonized by endophytic fungi underwent mycosis (Lestari et al. 2022; Sari et al. 2022).

Finally, the present research confirmed that isolates of *B. bassiana* isolated from soil and infected-host cadavers of Lepidoptera were the endophytic entomopathogenic fungus. All isolates (TaBrPGA, LtApPGA, and TaTtLH) of endophytic *B. bassiana* caused negative effect on the growth of *S. frugiperda* larvae. Endophytic *B. bassiana* applied in seed treatment could protect since the stored corn seeds and the young maize plant against *S. frugiperda*. So, *B. bassiana* could be recommended to protect the stored corn seeds.

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