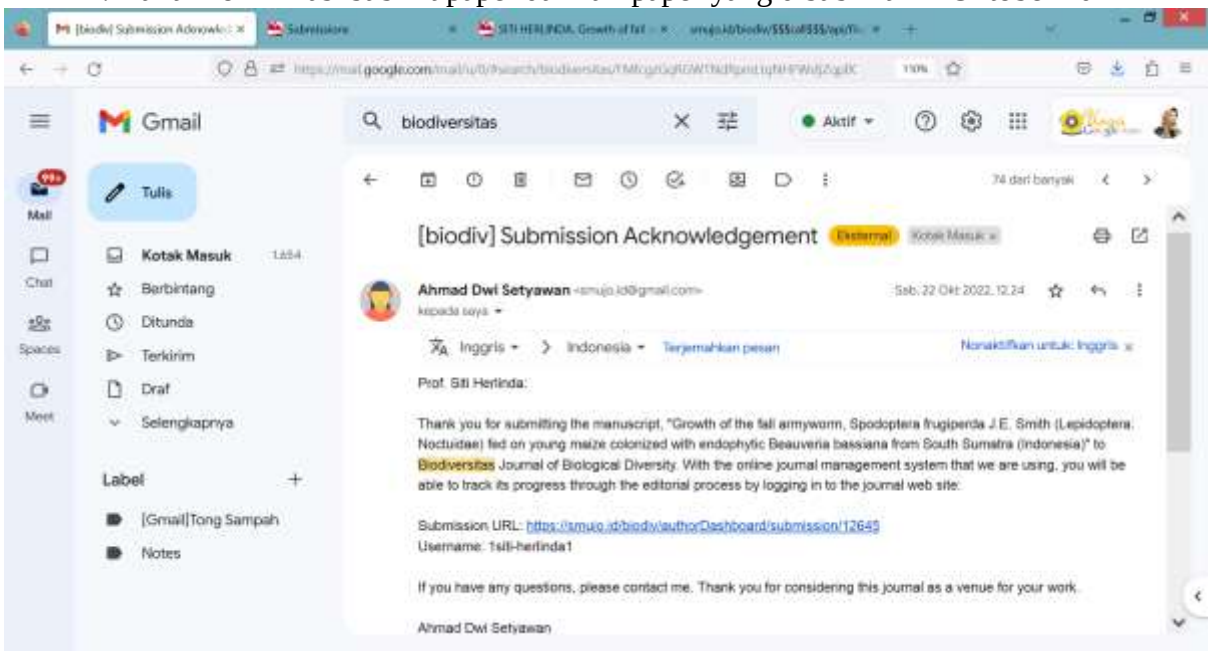
BUKTI KORESPONDENSI
ARTIKEL JURNAL INTERNASIONAL BEREPUTASI

Judul artikel : 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
Jurnal : BIODIVERSITAS
Penulis: Detya Rosa Faddilah, Marieska Verawaty, Siti Herlinda

Bukti korespondensi

No.	Perihal	Tanggal
1.	Bukti Konfirmasi submit paper dan full paper yang disubmit	22 Oktober 2022
2.	Bukti konfirmasi review pertama dan hasil revisi pertama	12 Desember 2022
3.	Bukti konfirmasi paper accepted, uncorrected Proof dan hasil koreksi penulis	22 Desember 2022
4	Bukti tagihan untuk penerbitan artikel	22 Desember 2022

1. Bukti Konfirmasi submit paper dan full paper yang disubmit 22 Oktober 2022



COVERING LETTER

Dear **Editor-in-Chief,**

I herewith enclosed a research article,

- [x] The submission has not been previously published, nor is it before another journal for consideration (or an explanation has been provided in Comments to the Editor).

- [x] The submission file is in OpenOffice, Microsoft Word (DOC, not DOCX), or RTF document file format.

- [x] The text is single-spaced; uses a 10-point font; employs italics, rather than underlining (except with URL addresses); and all illustrations, figures, and tables are placed within the text at the appropriate points, rather than at the end.

- [x] The text adheres to the stylistic and bibliographic requirements outlined in the Author Guidelines.

- [x] Most of the references come from current scientific journals (c. 80% published in the last 10 years), except for taxonomic papers.

- [x] Where available, DOIs for the references have been provided.

- [x] When available, a certificate for proofreading is included.

SUBMISSION CHECKLIST

Ensure that the following items are present:

The first corresponding author must be accompanied with contact details:

- [x] E-mail address
- [x] Full postal address (incl street name and number (location), city, postal code, state/province, country)
- [x] Phone and facsimile numbers (incl country phone code)

All necessary files have been uploaded, and contain:

- [x] Keywords
- [x] Running titles
- [x] All figure captions
- [x] All tables (incl title and note/description)

Further considerations

- [x] Manuscript has been "spell & grammar-checked" Better, if it is revised by a professional science editor or a native English speaker
- [x] References are in the correct format for this journal
- [x] All references mentioned in the Reference list are cited in the text, and vice versa
- [x] Colored figures are only used if the information in the text may be losing without those images
- [x] Charts (graphs and diagrams) are drawn in black and white images; use shading to differentiate

Title:

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

Author(s) name:

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

Address

(Fill in your institution's name and address, your personal cellular phone and email)

[1]Graduate Program of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya. Palembang 30139, South Sumatera, Indonesia.
[2]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya, Indralaya 30662, South Sumatra, Indonesia
[3]Department of Plant Protection, Faculty of Agriculture, Universitas Sriwijaya, Indralaya 30662, South Sumatra, Indonesia. Tel. +62-0711-580059 Fax. +62-0711-580276, ♥email: sitiherlinda@unsri.ac.id
[4]Research Center for Sub-optimal Lands (PUR-PLSO), Universitas Sriwijaya, Palembang 30139, South Sumatera, Indonesia.

For possibility publication on the journal:

(fill in *Biodiversitas* or *Nusantara Bioscience* or *mention the others*)

- [x] Biodiversitas Journal of Biological Diversity
- [ ] Nusantara Bioscience
- [ ] Prosiding Seminar Nasional Masyarakat Biodiversitas Indonesia
- [ ] Asian Journal of Agriculture
- [ ] Asian Journal of Ethnobiology
- [ ] Asian Journal of Forestry
- [ ] Asian Journal of Natural Product Biochemistry
- [ ] Asian Journal of Tropical Biotechnology
- [ ] International Journal of Bonorowo Wetlands
- [ ] Cell Biology and Development
- [ ] Indo Pacific Journal of Ocean Life
- [ ] International Journal of Tropical Drylands

Novelty:

(state your claimed novelty of the findings versus current knowledge)

Novelty of the finding that the *Beauveria bassiana* isolates (TaBrPGA, LtApPGA, and TaTtLH) isolated from soil and infected-host cadavers of Lepidoptera could be corn endophytes. The endophytic *B. bassiana* in seed treatment could retard the growth of *S. frugiperda* larvae, pupae, and adults. The endophytic *B. bassiana* could kill the pupae and adults of *S. frugiperda*, and decrease the pupae and adult emergence.

Statements:

This manuscript has not been published and is not under consideration for publication to any other journal or any other type of publication (including web hosting) either by me or any of my co-authors.
Author(s) has been read and agree to the Ethical Guidelines.

List of five potential reviewers
(Fill in names of five potential reviewers **that agree to review your manuscpt** and their **email** addresses. He/she should have Scopus ID and come from different institution with the authors; and from at least three different countries)

1. Prof. I Wayan Supartha, Faculty of Agriculture, Universitas Udayana, Indonesia email: yansupartha@yahoo.com
2. Prof. Shahabuddin Saleh, Faculty of Agriculture, Tadulako University, Indonesia shahabsaleh@gmail.com
3. Prof. Itji Diana Daud, Faculty of Agriculture, Universitas Hasanuddin, Indonesia, email: itfir@yahoo.com
4. Prof. Abu Hassan Ahmad, School of Biological Sciences, Universiti Sains Malaysia, Malaysia, e-mail: stegoculex@gmail.com
5. Prof. Ouiza Ourrad, Faculty of Biological Sciences and Agronomical Sciences, Mouloud Mammeri University, Algeria, email: ouiza.ourrad@ummto.dz

Place and date:

21 October 2022

Sincerely yours,
(fill in your name, no need scanned autograph)

Siti Herlinda

Growth of the fall armyworm, *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae) fed on young maize colonized with endophytic *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. Palembang 30139, South Sumatera, Indonesia.

[2]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya. Indralaya 30662, South Sumatra, Indonesia

[3]Department of Plant Protection, Faculty of Agriculture, Universitas Sriwijaya. Indralaya 30662, South Sumatra, Indonesia.

Tel./fax. +62-711-580059, ♥email: sitiherlinda@unsri.ac.id

[4]Research Center for Sub-optimal Lands (PUR-PLSO), Universitas Sriwijaya, Palembang 30139, South Sumatera, Indonesia.

Manuscript received: 21 October 2022. Revision accepted: .....

Abstract. There is no information about the seed treated effect of the endophytic fungi isolated from soil and infected-host cadavers on the growth of *Spodoptera frugiperda*. The research aimed to evaluate the the seed treated effect of the 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 *B. bassiana* isolates isolated from soil and infected-host cadavers of Lepidoptera were the endophytic entomopathogenic fungus. All isolates of the endophytic *B. bassiana* caused negative effects on the growth of *S. frugiperda* larvae. TaTtLH isolate of *B. bassiana* was the most pathogenic isolates (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. *B. bassiana* could decrease 86% of adult emergence. *B. bassiana* applied in seed treatment could protect since the stored corn seeds and the young maize plant against *S. frugiperda*. So, the endophytic *B. bassiana* could be recommended to protect the stored corn seeds.

Key words: endophytes, entomopathogen, neonate larvae, seed treatment, *Zea mays*

Abbreviations (if any): -

Running title: Growth of *Spodoptera frugiperda* with endophytic *Beauveria bassiana*

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 whole 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 enter Africa in 2016 (Goergen et al., 2016). 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, the 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 millions per year in Africa (Harrison et al., 2019). In Indonesia, the FAW severely attacked corn and caused damage reaching 100% (Herlinda et al., 2022; Mukkun et al., 2021). The FAW larvae destructs corn or other crops by eating young leaves, stems, flowers, fruits, and growing points (Montezano et al., 2018; Herlinda et al., 2022). In the morning, the larvae 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).

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

In the field, it is difficult to control the FAW larvae by topical spraying of the EPF because the 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 the 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). The endophytic fungal *B. bassiana* isolated from plants from South Sumatra that was inoculated by seed treatment could only result 22.67% of the FAW larva mortality (Herlinda et al., 2021). The other previous experiment showed that the 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*. Furthermore, to confirm that the fungi isolated from soil and infected-host cadavers are the endophytic entomopathogens needs to be investigated. 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 research aimed to evaluate the the seed treated effect of the 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 have been mass-reared in the laboratory few years ago (Herlinda et al., 2020) and the species of *S. frugiperda* cultured was identified molecularly (Herlinda et al., 2022). The FAW were 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 given sterile soil (5 cm in thickness) used for pupal habitat. Within the PVC container, the 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. oviposition

The ability of endophytic fungal colonization in the young maize plant tissue

The three isolates of *B. bassiana* used for current bioassay were from the collection of the Laboratory of Entomology. B. bassiana isolates were identified molecularly (Herlinda et al., 2021). The fungal isolates were group in 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 the maize seeds. The fungal isolates used were grown on medium of SDA (sabouraud dextrose agar) and incubated for 2 weeks. The 45 maize seeds were first surface sterilized (Russo et al., 2020), and then they were dipped in 10 ml of fungal suspension for 24 hours with concentration of 1 x 10^8 conidia ml^{-1} while the untreated seeds (control) were submerged with 10 ml of sterilized water. The treated and control seeds were cultured in the hydroponic medium (Novianti et al., 2020). To detect that the fungal isolates have colonized the maize plant tissues, the tip leaves of 7 and 14-day-old plants were cut (1 cm length). Then the tip leaves were surface-sterilized by dipping them in 70% ethanol and sodium hypochlorite rinsed twice in sterile distilled water (Russo et al., 2020). The tip leaves were grown onto agar medium (SDA) to stimulate mycelia to grow out. The final rinse water was grown onto the agar medium, if no fungal mycelia was found, it indicated that the surface-sterilization effectively eradicated the epiphytic microorganisms. So, the fungus found on the agar medium was endophytic fungus or endophyte. The rest maize plant leaves were given to the first instar neonate larvae (hatching within 24 hours) of *S. frugiperda* for bioassays below.

Table 1. Isolates of endophytic-entomopathogenic fungi from South Sumatra, Indonesia used in this research

Location (village, district/city)	Isolate origin	Altitude (m)	Fungal species	Fungal isolate 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 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.

The maize leaves used in current research were obtained from maize seedling colonized with endophytic *B. bassiana* via seed treatment. The leaves colonized by the endophytic fungus were provided to the first instar neonate larvae of *S. frugiperda* while non-treated leaves were given as feed by the larvae of control. A hundred neonate larvae were provided with 15 treated leaves or untreated ones for control for 12 hours or until the leaves were eaten. Then, the 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. This research used a completely randomized block design with the 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* were recorded daily. The morphology of the unhatched eggs, the unhealthy larvae and pupae, and the dead larvae and pupae were observed every day. The behavior of the unhealthy larvae were 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. frugiperga* from each treatment were analyzed using analysis of variance (ANOVA). 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

The endophytic fungal colonization in the young maize tissue

All *B. bassiana* isolates (TaBrPGA, LtApPGA, and TaTtLH) that were assessed to colonize maize plant tissue confirmed as a fungal endophyte. The mycelia of three fungal isolates were able to colonize within the maize tissue, and the mycelia of those were not found whitin the untreated maize tisssue (control). All *B. bassiana* isolates could colonize the leaves of the treated maize plant when the leaves were cultured on the SDA medium. All the leaves of the treated maize plant were overgrown and covered by the fungus (Figure 1), but the fungal mycelia were not found on the final flushing water and on leaves of control maize. A percentage of *B. bassiana* colonization whitin 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 no significant differences (Table 2). However, the colonization were significant difference between those by *B. bassiana* isolates and control. The colonization percentage was resulted from from the number of leaf tissue overgrown with fungus divided by the number of leaf tissue observed x 100. The obtained data confirmed that the *B. bassiana* isolates (TaBrPGA, LtApPGA, and TaTtLH) isolated from soil and infected-host cadavers are the endophytic entomopathogenic fungus.

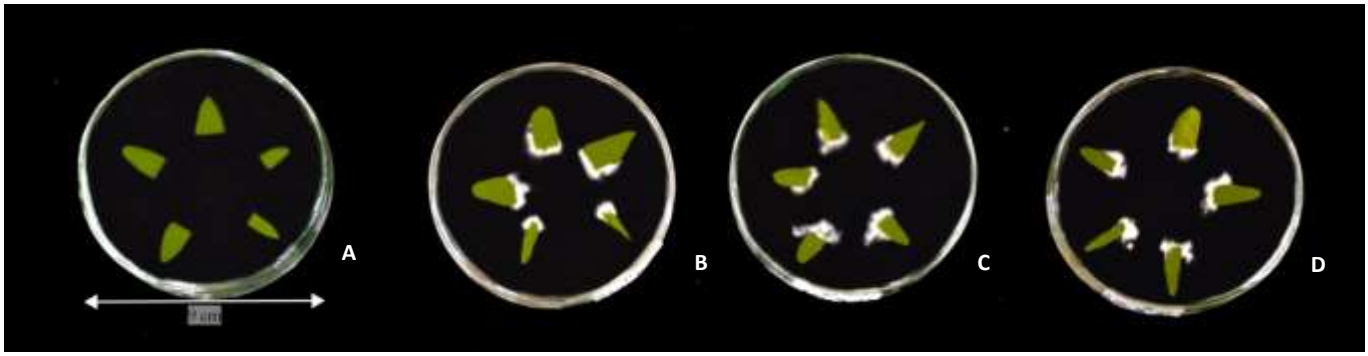


Figure 1. Colony morphology of endophytic fungi from the leaves of maize where seed treatment was given and control (untreated seeds): Control (A), *Beauveria bassiana* of TaBrPGA isolate (B), LtApPGA (C), and TaTtLH isolates (D)

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

		Mean colonization (%)	
Isolate	Species	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. Original data were transformed using Arcsin transformation prior to statistical analysis

Effect of fungal colonized maize on *Spodoptera frugiperda* growth

The first instar neonate larvae (hatching within 24 hours) fed on *B. bassiana* colonized maize leaves caused the 2nd, 3rd, 4th, 5th, and 6th larvae significantly reducing their leaf area eaten. However, at the 1st larvae, leaf area eaten by *S. frugiperda* larvae fed on young maize colonized with *B. bassiana* of TaBrPGA, LtApPGA, and TaTtLH isolates were not significantly different from those of control. At the 2nd, 3rd, 4th, 5th, and 6 larvae, the leaf area eaten by *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 current data showed that all *B. bassiana* isolates reduced the *S. frugiperda* larvae's appetite.

Fecal weight of *S. frugiperda* larvae fed on *B. bassiana* colonized maize leaves mostly reduced significantly. However, at the 2nd and 3rd larvae, all isolates of *B. bassiana* have not decreased larvae fecal weight yet. At 4th, 5th, and 6th larvae, their fecal weight of larvae fed on *B. bassiana* colonized maize leaves were significantly lighter than those of control (P<0.0001) (Table 4), but their fecal weight of those treated larvae were not significantly different among isolates. So, all isolates of *B. bassiana* could reduced the fecal weight of *S. frugiperda* larvae.

The first instar neonate larvae fed on *B. bassiana* colonized maize leaves caused all instar larvae significantly decreasing their larvae weight. At the 1st and 2nd larvae, all isolates of *B. bassiana* caused significantly lower larvae weight than the untreated ones (control), but the effect among isolates was not significantly different. The older the larvae was, 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 were significantly different from those colonized by LtApPGA isolates and control. However, all isolates of *B. bassiana* could decline significantly the 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 the corn leaves inoculated with the fungus were shorter than the 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 the 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, the obtained study highlighted that TaTtLH isolate was the most pathogenic isolates among the 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 reduce significantly 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 *Beauveria bassiana* of TaBrPGA, LtApPGA, and TaTtLH isolates

Isolate	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. Original data were transformed using Square Root transformation prior to statistical analysis

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

Isolate	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. Original data were transformed using Square Root transformation prior to statistical analysis

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

Isolate	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. Original data were transformed using Square Root transformation prior to statistical analysis

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

Isolate	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. Original data were transformed using Square Root transformation prior to statistical analysis

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

Isolate	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. Original data were transformed using Arcsin transformation prior to statistical analysis

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

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. Original data pupae and adult emergence were transformed using Arcsin transformation before statistical analysis and using Square Root transformation before statistical analysis of sex ratio.

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

The first instar neonate larvae of *S. frugiperda* fed on the *B. bassiana* colonized maize leaves could produce mycosis on their larvae, pupae, and the adults. The mycosis on the infected larvae began with the larvae color and behavior changed. The fungal infected larvae were less active and their appetite decreased as evidenced by the lower leaf area eaten compared to the control (Table 3). The fungal infected and sick larvae had unique symptoms, such as their size was smaller than the healthy ones and their color became darker than the healthy ones. About 4−6 days after feeding on colonized maize leaves, the larvae began to die. The cadaver from the infected larvae became shrunken and hardened and appeared mummification. When the cadaver was grown onto water-agar medium, after being incubated for 4−6 days, the white conidia and mycelia of *B. bassiana* began to emerge from the cadaver body, then, the conidia and mycelia could fully cover the cadaver body after being incubated for more than 10 days (Figure 2). The conidia isolated from the cadaver were grown onto SDA medium, the fungus grown 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). Morphology of hyphae of all isolates had hyaline color, and their conidia were globose, and the mycelia were hyaline. The obtained data showed that the larvae cadaver produced the *B. bassiana* conidia and mycelia. So, the larvae were considered to have died from mycosis.

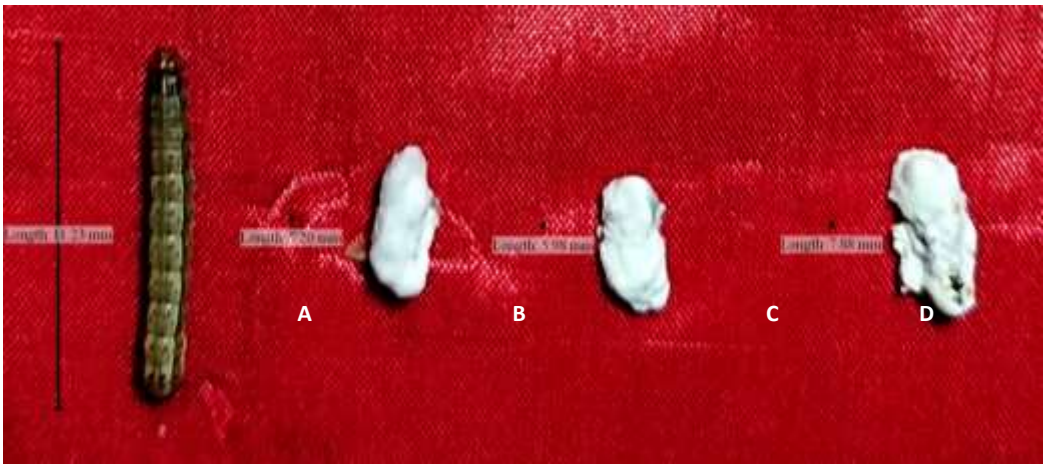


Figure 2. The cadavers from larvae fed on maize leaves uncolonized with fungi or control (A), and the cadavers from larvae fed on maize leaves colonized by *Beauveria bassiana* of TaBrPGA isolate (B), LtApPGA (C), and TaTtLH isolates (D)

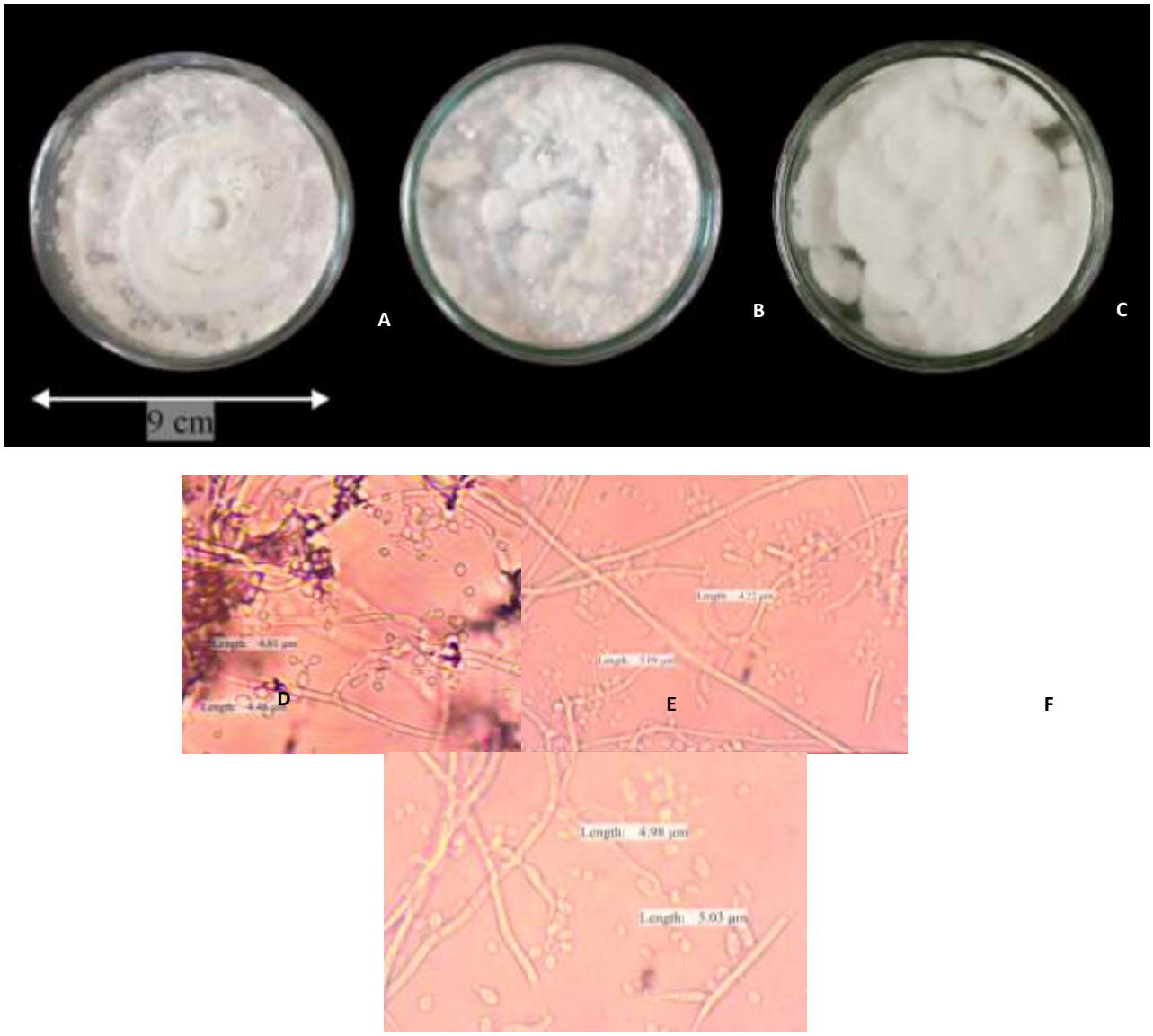


Figure 3. Colony morphology of endophytic fungi isolated from the cadavers, and cultured on SDA media (above) and the conidial and hyphal morphology (below) of the fungi: *Beauveria bassiana* of TaBrPGA isolate (A and D), LtApPGA (B and E), and TaTtLH isolates (C and F)

Some fungal infected larvae that were survival could become abnormal larvae, and some of them got into pupae and adult stage (Table 8). The abnormal larvae had smaller and shrinkage body with darker in color than the healthy or normal ones (Figure 4). The abnormal larvae had harder integument than those of healthy larvae. The survival infected larvae that reached pupae stage could also produce abnormal pupae. The symptoms of the abnormal pupae were similar to the abnormal larvae with the smaller and shrinkage body and darker in color compared to the healthy pupae (Figure 5). The abnormal pupae also had harder integument compared to normal pupae. The infected pupae that were survival could produce the abnomal adults. The abnormal adults underwent smaller and malformed body with folded wings or abnormal wings that caused the adults becoming inability to fly (Figure 6).

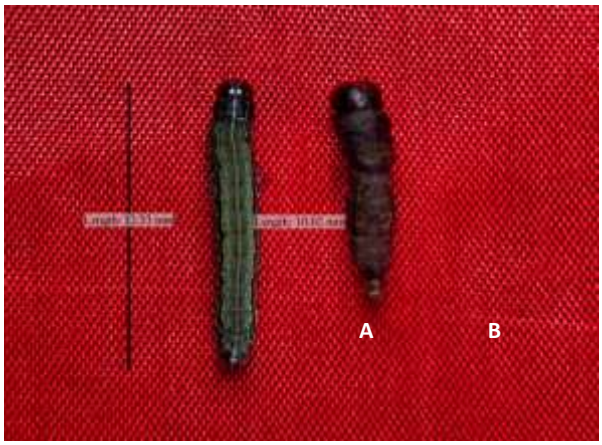


Figure 4. Larval *Spodoptera frugiperda*: healthy larvae of control (A) and larvae infected by endophytic *Beauveria bassiana* (B)

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

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

Discussion

The current study confirmed that the *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 finding highlighted

that the fungus, *B. bassiana* isolated from soil and infected-host cadavers confirm as a fungal endophyte. *B. bassiana* isolated from *S. frugiperda* larvae have been reported that could be as a fungal endophyte (Sari et al., 2022). Previous study also found that endophytic *B. bassiana,* and *M. anisopliae* isolated from maize and red pepper, respectively were able to colonize the maize when inoculated by seed treatment, and at 14 days after fungal seed treatment, they could colonize 100% of leaves (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 the plant tissues could be more than 14 days and found within leaves of tomatoes up to 30 days after inoculation (Carolina et al., 2020). In the present study, all isolates of *B. bassiana* could colonize the young maize leaf tissue (80−100%). The ability of the fungus colonized the 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 the colonized young maize were beneficial for early prevention from *S. frugiperda* larvae (Lestari et al., 2022). Furthermore, the *S. frugiperda* larvae that always hide within the corn midribs were effectively controlled by using the endophytic fungi (Sari et al., 2022).

The leaf area eaten by the 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 the *S. frugiperda* larvae's appetite. The fecal weight of *S. frugiperda* larvae treated with all isolates of *B. bassiana* also decreased significantly. The reduction of leaf area eaten by the larvae of *S. frugiperda* fed on *B. bassiana* colonized maize leaves caused the weight and body length of all instar larvae significantly to decline. So, all isolates of *B. bassiana* caused negative effects on the growth of *S. frugiperda* larvae.

Furthermore, the reduction of leaf area eaten by *S. frugiperda* larvae induced the increase of larvae mortality. All *B. bassiana* isolates caused the mortality of all instar larvae of *S. frugiperda* to increase significantly. The highest mortality of the 6th larvae reached 73% of mortality induced by TaTtLH isolate treatment. So, TaTtLH isolate of *B. bassiana* was the most pathogenic isolates among the other isolates of *B. bassiana*. 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. This current study was successful in raising mortality (73%) of *S. frugiperda* larvae by increasing conidial suspension (1×10^8 conidia mL^{-1}) of the endophytic *B. bassiana*. The commercial strains *B. bassiana* Bb-18 at 1×10^8 conidia mL^{-1} could kill 87% of the *S. frugiperda* larvae, however the fungus was applied using the soil drench method not by seed treatment (Ramos et al., 2020). The seed treatment method for applying the endophytic B. bassiana is more advantageous because the fungus could protect plants early since the stored corn seeds. So, the endophytic *B. bassiana* could be recommended to protect the stored corn seeds.

In addition to killing the larvae of *S. frugiperda*, the endophytic *B. bassiana* could kill the 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 the first instar neonate larvae fed on *B. bassiana* colonized maize leaves could induce 14% of adult emergence. So, the 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 the 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 the endophytic fungi on growth of *S. frugiperda* began with the reduction of leaf area consumed by the larvae of *S. frugiperda*. The *S. 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* (Russo et al., 2020). The toxins secreted by blastospores were toxic to the larvae and killed the larvae (Mancillas-Paredes et al., 2019). The secondary metabolites *in planta* were also toxic and resulted antibiosis and feeding deterrence for the 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 current study found that the 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 the endophytic fungi underwent mycosis (Lestari et al., 2022; Sari et al., 2022).

Finally, the research confirmed that *B. bassiana* isolates isolated from soil and infected-host cadavers of Lepidoptera were the endophytic entomopathogenic fungus. All isolates (TaBrPGA, LtApPGA, and TaTtLH) of the endophytic *B. bassiana* caused negative effects on the growth of *S. frugiperda* larvae. TaTtLH isolate of *B. bassiana* was the most pathogenic isolates (73% of larvae mortality) among the other isolates. The endophytic *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. The endophytic *B. bassiana* could decrease 86% of adult emergence. The endophytic *B. bassiana* applied in seed treatment could protect since the stored corn seeds and the young maize plant against *S. frugiperda*. So, the endophytic *B. bassiana* could be recommended to protect the stored corn seeds.

ACKNOWLEDGEMENTS

This research was funded by the DIPA of Universitas Sriwijaya 2021, contract number: SP DIPA-023.17.2.677515/2021, November 23th, 2020, in line with Rector's Decree Number: 0014/UN9/SK.LP2M.PT/2021, May 25th, 2021, chaired by Siti Herlinda. We would like to thank the Rector of Universitas Sriwijaya for funding this research.

REFERENCES

Carolina A, Silva L, Silva GA, Henrique P, Abib N, Carolino AT, et al. (2020). Endophytic colonization of tomato plants by the entomopathogenic fungus Beauveria bassiana for controlling the South American tomato pinworm, *Tuta absoluta*. CABI Agric Biosci 1: 1–9. DOI: 10.1186/s43170-020-00002-x.

Early R, González-Moreno P, Murphy ST, Day R. (2018). Forecasting the global extent of invasion of the cereal pest *Spodoptera frugiperda*, the fall armyworm. NeoBiota 40: 25–50. DOI: http://hdl.handle.net/10871/34861.

Ganiger PC, Yeshwanth HM, Muralimohan K, Vinay N, Kumar AR V., Chandrashekara K. (2018). Occurrence of the new invasive pest, fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), in the maize fields of Karnataka, India. Curr Sci 115: 621–623.

Goergen G, Kumar PL, Sankung SB, Togola A, Tam M. (2016). First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (J E Smith) (Lepidoptera , Noctuidae), a new alien invasive pest in West and Central Africa. PLoS One 11: 1–9.

De Groote H, Kimenju SC, Munyua B, Palmas S, Kassie M, Bruce A. (2020). Spread and impact of fall armyworm (*Spodoptera frugiperda* J.E. Smith) in maize production areas of Kenya. Agric Ecosyst Environ 292: 1–10. DOI: 10.1016/j.agee.2019.106804.

Gustianingtyas M, Herlinda S, Suwandi S. (2021). The endophytic fungi from South Sumatra (Indonesia) and their pathogenecity against the new invasive fall armyworm, *Spodoptera frugiperda*. Biodiversitas 22: 1051–1062. doi: 10.13057/biodiv/d220262.

Harrison RD, Thierfelder C, Baudron F, Chinwada P, Midega C, Scha U, et al. (2019). Agro-ecological options for fall armyworm (*Spodoptera frugiperda* JE Smith) management: Providing low-cost, smallholder friendly solutions to an invasive pest. J Environ Manage 243: 318–330. DOI: 10.1016/j.jenvman.2019.05.011.

Herlinda S, Gustianingtyas M, Suwandi S, Suharjo R, Sari JMP, Lestari RP. (2021). Endophytic fungi confirmed as entomopathogens of the new invasive pest, the fall armyworm, *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae), infesting maize in South Sumatra, Indonesia. Egypt J Biol Pest Control 31: 1–13. DOI: 10.1186/s41938-021-00470-x.

Herlinda S, Octariati N, Suwandi S, Hasbi. (2020). Exploring entomopathogenic fungi from South Sumatra (Indonesia) soil and their pathogenicity against a new invasive maize pest, *Spodoptera frugiperda*. Biodiversitas 21: 2955–2965. DOI: 10.13057/biodiv/d210711.

Herlinda S, Suharjo R, Sinaga ME, Fawwazi F, Suwandi S. (2022). First report of occurrence of corn and rice strains of fall armyworm, *Spodoptera frugiperda* in South Sumatra, Indonesia and its damage in maize. J Saudi Soc Agric Sci 21: 412–419. DOI: 10.1016/j.jssas.2021.11.003.

Jaber LR, Ownley BH. (2018). Can we use entomopathogenic fungi as endophytes for dual biological control of insect pests and plant pathogens? Biol Control 116: 36–45. DOI: 10.1016/j.biocontrol.2017.01.018.

Kumela T, Simiyu J, Sisay B, Likhayo P, Gohole L, Tefera T. (2018). Farmers knowledge, perceptions, and management practices of the new invasive pest, fall armyworm (*Spodoptera frugiperda*) in Ethiopia and Kenya. Int J Pest Manag 65: 1–9. DOI: 10.1080/09670874.2017.1423129.

Lestari YA, Verawaty M, Herlinda S. (2022). Development of *Spodoptera frugiperda* fed on young maize plant's fresh leaves inoculated with endophytic fungi from South Sumatra, Indonesia. Biodi 23: 5056–5063. DOI: 10.13057/biodiv/d231012.

Lira AC de, Mascarin GM, Júnior ID. (2020). Microsclerotia production of *Metarhizium* spp. for dual role as plant biostimulant and control of Spodoptera frugiperda through corn seed coating. Fungal Biol 124: 689–699. DOI: 10.1016/j.funbio.2020.03.011.

Maharani Y, Dewi VK, Puspasari LT, Rizkie L, Hidayat Y, Dono D. (2019). Cases of fall army worm *Spodoptera frugiperda* J. E. Smith (Lepidoptera: Noctuidae) attack on maize in Bandung, Garut and Sumedang District, West Java. Crop - J Plant Prot 2: 38. doi: 10.24198/cropsaver.v2i1.23013.

Mancillas-Paredes JM, Hernández-Sánchez H, Jaramillo-Flores ME, García-Gutiérrez C. (2019). Proteases and chitinases induced in *Beauveria bassiana* during infection by *Zabrotes subfasciatus*. Southwest Entomol 44: 125–137. DOI: 10.3958/059.044.0114.

Montezano DG, Specht A, Sosa-gómez DR, Brasília U De. (2018). Host plants of Spodoptera frugiperda (Lepidoptera : Noctuidae) in the Americas. African Entomol 26: 286–300. DOI: 10.4001/003.026.0286.

Mukkun L, Kleden YL, Simamora AV. (2021). Detection of *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) in maize field in East Flores District, East Nusa Tenggara Province, Indonesia. Intl J Trop Drylands 5: 20–26. DOI: 10.13057/tropdrylands/t050104.

Novianti V, Indradewa D, Maryani, Rachmawati D. (2020). Selection of local swamp rice cultivars from Kalimantan (Indonesia) tolerant to iron stress during vegetative stage. Biodiversitas 21: 5650–5661. DOI: 10.13057/biodiv/d211210.

Otim MH, Tay WT, Walsh TK, Kanyesigye D, Adumo S, Abongosi J, et al. (2018). Detection of sister-species in invasive populations of the fall armyworm *Spodoptera frugiperda* (Lepidoptera : Noctuidae) from Uganda. PLoS One 13: 1–18. DOI: 10.1371/journal.pone.0194571.

Quesada-moraga E, Vey A. (2004). Bassiacridin, a protein toxic for locusts secreted by the entomopathogenic fungus *Beauveria bassiana*. Mycol Res 108: 441–452. DOI: 10.1017/S0953756204009724.

Ramanujam B, Poornesha B, Shylesha AN. (2020). Effect of entomopathogenic fungi against invasive pest *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera : Noctuidae) in maize. Egypt J Biol Pest Control 30: 1–5. DOI: 10.1186/s41938-020-00291-4.

Ramos Y, Taibo AD, Jiménez JA, Portal O. (2020). Endophytic establishment of Beauveria bassiana and Metarhizium anisopliae in maize plants and its effect against *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) larvae. Egypt J Biol Pest Control 30: 1–6. DOI: 10.1186/s41938-020-00223-2.

Russo ML, Jaber LR, Scorsetti AC, Vianna F, Cabello MN, Pelizza SA. (2020). Effect of entomopathogenic fungi introduced as corn endophytes on the development, reproduction, and food preference of the invasive fall armyworm *Spodoptera frugiperda*. J Pest Sci (2004) 93: 1–12. DOI: 10.1007/s10340-020-01302-x.

Safavi SA. (2012). In vitro and in vivo induction, and characterization of Beauvericin isolated from *Beauveria bassiana* and its bioassay on *Galleria mellonell*a larvae. J Agric Sci Technol 15: 1–10.

Sari JMP, Herlinda S, Suwandi S. (2022). Endophytic fungi from South Sumatra (Indonesia) in seed-treated corn seedlings Affecting development of the fall armyworm, *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae). Egypt J Biol Pest Control 32: 1–11. DOI: 10.1186/s41938-022-00605-8.

Sartiami D, Dadang, Harahap I, Kusumah Y, Anwar R. (2020). First record of fall armyworm (Spodoptera frugiperda) in Indonesia and its occurence in three provinces. In: IOP Conf. Ser.: Earth Environ. Sci. 468 012021. pp 1–8. DOI: 10.1088/1755-1315/468/1/012021.

Sisay B, Simiyu J, Mendesil E, Likhayo P, Ayalew G, Mohamed S, et al. (2019). Fall armyworm, *Spodoptera frugiperda* infestations in East Africa: Assessment of damage and parasitism. Insects 10: 1–10. DOI: 10.3390/insects10070195.

Supartha IW, Susila IW, Sunari AAAAS, Mahaputra IGF, Yudha IKW, Wiradana PA. (2021). Damage characteristics and distribution patterns of invasive pest, *Spodoptera frugiperda* (J.E Smith) (Lepidoptera: Noctuidae) on maize crop in Bali, Indonesia. Biodiversitas 22: 3378–3387. DOI: 10.13057/biodiv/d2206xx.

Vidal S, Jaber LR. (2015). Entomopathogenic fungi as endophytes: Plant-endophyte-herbivore interactions and prospects for use in biological control. Curr Sci 109: 46–54.

Zhang D, Xiao Y, Xu P, Yang X, Wu Q, Wu K. (2021). Insecticide resistance monitoring for the invasive populations of fall armyworm, *Spodoptera frugiperda* in China. J Integr Agric 20: 783–791. DOI: 10.1016/S2095-3119(20)63392-5.

SUBMISSION CHECKLIST

Ensure that the following items are present:

The first corresponding author must be accompanied with contact details: **Give mark (X)**

• E-mail address	x
• Full postal address (incl street name and number (location), city, postal code, state/province, country)	x
• Phone and facsimile numbers (incl country phone code)	x

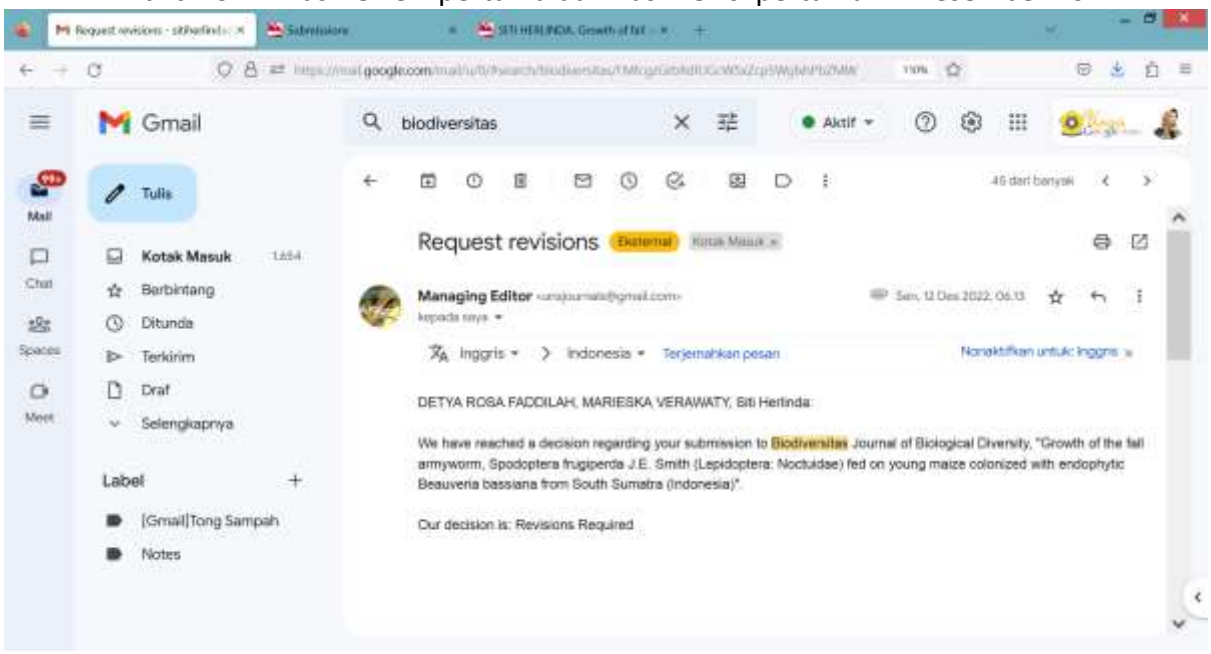
All necessary files have been uploaded, and contain:

• Keywords	x
• Running titles	x
• All figure captions	x
• All tables (incl title and note/description)	x

Further considerations

• Manuscript has been "spell & grammar-checked" Better, if it is revised by a professional science editor or a native English speaker	x
• References are in the correct format for this journal	x
• All references mentioned in the Reference list are cited in the text, and vice versa	x
• Colored figures are only used if the information in the text may be losing without those images	x
• Charts (graphs and diagrams) are drawn in black and white images; use shading to differentiate	x

2. Bukti konfirmasi review pertama dan hasil revisi pertama 12 Desember 2022



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

Abstract. 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 fungus. 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. *B. bassiana* could decrease 86% of adult emergence. *B. bassiana* applied in seed treatment could protect since the 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: Endophytes, entomopathogen, neonate larvae, *Spodoptera frugiperda*, seed treatment, corn

Abbreviations (if any): -

Running title: Growth of *Spodoptera frugiperda* with endophytic *Beauveria bassiana*

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 is 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 the 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 millions per year in Africa (Harrison et al. 2019). In Indonesia, FAW severely attacked corn and caused up to100% damage (Herlinda et al. 2022; 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. 2022). 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). 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: Hypomycetes) 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). *B. bassiana* isolated from plants from South Sumatra that was 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 have been mass-reared in the laboratory few years ago (Herlinda et al. 2020) and *S. frugiperda* was identified molecularly (Herlinda et al. 2022). The FAW were 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. 45 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 soloution and rinsed twice with sterile distilled water (Russo et al. 2020). The tips were grown onto agar medium (SDA). 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.

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)

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.

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* were 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 were 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. frugiperga* from each treatment were analyzed using analysis of variance (ANOVA). 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 colonization percentage was resulted from from the number of leaf tissue overgrown with fungus divided by the number of leaf tissue observed x 100. The result confirmed that *B. bassiana* isolates (TaBrPGA, LtApPGA, and TaTtLH) isolated from soil and infected-host cadavers was an endophytic entomopathogenic fungus.

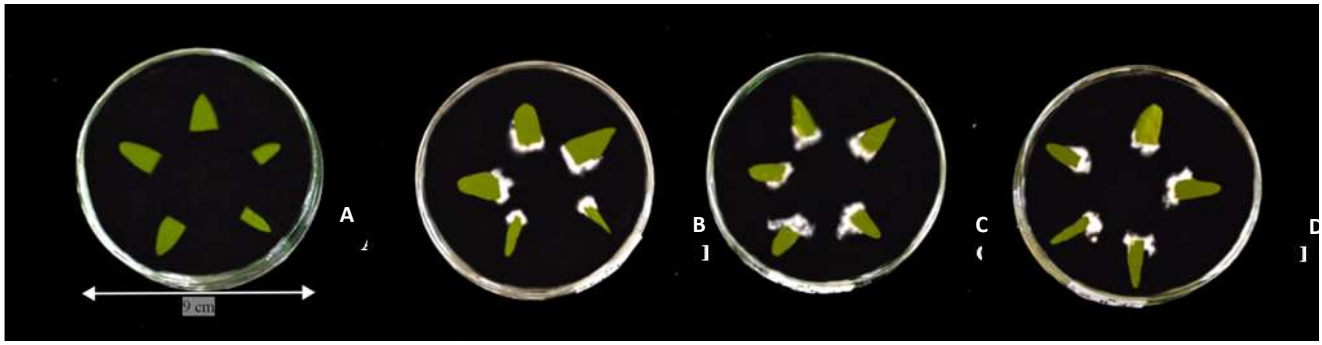


Figure 1. Colony morphology of endophytic fungi from the leaves of maize : Control (A), *Beauveria bassiana* of TaBrPGA isolate (B), LtApPGA (C), and TaTtLH isolates (D)

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

		Mean colonization (%)	
Isolates	Species	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. Original data were transformed using Arcsin transformation prior to statistical analysis

Effect of fungal colonization maize on *Spodoptera 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 fed on *B. bassiana* colonized maize leaves caused all instar larvae significantly decreasing their larvae 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 was, 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. Original data were transformed using Square Root transformation prior to statistical analysis

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. Original data were transformed using Square Root transformation prior to statistical analysis

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*	4.04~~b~~	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. Original data were transformed using Square Root transformation prior to statistical analysis

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. Original data were transformed using Square Root transformation prior to statistical analysis

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. Original data pupae and adult emergence were transformed using Arcsin transformation before statistical analysis and using Square Root transformation before statistical analysis of sex ratio.

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 zhe 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.

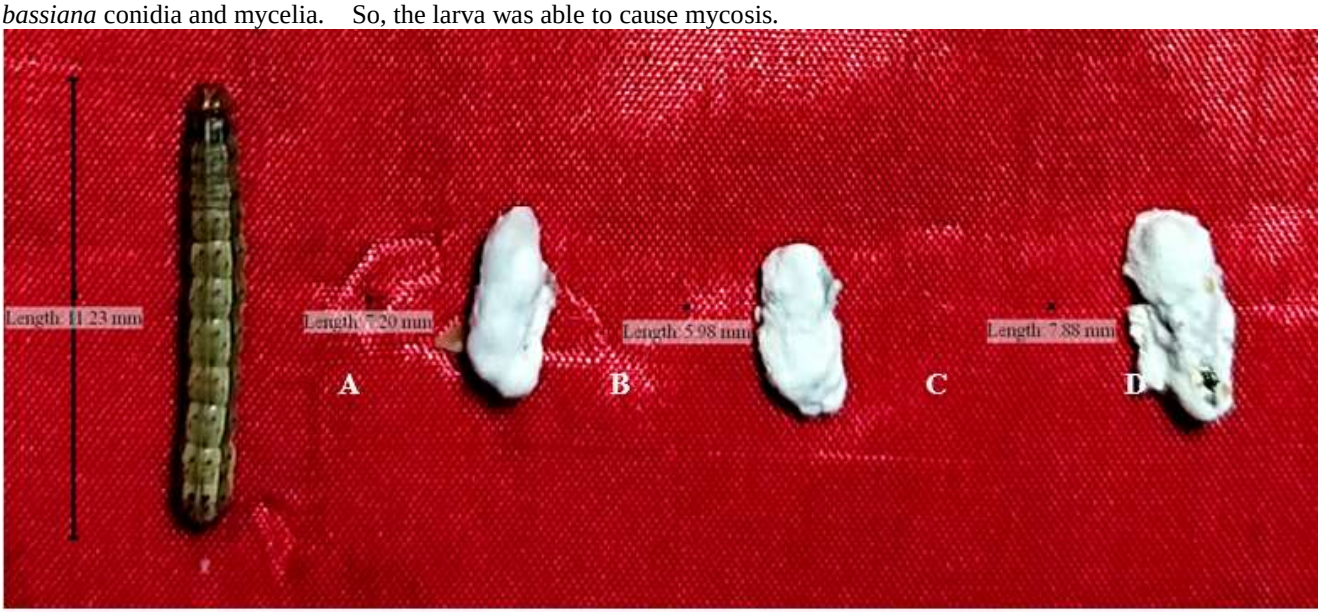


Figure 2. The cadavers from larvae fed on maize leaves uncolonized with fungi or control (A), and cadavers from larvae fed on maize leaves colonized by *Beauveria bassiana* of TaBrPGA isolate (B), LtApPGA (C), and TaTtLH isolates (D)

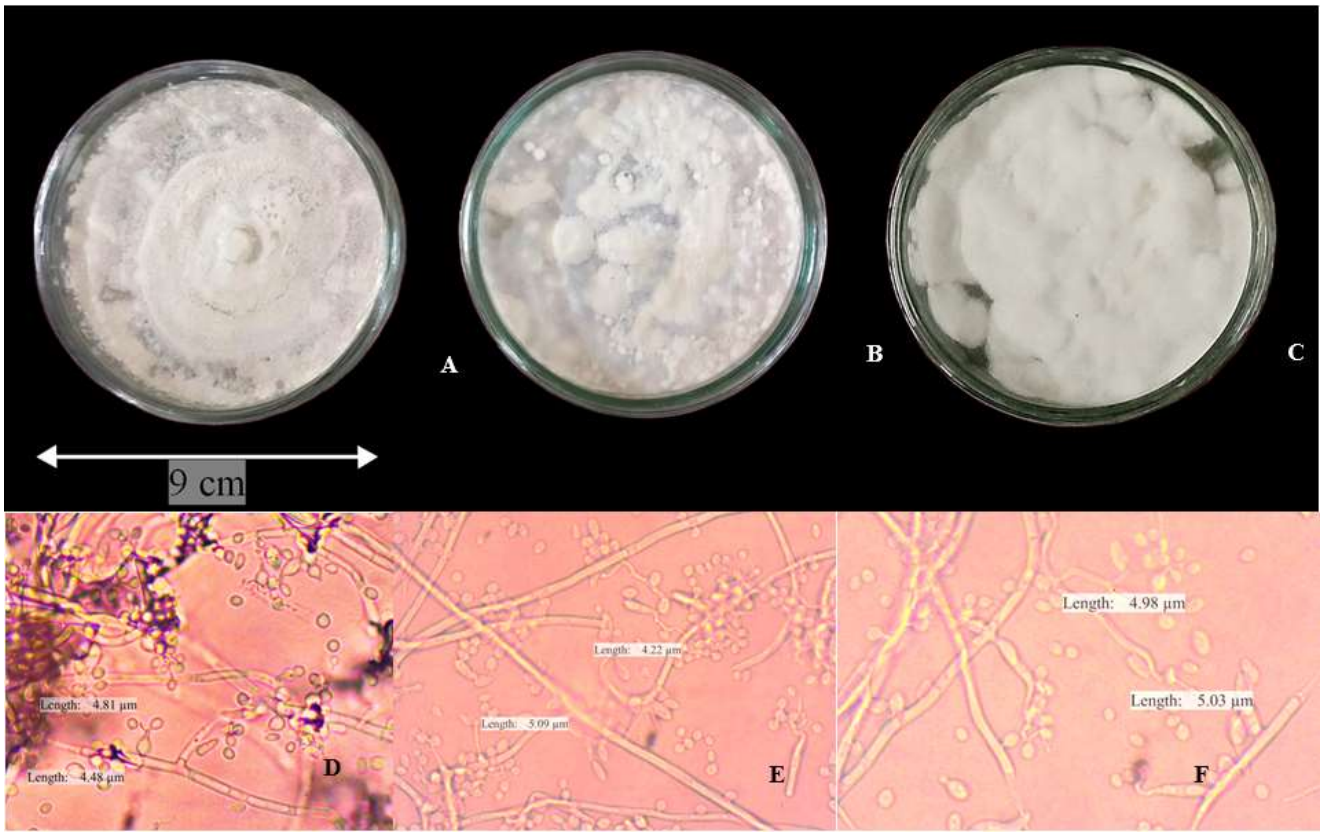


Figure 3. Colony morphology of endophytic fungi on SDA media (above) isolated from cadavers, and conidial and hyphal morphology (below) of fungi: *Beauveria bassiana* of TaBrPGA isolate (A and D), LtApPGA (B and E), and TaTtLH isolate (C and F)

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).

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)

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 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 (Carolina 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 are 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.

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 (.....). 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*. *S. 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* (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.

ACKNOWLEDGEMENTS

This research was funded by the DIPA of Universitas Sriwijaya 2021, contract number: SP DIPA-023.17.2.677515/2021, November 23th, 2020, in line with Rector's Decree Number: 0014/UN9/SK.LP2M.PT/2021, May 25th, 2021, chaired by Siti Herlinda. We would like to thank the Rector of Universitas Sriwijaya for funding this research.

REFERENCES

Carolina A, Silva L, Silva GA, Henrique P, Abib N, Carolino AT, et al. 2020. Endophytic colonization of tomato plants by the entomopathogenic fungus Beauveria bassiana for controlling the South American tomato pinworm, *Tuta absoluta*. CABI Agric Biosci 1: 1–9. DOI: 10.1186/s43170-020-00002-x.

Early R, González-Moreno P, Murphy ST, Day R. 2018. Forecasting the global extent of invasion of the cereal pest *Spodoptera frugiperda*, the fall armyworm. NeoBiota 40: 25–50. DOI: http://hdl.handle.net/10871/34861.

Ganiger PC, Yeshwanth HM, Muralimohan K, Vinay N, Kumar AR V, Chandrashekara K. 2018. Occurrence of the new invasive pest, fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), in the maize fields of Karnataka, India. Curr Sci 115: 621–623.

Goergen G, Kumar PL, Sankung SB, Togola A, Tam M. 2016. First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (J E Smith) (Lepidoptera , Noctuidae), a new alien invasive pest in West and Central Africa. PLoS One 11: 1–9.

De Groote H, Kimenju SC, Munyua B, Palmas S, Kassie M, Bruce A. 2020. Spread and impact of fall armyworm (*Spodoptera frugiperda* J.E. Smith) in maize production areas of Kenya. Agric Ecosyst Environ 292: 1–10. DOI: 10.1016/j.agee.2019.106804.

Gustianingtyas M, Herlinda S, Suwandi S. 2021. The endophytic fungi from South Sumatra (Indonesia) and their pathogenecity against the new invasive fall armyworm, *Spodoptera frugiperda*. Biodiversitas 22: 1051–1062. doi: 10.13057/biodiv/d220262.

Harrison RD, Thierfelder C, Baudron F, Chinwada P, Midega C, Scha U, et al. 2019. Agro-ecological options for fall armyworm (*Spodoptera frugiperda* JE Smith) management: Providing low-cost, smallholder friendly solutions to an invasive pest. J Environ Manage 243: 318–330. DOI: 10.1016/j.jenvman.2019.05.011.

Herlinda S, Gustianingtyas M, Suwandi S, Suharjo R, Sari JMP, Lestari RP. 2021. Endophytic fungi confirmed as entomopathogens of the new invasive pest, the fall armyworm, *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae), infesting maize in South Sumatra, Indonesia. Egypt J Biol Pest Control 31: 1–13. DOI: 10.1186/s41938-021-00470-x.

Herlinda S, Octariati N, Suwandi S, Hasbi. 2020. Exploring entomopathogenic fungi from South Sumatra (Indonesia) soil and their pathogenicity against a new invasive maize pest, *Spodoptera frugiperda*. Biodiversitas 21: 2955–2965. DOI: 10.13057/biodiv/d210711.

Herlinda S, Suharjo R, Sinaga ME, Fawwazi F, Suwandi S. 2022. First report of occurrence of corn and rice strains of fall armyworm, *Spodoptera frugiperda* in South Sumatra, Indonesia and its damage in maize. J Saudi Soc Agric Sci 21: 412–419. DOI: 10.1016/j.jssas.2021.11.003.

Jaber LR, Ownley BH. 2018. Can we use entomopathogenic fungi as endophytes for dual biological control of insect pests and plant pathogens? Biol Control 116: 36–45. DOI: 10.1016/j.biocontrol.2017.01.018.

Kumela T, Simiyu J, Sisay B, Likhayo P, Gohole L, Tefera T. 2018. Farmers knowledge, perceptions, and management practices of the new invasive pest, fall armyworm (*Spodoptera frugiperda*) in Ethiopia and Kenya. Int J Pest Manag 65: 1–9. DOI: 10.1080/09670874.2017.1423129.

Lestari YA, Verawaty M, Herlinda S. 2022. Development of *Spodoptera frugiperda* fed on young maize plant's fresh leaves inoculated with endophytic fungi from South Sumatra, Indonesia. Biodi 23: 5056–5063. DOI: 10.13057/biodiv/d231012.

Lira AC de, Mascarin GM, Júnior ID. 2020. Microsclerotia production of *Metarhizium* spp. for dual role as plant biostimulant and control of Spodoptera frugiperda through corn seed coating. Fungal Biol 124: 689–699. DOI: 10.1016/j.funbio.2020.03.011.

Maharani Y, Dewi VK, Puspasari LT, Rizkie L, Hidayat Y, Dono D. 2019. Cases of fall army worm *Spodoptera frugiperda* J. E. Smith (Lepidoptera: Noctuidae) attack on maize in Bandung, Garut and Sumedang District, West Java. Crop - J Plant Prot 2: 38. doi: 10.24198/cropsaver.v2i1.23013.

Mancillas-Paredes JM, Hernández-Sánchez H, Jaramillo-Flores ME, García-Gutiérrez C. 2019. Proteases and chitinases induced in *Beauveria bassiana* during infection by *Zabrotes subfasciatus*. Southwest Entomol 44: 125–137. DOI: 10.3958/059.044.0114.

Montezano DG, Specht A, Sosa-gómez DR, Brasília U De. 2018. Host plants of Spodoptera frugiperda (Lepidoptera : Noctuidae) in the Americas. African Entomol 26: 286–300. DOI: 10.4001/003.026.0286.

Mukkun L, Kleden YL, Simamora AV. 2021. Detection of *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) in maize field in East Flores District, East Nusa Tenggara Province, Indonesia. Intl J Trop Drylands 5: 20–26. DOI: 10.13057/tropdrylands/t050104.

Novianti V, Indradewa D, Maryani, Rachmawati D. 2020. Selection of local swamp rice cultivars from Kalimantan (Indonesia) tolerant to iron stress during vegetative stage. Biodiversitas 21: 5650–5661. DOI: 10.13057/biodiv/d211210.

Otim MH, Tay WT, Walsh TK, Kanyesigye D, Adumo S, Abongosi J, et al. 2018. Detection of sister-species in invasive populations of the fall armyworm *Spodoptera frugiperda* (Lepidoptera : Noctuidae) from Uganda. PLoS One 13: 1–18. DOI: 10.1371/journal.pone.0194571.

Quesada-moraga E, Vey A. 2004. Bassiacridin, a protein toxic for locusts secreted by the entomopathogenic fungus *Beauveria bassiana*. Mycol Res 108: 441–452. DOI: 10.1017/S0953756204009724.

Ramanujam B, Poornesha B, Shylesha AN. 2020. Effect of entomopathogenic fungi against invasive pest *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera : Noctuidae) in maize. Egypt J Biol Pest Control 30: 1–5. DOI: 10.1186/s41938-020-00291-4.

Ramos Y, Taibo AD, Jiménez JA, Portal O. 2020. Endophytic establishment of Beauveria bassiana and Metarhizium anisopliae in maize plants and its effect against *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) larvae. Egypt J Biol Pest Control 30: 1–6. DOI: 10.1186/s41938-020-00223-2.

Russo ML, Jaber LR, Scorsetti AC, Vianna F, Cabello MN, Pelizza SA. 2020. Effect of entomopathogenic fungi introduced as corn endophytes on the development, reproduction, and food preference of the invasive fall armyworm *Spodoptera frugiperda*. J Pest Sci 2004 93: 1–12. DOI: 10.1007/s10340-020-01302-x.

Safavi SA. 2012. In vitro and in vivo induction, and characterization of Beauvericin isolated from *Beauveria bassiana* and its bioassay on *Galleria mellonell*a larvae. J Agric Sci Technol 15: 1–10.

Sari JMP, Herlinda S, Suwandi S. 2022. Endophytic fungi from South Sumatra (Indonesia) in seed-treated corn seedlings Affecting development of the fall armyworm, *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae). Egypt J Biol Pest Control 32: 1–11. DOI: 10.1186/s41938-022-00605-8.

Sartiami D, Dadang, Harahap I, Kusumah Y, Anwar R. 2020. First record of fall armyworm (Spodoptera frugiperda) in Indonesia and its occurence in three provinces. In: IOP Conf. Ser.: Earth Environ. Sci. 468 012021. pp 1–8. DOI: 10.1088/1755-1315/468/1/012021.

Sisay B, Simiyu J, Mendesil E, Likhayo P, Ayalew G, Mohamed S, et al. 2019. Fall armyworm, *Spodoptera frugiperda* infestations in East Africa: Assessment of damage and parasitism. Insects 10: 1–10. DOI: 10.3390/insects10070195.

Supartha IW, Susila IW, Sunari AAAAS, Mahaputra IGF, Yudha IKW, Wiradana PA. 2021. Damage characteristics and distribution patterns of invasive pest, *Spodoptera frugiperda* (J.E Smith) (Lepidoptera: Noctuidae) on maize crop in Bali, Indonesia. Biodiversitas 22: 3378–3387. DOI: 10.13057/biodiv/d2206xx.

Vidal S, Jaber LR. 2015. Entomopathogenic fungi as endophytes: Plant-endophyte-herbivore interactions and prospects for use in biological control. Curr Sci 109: 46–54.

Zhang D, Xiao Y, Xu P, Yang X, Wu Q, Wu K. 2021. Insecticide resistance monitoring for the invasive populations of fall armyworm, *Spodoptera frugiperda* in China. J Integr Agric 20: 783–791. DOI: 10.1016/S2095-3119(20)63392-5.

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)

Abstract. 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 fungus. 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. *B. bassiana* could decrease 86% of adult emergence. *B. bassiana* applied in seed treatment could protect since the 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: Endophytes, entomopathogen, neonate larvae, *Spodoptera frugiperda*, seed treatment, corn

Abbreviations (if any): -

Running title: Growth of *Spodoptera frugiperda* with endophytic *Beauveria bassiana*

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 is 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 the 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 millions per year in Africa (Harrison et al. 2019). In Indonesia, FAW severely attacked corn and caused up to100% 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: Hypomycetes) 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). *B. bassiana* isolated from plants from South Sumatra that was 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 have been mass-reared in the laboratory few years ago (Herlinda et al. 2020) and *S. frugiperda* was identified molecularly (Herlinda et al. 2022a). The FAW were 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 soloution 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 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..

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)

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.

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* were 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 were 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. frugiperga* 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 was an endophytic entomopathogenic fungus.

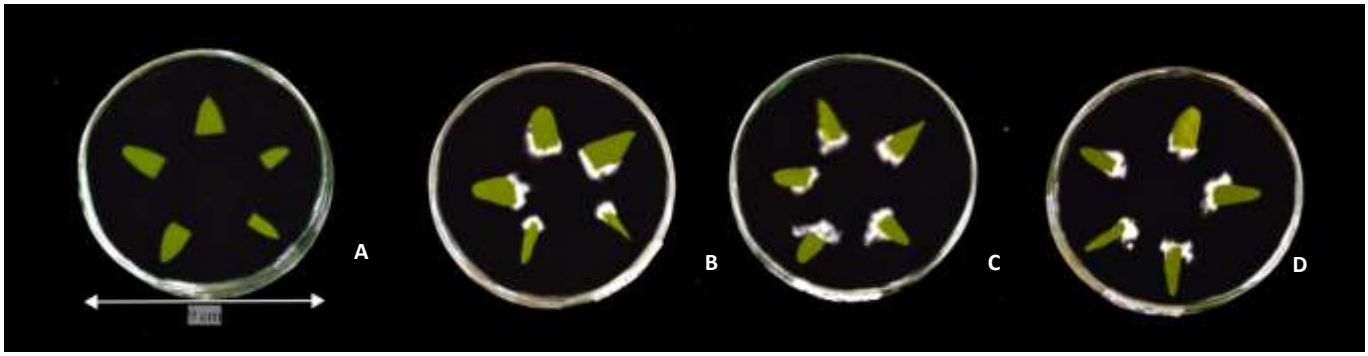


Figure 1. Colony morphology of endophytic fungi from the leaves of maize: Control (A), *Beauveria bassiana* of TaBrPGA isolate (B), LtApPGA (C), and TaTtLH isolates (D)

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

		Mean colonization (%)	
Isolates	Species	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 *Spodoptera 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 was, 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.

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.

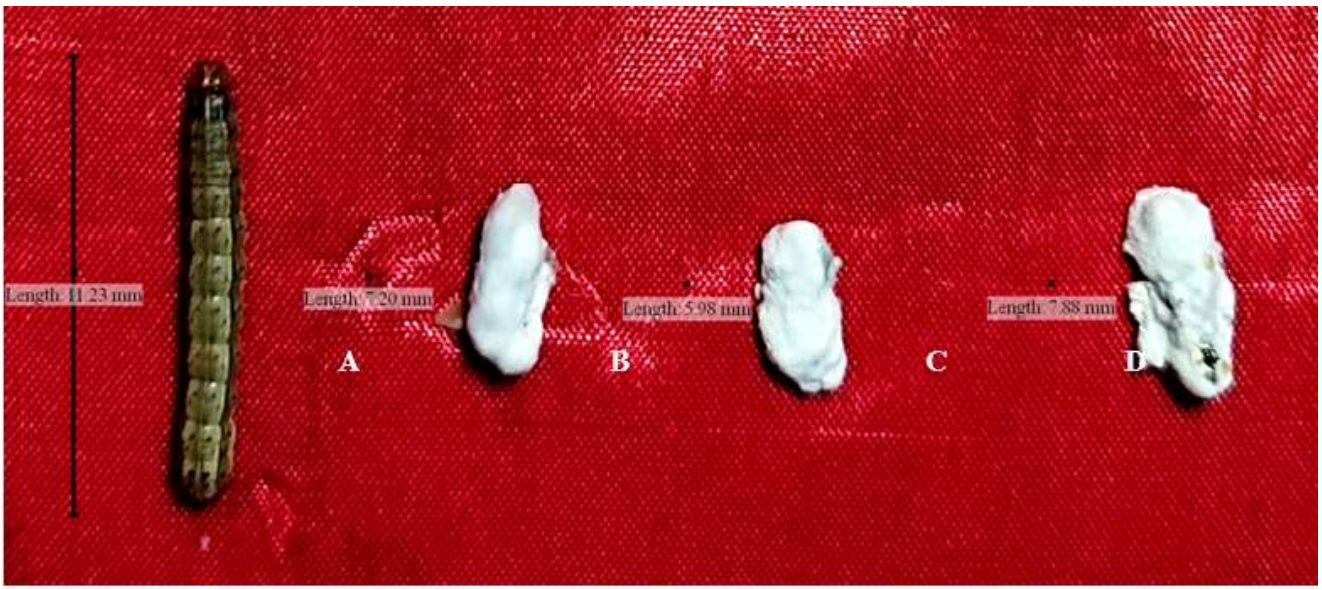


Figure 2. The cadavers from larvae fed on maize leaves uncolonized with fungi or control (A), and cadavers from larvae fed on maize leaves colonized by *Beauveria bassiana* of TaBrPGA isolate (B), LtApPGA (C), and TaTtLH isolates (D)

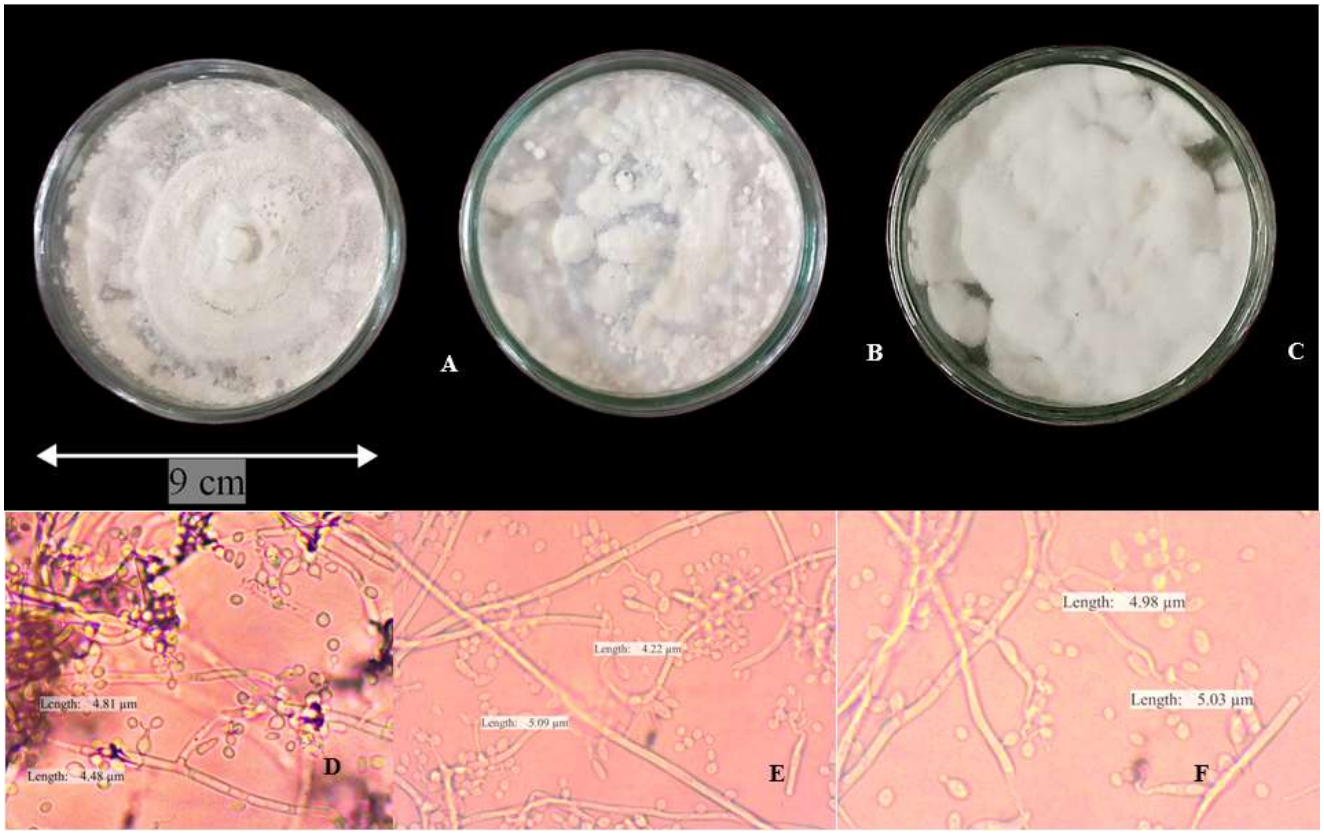


Figure 3. Colony morphology of endophytic fungi on SDA media (above) isolated from cadavers, and conidial and hyphal morphology (below) of fungi: *Beauveria bassiana* of TaBrPGA isolate (A and D), LtApPGA (B and E), and TaTtLH isolate (C and F)

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).

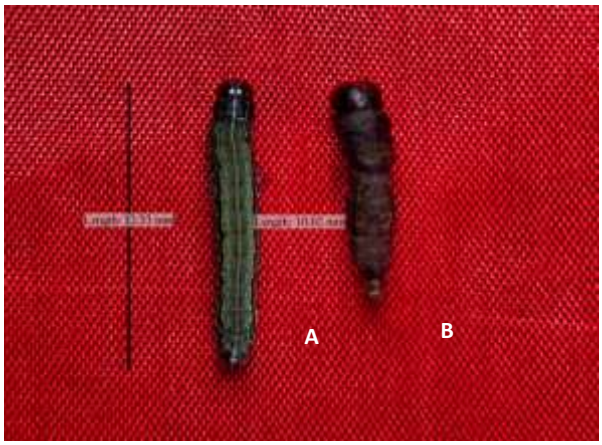


Figure 4. Larval *Spodoptera frugiperda*: healthy larvae (A) and larvae infected by endophytic *Beauveria bassiana* (B)

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)

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 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 (Carolina 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 are 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.

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.* *S. 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.

ACKNOWLEDGEMENTS

This research was funded by the DIPA of Universitas Sriwijaya 2021, contract number: SP DIPA-023.17.2.677515/2021, November 23th, 2020, in line with Rector's Decree Number: 0014/UN9/SK.LP2M.PT/2021, May 25th, 2021, chaired by Siti Herlinda. We would like to thank the Rector of Universitas Sriwijaya for funding this research.

REFERENCES

Carolina A, Silva L, Silva GA, Henrique P, Abib N, Carolino AT, et al. 2020. Endophytic colonization of tomato plants by the entomopathogenic fungus Beauveria bassiana for controlling the South American tomato pinworm, *Tuta absoluta*. CABI Agric Biosci 1: 1–9. DOI: 10.1186/s43170-020-00002-x.

Early R, González-Moreno P, Murphy ST, Day R. 2018. Forecasting the global extent of invasion of the cereal pest *Spodoptera frugiperda*, the fall armyworm. NeoBiota 40: 25–50. DOI: http://hdl.handle.net/10871/34861.

Ganiger PC, Yeshwanth HM, Muralimohan K, Vinay N, Kumar AR V, Chandrashekara K. 2018. Occurrence of the new invasive pest, fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), in the maize fields of Karnataka, India. Curr Sci 115: 621–623.

Goergen G, Kumar PL, Sankung SB, Togola A, Tam M. 2016. First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (J E Smith) (Lepidoptera , Noctuidae), a new alien invasive pest in West and Central Africa. PLoS One 11: 1–9.

De Groote H, Kimenju SC, Munyua B, Palmas S, Kassie M, Bruce A. 2020. Spread and impact of fall armyworm (*Spodoptera frugiperda* J.E. Smith) in maize production areas of Kenya. Agric Ecosyst Environ 292: 1–10. DOI: 10.1016/j.agee.2019.106804.

Gustianingtyas M, Herlinda S, Suwandi S. 2021. The endophytic fungi from South Sumatra (Indonesia) and their pathogenecity against the new invasive fall armyworm, *Spodoptera frugiperda*. Biodiversitas 22: 1051–1062. doi: 10.13057/biodiv/d220262.

Harrison RD, Thierfelder C, Baudron F, Chinwada P, Midega C, Scha U, et al. 2019. Agro-ecological options for fall armyworm (*Spodoptera frugiperda* JE Smith) management: Providing low-cost, smallholder friendly solutions to an invasive pest. J Environ Manage 243: 318–330. DOI: 10.1016/j.jenvman.2019.05.011.

Herlinda S, Gustianingtyas M, Suwandi S, Suharjo R, Sari JMP, Lestari RP. 2021. Endophytic fungi confirmed as entomopathogens of the new invasive pest, the fall armyworm, *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae), infesting maize in South Sumatra, Indonesia. Egypt J Biol Pest Control 31: 1–13. DOI: 10.1186/s41938-021-00470-x.

Herlinda S, Gustianingtyas M, Suwandi S, Suharjo R, Sari JMP, Suparman, Hamidson H, Hasyim H. 2022. Endophytic fungi from South Sumatra (Indonesia) in seed-treated corn suppressing *Spodoptera frugiperda* growth. Biodiversitas 23: 6013–6020. DOI: 10.13057/biodiv/d231156.

Herlinda S, Octariati N, Suwandi S, Hasbi. 2020. Exploring entomopathogenic fungi from South Sumatra (Indonesia) soil and their pathogenicity against a new invasive maize pest, *Spodoptera frugiperda*. Biodiversitas 21: 2955–2965. DOI: 10.13057/biodiv/d210711.

Herlinda S, Suharjo R, Sinaga ME, Fawwazi F, Suwandi S. 2022. First report of occurrence of corn and rice strains of fall armyworm, *Spodoptera frugiperda* in South Sumatra, Indonesia and its damage in maize. J Saudi Soc Agric Sci 21: 412–419. DOI: 10.1016/j.jssas.2021.11.003.

Jaber LR, Ownley BH. 2018. Can we use entomopathogenic fungi as endophytes for dual biological control of insect pests and plant pathogens? Biol Control 116: 36–45. DOI: 10.1016/j.biocontrol.2017.01.018.

Kumela T, Simiyu J, Sisay B, Likhayo P, Gohole L, Tefera T. 2018. Farmers knowledge, perceptions, and management practices of the new invasive pest, fall armyworm (*Spodoptera frugiperda*) in Ethiopia and Kenya. Int J Pest Manag 65: 1–9. DOI: 10.1080/09670874.2017.1423129.

Lestari YA, Verawaty M, Herlinda S. 2022. Development of *Spodoptera frugiperda* fed on young maize plant's fresh leaves inoculated with endophytic fungi from South Sumatra, Indonesia. Biodi 23: 5056–5063. DOI: 10.13057/biodiv/d231012.

Lira AC de, Mascarin GM, Júnior ID. 2020. Microsclerotia production of *Metarhizium* spp. for dual role as plant biostimulant and control of Spodoptera frugiperda through corn seed coating. Fungal Biol 124: 689–699. DOI: 10.1016/j.funbio.2020.03.011.

Maharani Y, Dewi VK, Puspasari LT, Rizkie L, Hidayat Y, Dono D. 2019. Cases of fall army worm *Spodoptera frugiperda* J. E. Smith (Lepidoptera: Noctuidae) attack on maize in Bandung, Garut and Sumedang District, West Java. Crop - J Plant Prot 2: 38. doi: 10.24198/cropsaver.v2i1.23013.

Mancillas-Paredes JM, Hernández-Sánchez H, Jaramillo-Flores ME, García-Gutiérrez C. 2019. Proteases and chitinases induced in *Beauveria bassiana* during infection by *Zabrotes subfasciatus*. Southwest Entomol 44: 125–137. DOI: 10.3958/059.044.0114.

Montezano DG, Specht A, Sosa-gómez DR, Brasília U De. 2018. Host plants of Spodoptera frugiperda (Lepidoptera : Noctuidae) in the Americas. African Entomol 26: 286–300. DOI: 10.4001/003.026.0286.

Mukkun L, Kleden YL, Simamora AV. 2021. Detection of *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) in maize field in East

Flores District, East Nusa Tenggara Province, Indonesia. Intl J Trop Drylands 5: 20–26. DOI: 10.13057/tropdrylands/t050104.

Novianti V, Indradewa D, Maryani, Rachmawati D. 2020. Selection of local swamp rice cultivars from Kalimantan (Indonesia) tolerant to iron stress during vegetative stage. Biodiversitas 21: 5650–5661. DOI: 10.13057/biodiv/d211210.

Otim MH, Tay WT, Walsh TK, Kanyesigye D, Adumo S, Abongosi J, et al. 2018. Detection of sister-species in invasive populations of the fall armyworm *Spodoptera frugiperda* (Lepidoptera : Noctuidae) from Uganda. PLoS One 13: 1–18. DOI: 10.1371/journal.pone.0194571.

Quesada-moraga E, Vey A. 2004. Bassiacridin, a protein toxic for locusts secreted by the entomopathogenic fungus *Beauveria bassiana*. Mycol Res 108: 441–452. DOI: 10.1017/S0953756204009724.

Ramanujam B, Poornesha B, Shylesha AN. 2020. Effect of entomopathogenic fungi against invasive pest *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera : Noctuidae) in maize. Egypt J Biol Pest Control 30: 1–5. DOI: 10.1186/s41938-020-00291-4.

Ramos Y, Taibo AD, Jiménez JA, Portal O. 2020. Endophytic establishment of Beauveria bassiana and Metarhizium anisopliae in maize plants and its effect against *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) larvae. Egypt J Biol Pest Control 30: 1–6. DOI: 10.1186/s41938-020-00223-2.

Russo ML, Jaber LR, Scorsetti AC, Vianna F, Cabello MN, Pelizza SA. 2020. Effect of entomopathogenic fungi introduced as corn endophytes on the development, reproduction, and food preference of the invasive fall armyworm *Spodoptera frugiperda*. J Pest Sci 2004 93: 1–12. DOI: 10.1007/s10340-020-01302-x.

Safavi SA. 2012. In vitro and in vivo induction, and characterization of Beauvericin isolated from *Beauveria bassiana* and its bioassay on *Galleria mellonell*a larvae. J Agric Sci Technol 15: 1–10.

Sari JMP, Herlinda S, Suwandi S. 2022. Endophytic fungi from South Sumatra (Indonesia) in seed-treated corn seedlings Affecting development of the fall armyworm, *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae). Egypt J Biol Pest Control 32: 1–11. DOI: 10.1186/s41938-022-00605-8.

Sartiami D, Dadang, Harahap I, Kusumah Y, Anwar R. 2020. First record of fall armyworm (Spodoptera frugiperda) in Indonesia and its occurence in three provinces. In: IOP Conf. Ser.: Earth Environ. Sci. 468 012021. pp 1–8. DOI: 10.1088/1755-1315/468/1/012021.

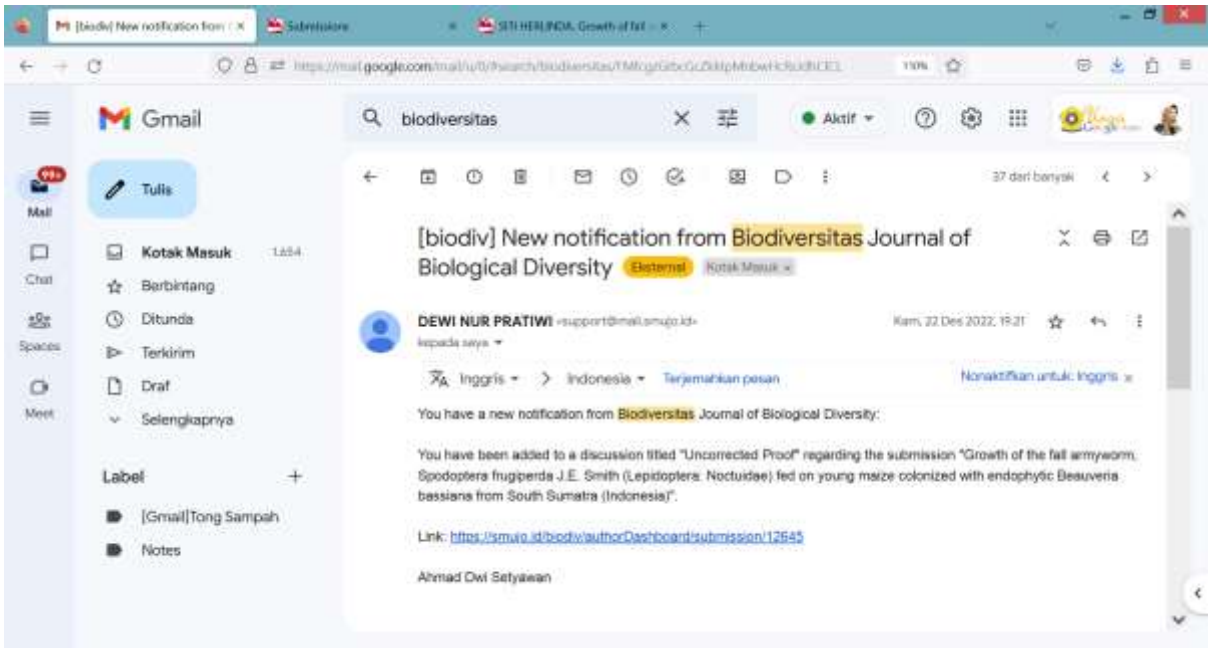
Sisay B, Simiyu J, Mendesil E, Likhayo P, Ayalew G, Mohamed S, et al. 2019. Fall armyworm, *Spodoptera frugiperda* infestations in East Africa: Assessment of damage and parasitism. Insects 10: 1–10. DOI: 10.3390/insects10070195.

Supartha IW, Susila IW, Sunari AAAAS, Mahaputra IGF, Yudha IKW, Wiradana PA. 2021. Damage characteristics and distribution patterns of invasive pest, *Spodoptera frugiperda* (J.E Smith) (Lepidoptera: Noctuidae) on maize crop in Bali, Indonesia. Biodiversitas 22: 3378–3387. DOI: 10.13057/biodiv/d2206xx.

Vidal S, Jaber LR. 2015. Entomopathogenic fungi as endophytes: Plant-endophyte-herbivore interactions and prospects for use in biological control. Curr Sci 109: 46–54.

Zhang D, Xiao Y, Xu P, Yang X, Wu Q, Wu K. 2021. Insecticide resistance monitoring for the invasive populations of fall armyworm, *Spodoptera frugiperda* in China. J Integr Agric 20: 783–791. DOI: 10.1016/S2095-3119(20)63392-5.

3. Bukti konfirmasi paper accepted, uncorrected Proof dan hasil koreksi penulis 22 Desember 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)

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. Palembang 30139, South Sumatera, Indonesia.

[2]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya. Indralaya 30662, South Sumatra, Indonesia

[3]Department of Plant Protection, Faculty of Agriculture, Universitas Sriwijaya. Indralaya 30662, South Sumatra, Indonesia. Tel./fax. +62-711-580059, ♥email: sitiherlinda@unsri.ac.id

[4]Research Center for Sub-optimal Lands (PUR-PLSO), Universitas Sriwijaya, Palembang 30139, South Sumatera, Indonesia.

Manuscript received: xxx 2022. Revision accepted: xxx December 2022.

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: xxxx.* 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 fungus. 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. *B. bassiana* could decrease 86% of adult emergence. *B. bassiana* applied in seed treatment could protect since the 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: Endophytes, entomopathogen, neonate larvae, *Spodoptera frugiperda*, seed treatment, corn

BIODIVERSITAS ISSN: 1412-033X

Volume 23, Number 12, December 2022 E-ISSN: 2085-4722

Pages: xxxx DOI: 10.13057/biodiv/d2312xx

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 is 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 the 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 millions per year in Africa (Harrison et al. 2019). In Indonesia, FAW severely attacked corn and caused up to100% 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: Hypomycetes) 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). *B. bassiana* isolated from plants from South Sumatra that was 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 have been mass-reared in the laboratory few years ago (Herlinda et al. 2020) and *S. frugiperda* was identified molecularly (Herlinda et al. 2022a). The FAW were 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 soloution 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 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* were 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 were 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. frugiperga* 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 was an endophytic entomopathogenic fungus.

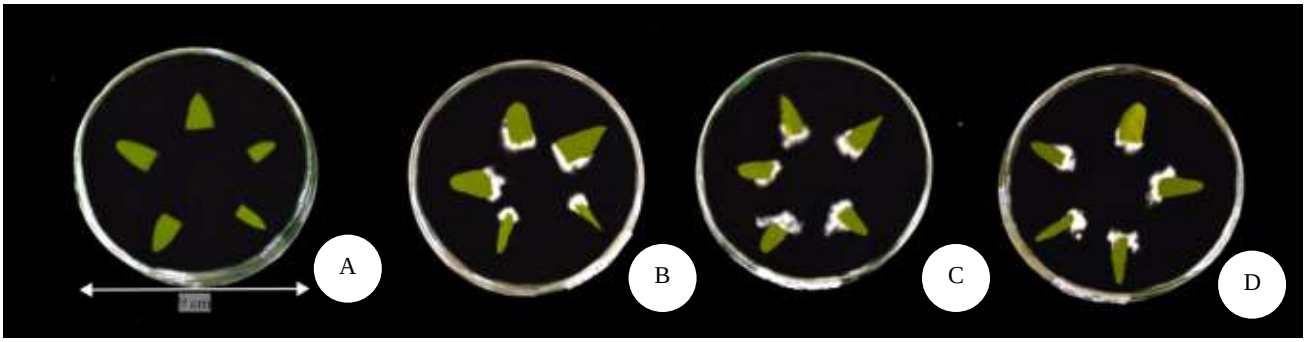


Figure 1. Colony morphology of endophytic fungi from the leaves of maize: Control (A), *Beauveria bassiana* of TaBrPGA isolate (B), LtApPGA (C), and TaTtLH isolates (D)

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

		Mean colonization (%)	
Isolates	**Species**	**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 *Spodoptera 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 was, 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

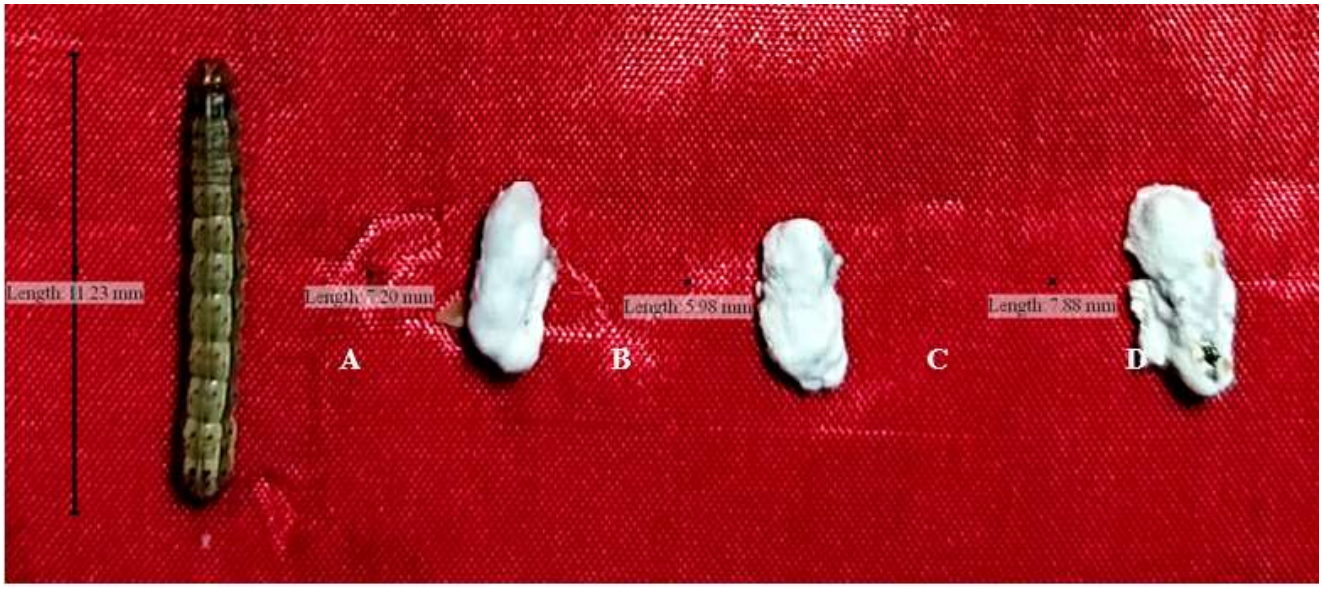


Figure 2. The cadavers from larvae fed on maize leaves uncolonized with fungi or control (A), and cadavers from larvae fed on maize leaves colonized by *Beauveria bassiana* of TaBrPGA isolate (B), LtApPGA (C), and TaTtLH isolates (D)

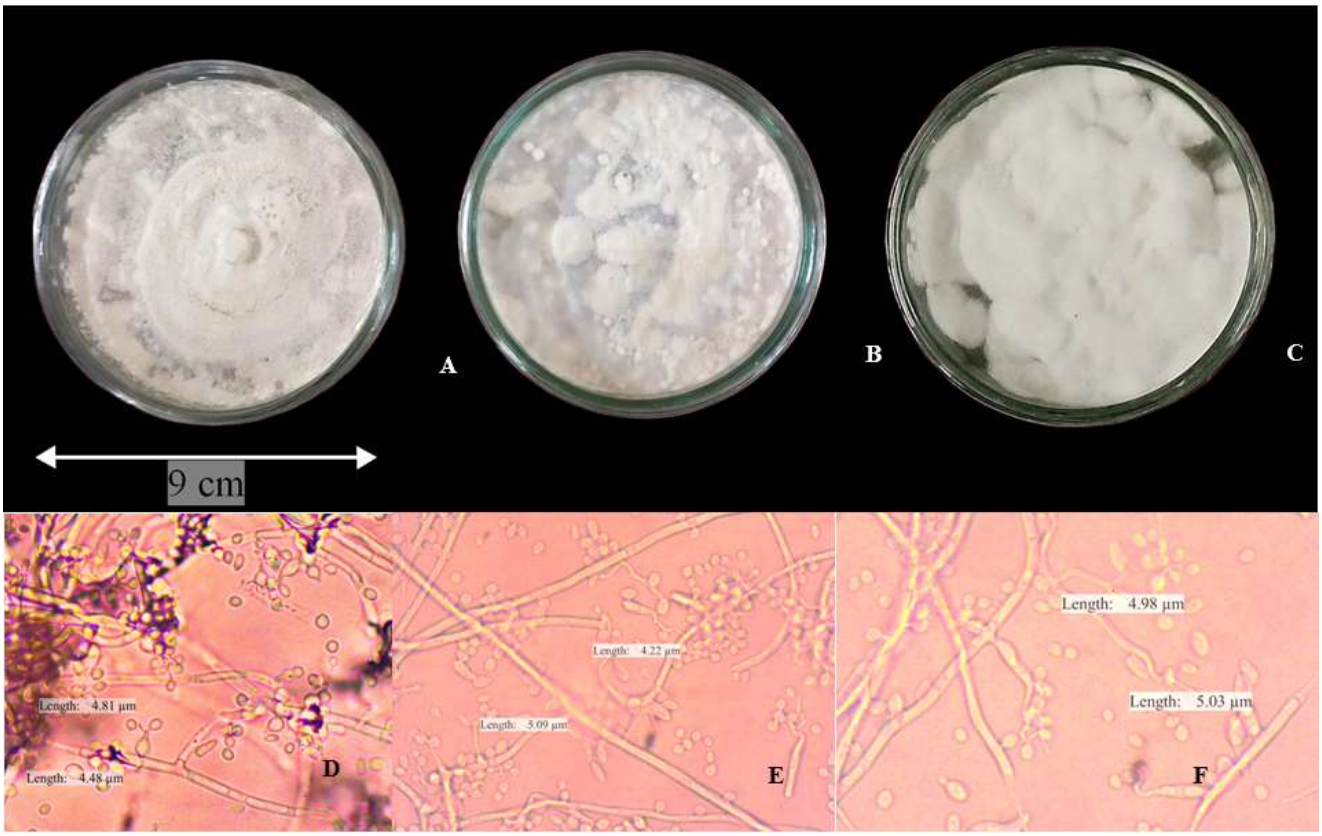


Figure 3. Colony morphology of endophytic fungi on SDA media (above) isolated from cadavers, and conidial and hyphal morphology (below) of fungi: *Beauveria bassiana* of TaBrPGA isolate (A and D), LtApPGA (B and E), and TaTtLH isolate (C and F)

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 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 (Carolina 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 are 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).

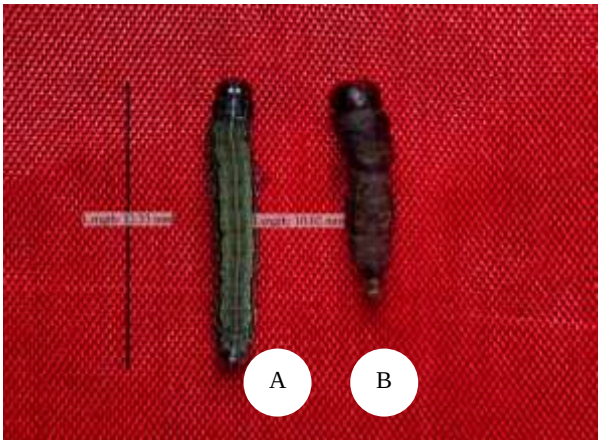


Figure 4. Larval *Spodoptera frugiperda*: healthy larvae (A) and larvae infected by endophytic *Beauveria bassiana* (B)

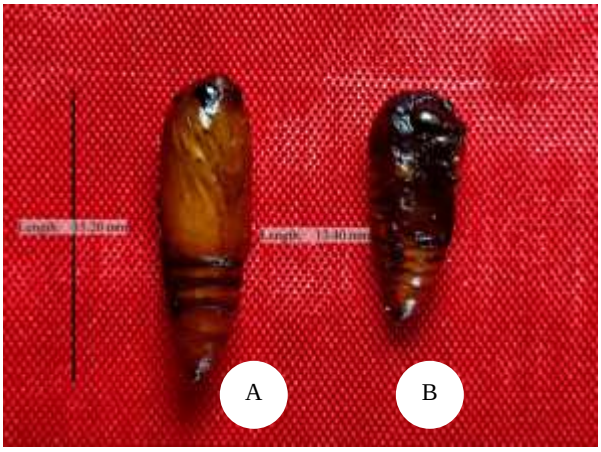


Figure 5. Pupal *Spodoptera frugiperda*: healthy pupae (A) and pupae infected by endophytic *Beauveria bassiana* (B)

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

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.

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. S.*

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.

ACKNOWLEDGEMENTS

This research was funded by the DIPA of Universitas Sriwijaya 2021, contract number: SP DIPA-023.17.2.677515/2021, November 23th, 2020, in line with Rector's Decree Number: 0014/UN9/SK.LP2M.PT/2021, May 25th, 2021, chaired by Siti Herlinda. We would like to thank the Rector of Universitas Sriwijaya for funding this research.

REFERENCES

Carolina A, Silva L, Silva GA, Henrique P, Abib N, Carolino AT, et al. 2020. Endophytic colonization of tomato plants by the entomopathogenic fungus Beauveria bassiana for controlling the South American tomato pinworm, *Tuta absoluta*. CABI Agric Biosci 1: 1-9. DOI: 10.1186/s43170-020-00002-x.

Early R, González-Moreno P, Murphy ST, Day R. 2018. Forecasting the global extent of invasion of the cereal pest *Spodoptera frugiperda*, the fall armyworm. NeoBiota 40: 25-50. DOI: http://hdl.handle.net/10871/34861.

Ganiger PC, Yeshwanth HM, Muralimohan K, Vinay N, Kumar AR V, Chandrashekara K. 2018. Occurrence of the new invasive pest, fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), in the maize fields of Karnataka, India. Curr Sci 115: 621-623.

Goergen G, Kumar PL, Sankung SB, Togola A, Tam M. 2016. First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (J E Smith) (Lepidoptera , Noctuidae), a new alien invasive pest in West and Central Africa. PLoS One 11: 1-9.

De Groote H, Kimenju SC, Munyua B, Palmas S, Kassie M, Bruce A. 2020. Spread and impact of fall armyworm (*Spodoptera frugiperda* J.E. Smith) in maize production areas of Kenya. Agric Ecosyst Environ 292: 1-10. DOI: 10.1016/j.agee.2019.106804.

Gustianingtyas M, Herlinda S, Suwandi S. 2021. The endophytic fungi from South Sumatra (Indonesia) and their pathogenecity against the new invasive fall armyworm, *Spodoptera frugiperda*. Biodiversitas 22: 1051-1062. doi: 10.13057/biodiv/d220262.

Harrison RD, Thierfelder C, Baudron F, Chinwada P, Midega C, Scha U, et al. 2019. Agro-ecological options for fall armyworm (*Spodoptera frugiperda* JE Smith) management: Providing low-cost, smallholder friendly solutions to an invasive pest. J Environ Manage 243: 318-330. DOI: 10.1016/j.jenvman.2019.05.011.

Herlinda S, Gustianingtyas M, Suwandi S, Suharjo R, Sari JMP, Lestari RP. 2021. Endophytic fungi confirmed as entomopathogens of the new invasive pest, the fall armyworm, *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae), infesting maize in South Sumatra, Indonesia. Egypt J Biol Pest Control 31: 1-13. DOI: 10.1186/s41938-021-00470-x.

Herlinda S, Gustianingtyas M, Suwandi S, Suharjo R, Sari JMP, Suparman, Hamidson H, Hasyim H. 2022. Endophytic fungi from South Sumatra (Indonesia) in seed-treated corn suppressing *Spodoptera frugiperda* growth. Biodiversitas 23: 6013-6020. DOI: 10.13057/biodiv/d231156.

Herlinda S, Octariati N, Suwandi S, Hasbi. 2020. Exploring entomopathogenic fungi from South Sumatra (Indonesia) soil and their pathogenicity against a new invasive maize pest, *Spodoptera frugiperda*. Biodiversitas 21: 2955-2965. DOI: 10.13057/biodiv/d210711.

Herlinda S, Suharjo R, Sinaga ME, Fawwazi F, Suwandi S. 2022. First report of occurrence of corn and rice strains of fall armyworm, *Spodoptera frugiperda* in South Sumatra, Indonesia and its damage in maize. J Saudi Soc Agric Sci 21: 412-419. DOI: 10.1016/j.jssas.2021.11.003.

Jaber LR, Ownley BH. 2018. Can we use entomopathogenic fungi as endophytes for dual biological control of insect pests and plant pathogens? Biol Control 116: 36-45. DOI: 10.1016/j.biocontrol.2017.01.018.

Kumela T, Simiyu J, Sisay B, Likhayo P, Gohole L, Tefera T. 2018. Farmers knowledge, perceptions, and management practices of the new invasive pest, fall armyworm (*Spodoptera frugiperda*) in Ethiopia and Kenya. Int J Pest Manag 65: 1-9. DOI: 10.1080/09670874.2017.1423129.

Lestari YA, Verawaty M, Herlinda S. 2022. Development of *Spodoptera frugiperda* fed on young maize plant's fresh leaves inoculated with endophytic fungi from South Sumatra, Indonesia. Biodi 23: 5056-5063. DOI: 10.13057/biodiv/d231012.

Lira AC de, Mascarin GM, Júnior ID. 2020. Microsclerotia production of *Metarhizium* spp. for dual role as plant biostimulant and control of Spodoptera frugiperda through corn seed coating. Fungal Biol 124: 689-699. DOI: 10.1016/j.funbio.2020.03.011.

Maharani Y, Dewi VK, Puspasari LT, Rizkie L, Hidayat Y, Dono D. 2019. Cases of fall army worm *Spodoptera frugiperda* J. E. Smith (Lepidoptera: Noctuidae) attack on maize in Bandung, Garut and Sumedang District, West Java. Crop - J Plant Prot 2: 38. doi: 10.24198/cropsaver.v2i1.23013.

Mancillas-Paredes JM, Hernández-Sánchez H, Jaramillo-Flores ME, García-Gutiérrez C. 2019. Proteases and chitinases induced in *Beauveria bassiana* during infection by *Zabrotes subfasciatus*. Southwest Entomol 44: 125-137. DOI: 10.3958/059.044.0114.

Montezano DG, Specht A, Sosa-gómez DR, Brasília U De. 2018. Host plants of Spodoptera frugiperda (Lepidoptera : Noctuidae) in the Americas. African Entomol 26: 286-300. DOI: 10.4001/003.026.0286.

Mukkun L, Kleden YL, Simamora AV. 2021. Detection of *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) in maize field in East Flores District, East Nusa Tenggara Province, Indonesia. Intl J Trop Drylands 5: 20-26. DOI: 10.13057/tropdrylands/t050104.

Novianti V, Indradewa D, Maryani, Rachmawati D. 2020. Selection of local swamp rice cultivars from Kalimantan (Indonesia) tolerant to iron stress during vegetative stage. Biodiversitas 21: 5650-5661. DOI: 10.13057/biodiv/d211210.

Otim MH, Tay WT, Walsh TK, Kanyesigye D, Adumo S, Abongosi J, et al. 2018. Detection of sister-species in invasive populations of the fall armyworm *Spodoptera frugiperda* (Lepidoptera : Noctuidae) from Uganda. PLoS One 13: 1-18. DOI: 10.1371/journal.pone.0194571.

Quesada-moraga E, Vey A. 2004. Bassiacridin, a protein toxic for locusts secreted by the entomopathogenic fungus *Beauveria bassiana*. Mycol Res 108: 441-452. DOI: 10.1017/S0953756204009724.

Ramanujam B, Poornesha B, Shylesha AN. 2020. Effect of entomopathogenic fungi against invasive pest *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera : Noctuidae) in maize. Egypt J Biol Pest Control 30: 1-5. DOI: 10.1186/s41938-020-00291-4.

Ramos Y, Taibo AD, Jiménez JA, Portal O. 2020. Endophytic establishment of Beauveria bassiana and Metarhizium anisopliae in maize plants and its effect against *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) larvae. Egypt J Biol Pest Control 30: 1-6. DOI: 10.1186/s41938-020-00223-2.

Russo ML, Jaber LR, Scorsetti AC, Vianna F, Cabello MN, Pelizza SA. 2020. Effect of entomopathogenic fungi introduced as corn endophytes on the development, reproduction, and food preference of the invasive fall armyworm *Spodoptera frugiperda*. J Pest Sci 2004 93: 1-12. DOI: 10.1007/s10340-020-01302-x.

Safavi SA. 2012. In vitro and in vivo induction, and characterization of Beauvericin isolated from *Beauveria bassiana* and its bioassay on *Galleria mellonell*a larvae. J Agric Sci Technol 15: 1-10.

Sari JMP, Herlinda S, Suwandi S. 2022. Endophytic fungi from South Sumatra (Indonesia) in seed- treated corn seedlings Affecting development of the fall armyworm, *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae). Egypt J Biol Pest Control 32: 1-11. DOI: 10.1186/s41938-022-00605-8.

Sartiami D, Dadang, Harahap I, Kusumah Y, Anwar R. 2020. First record of fall armyworm (Spodoptera frugiperda) in Indonesia and its occurence in three provinces. In: IOP Conf. Ser.: Earth Environ. Sci. 468 012021. pp 1-8. DOI: 10.1088/1755-1315/468/1/012021.

Sisay B, Simiyu J, Mendesil E, Likhayo P, Ayalew G, Mohamed S, et al. 2019. Fall armyworm, *Spodoptera frugiperda* infestations in East Africa: Assessment of damage and parasitism. Insects 10: 1-10. DOI: 10.3390/insects10070195.

Supartha IW, Susila IW, Sunari AAAAS, Mahaputra IGF, Yudha IKW, Wiradana PA. 2021. Damage characteristics and distribution patterns of invasive pest, *Spodoptera frugiperda* (J.E Smith) (Lepidoptera: Noctuidae) on maize crop in Bali, Indonesia. Biodiversitas 22: 3378-3387. DOI: 10.13057/biodiv/d2206xx.

Vidal S, Jaber LR. 2015. Entomopathogenic fungi as endophytes: Plant-endophyte-herbivore interactions and prospects for use in biological control. Curr Sci 109: 46-54.

Zhang D, Xiao Y, Xu P, Yang X, Wu Q, Wu K. 2021. Insecticide resistance monitoring for the invasive populations of fall armyworm, *Spodoptera frugiperda* in China. J Integr Agric 20: 783-791. DOI: 10.1016/S2095-3119(20)63392-5.

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. Palembang 30139, South Sumatera, Indonesia.

[2]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya. Indralaya 30662, South Sumatra, Indonesia

[3]Department of Plant Protection, Faculty of Agriculture, Universitas Sriwijaya. Indralaya 30662, South Sumatra, Indonesia. Tel./fax. +62-711-580059, ♥email: sitiherlinda@unsri.ac.id

[4]Research Center for Sub-optimal Lands (PUR-PLSO), Universitas Sriwijaya, Palembang 30139, South Sumatera, Indonesia.

Manuscript received: xxx 2022. Revision accepted: xxx December 2022.

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: xxxx*. 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 fungus. 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. *B. bassiana* could decrease 86% of adult emergence. *B. bassiana* applied in seed treatment could protect since the 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: Endophytes, entomopathogen, neonate larvae, *Spodoptera frugiperda*, seed treatment, corn

BIODIVERSITAS ISSN: 1412-033X

Volume 23, Number 12, December 2022 E-ISSN: 2085-4722

Pages: xxxx DOI: 10.13057/biodiv/d2312xx

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 is 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 the 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 millions per year in Africa (Harrison et al. 2019). In Indonesia, FAW severely attacked corn and caused up to100% 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: Hypomycetes) 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). *B. bassiana* isolated from plants from South Sumatra that was 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 have been mass-reared in the laboratory few years ago (Herlinda et al. 2020) and *S. frugiperda* was identified molecularly (Herlinda et al. 2022a). The FAW were 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 soloution 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 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* were 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 were 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. frugiperga* 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 was an endophytic entomopathogenic fungus.

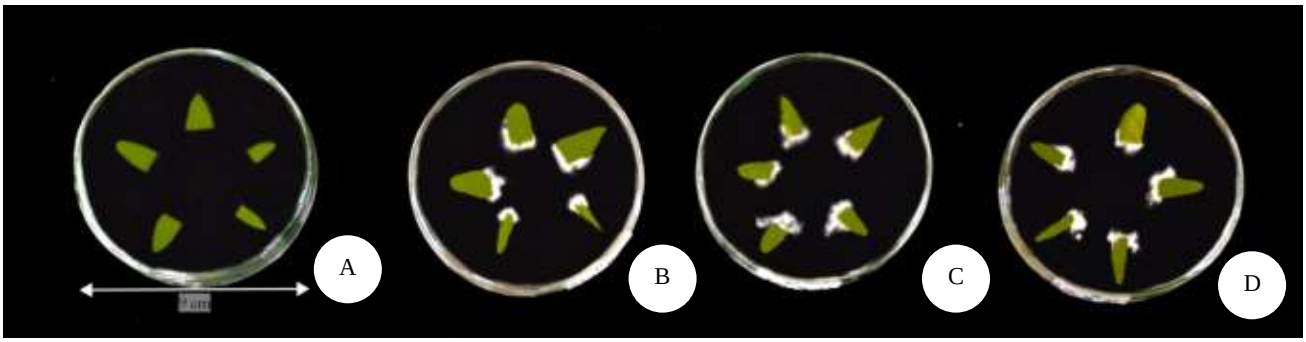


Figure 1. Colony morphology of endophytic fungi from the leaves of maize: Control (A), *Beauveria bassiana* of TaBrPGA isolate (B), LtApPGA (C), and TaTtLH isolates (D)

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

		Mean colonization (%)	
Isolates	**Species**	**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 *Spodoptera 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 was, 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

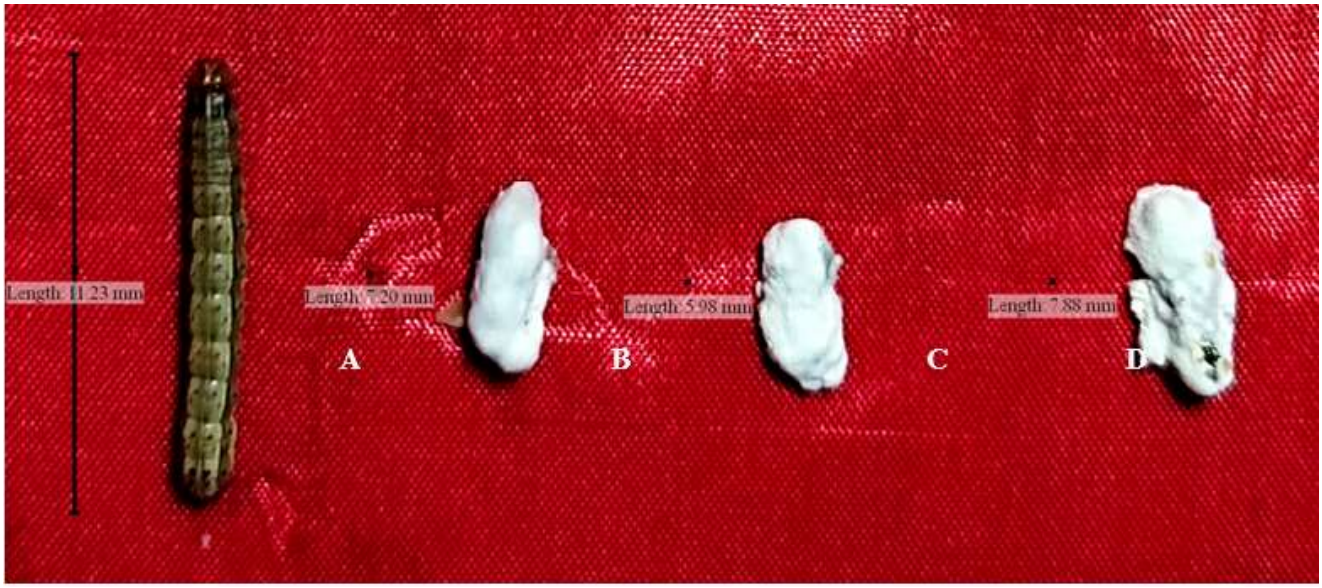


Figure 2. The cadavers from larvae fed on maize leaves uncolonized with fungi or control (A), and cadavers from larvae fed on maize leaves colonized by *Beauveria bassiana* of TaBrPGA isolate (B), LtApPGA (C), and TaTtLH isolates (D)

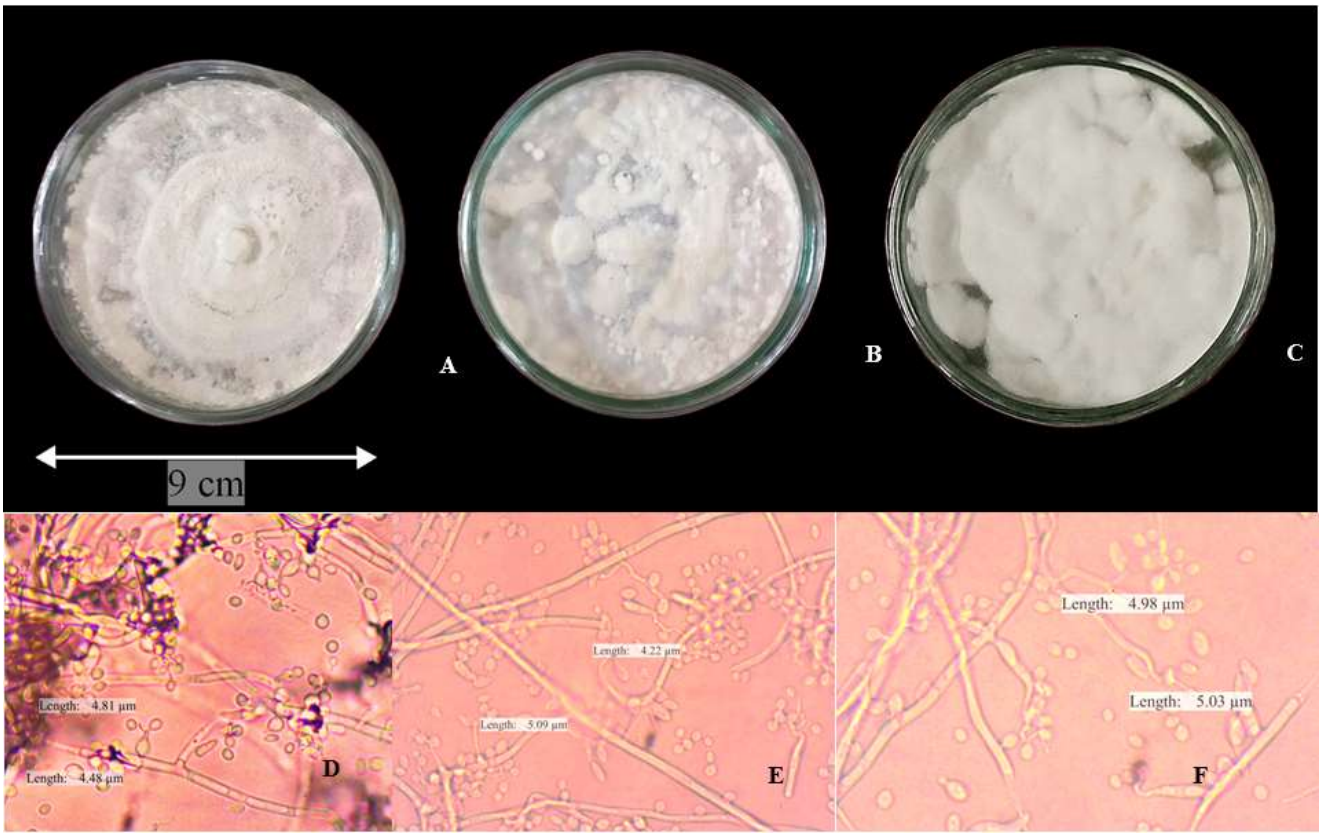


Figure 3. Colony morphology of endophytic fungi on SDA media (above) isolated from cadavers, and conidial and hyphal morphology (below) of fungi: *Beauveria bassiana* of TaBrPGA isolate (A and D), LtApPGA (B and E), and TaTtLH isolate (C and F)

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 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 (Carolina 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 are 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).

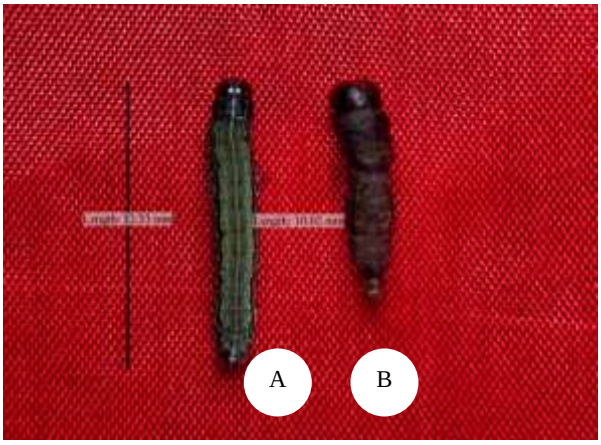


Figure 4. Larval *Spodoptera frugiperda*: healthy larvae (A) and larvae infected by endophytic *Beauveria bassiana* (B)

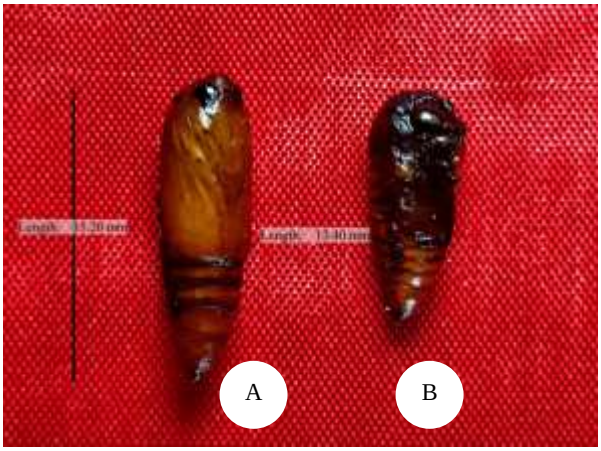


Figure 5. Pupal *Spodoptera frugiperda*: healthy pupae (A) and pupae infected by endophytic *Beauveria bassiana* (B)

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

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.

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*. *S. 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.

ACKNOWLEDGEMENTS

This research was funded by the DIPA of Universitas Sriwijaya 2021, contract number: SP DIPA-023.17.2.677515/2021, November 23th, 2020, in line with Rector's Decree Number: 0014/UN9/SK.LP2M.PT/2021, May 25th, 2021, chaired by Siti Herlinda. We would like to thank the Rector of Universitas Sriwijaya for funding this research.

REFERENCES

Carolina A, Silva L, Silva GA, Henrique P, Abib N, Carolino AT, et al. 2020. Endophytic colonization of tomato plants by the entomopathogenic fungus Beauveria bassiana for controlling the South American tomato pinworm, *Tuta absoluta*. CABI Agric Biosci 1: 1-9. DOI: 10.1186/s43170-020-00002-x.

Early R, González-Moreno P, Murphy ST, Day R. 2018. Forecasting the global extent of invasion of the cereal pest *Spodoptera frugiperda*, the fall armyworm. NeoBiota 40: 25-50. DOI: http://hdl.handle.net/10871/34861.

Ganiger PC, Yeshwanth HM, Muralimohan K, Vinay N, Kumar AR V, Chandrashekara K. 2018. Occurrence of the new invasive pest, fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), in the maize fields of Karnataka, India. Curr Sci 115: 621-623.

Goergen G, Kumar PL, Sankung SB, Togola A, Tam M. 2016. First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (J E Smith) (Lepidoptera , Noctuidae), a new alien invasive pest in West and Central Africa. PLoS One 11: 1-9.

De Groote H, Kimenju SC, Munyua B, Palmas S, Kassie M, Bruce A. 2020. Spread and impact of fall armyworm (*Spodoptera frugiperda* J.E. Smith) in maize production areas of Kenya. Agric Ecosyst Environ 292: 1-10. DOI: 10.1016/j.agee.2019.106804.

Gustianingtyas M, Herlinda S, Suwandi S. 2021. The endophytic fungi from South Sumatra (Indonesia) and their pathogenecity against the new invasive fall armyworm, *Spodoptera frugiperda*. Biodiversitas 22: 1051-1062. doi: 10.13057/biodiv/d220262.

Harrison RD, Thierfelder C, Baudron F, Chinwada P, Midega C, Scha U, et al. 2019. Agro-ecological options for fall armyworm (*Spodoptera frugiperda* JE Smith) management: Providing low-cost, smallholder friendly solutions to an invasive pest. J Environ Manage 243: 318-330. DOI: 10.1016/j.jenvman.2019.05.011.

Herlinda S, Gustianingtyas M, Suwandi S, Suharjo R, Sari JMP, Lestari RP. 2021. Endophytic fungi confirmed as entomopathogens of the new invasive pest, the fall armyworm, *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae), infesting maize in South Sumatra, Indonesia. Egypt J Biol Pest Control 31: 1-13. DOI: 10.1186/s41938-021-00470-x.

Herlinda S, Gustianingtyas M, Suwandi S, Suharjo R, Sari JMP, Suparman, Hamidson H, Hasyim H. 2022. Endophytic fungi from South Sumatra (Indonesia) in seed-treated corn suppressing *Spodoptera frugiperda* growth. Biodiversitas 23: 6013-6020. DOI: 10.13057/biodiv/d231156.

Herlinda S, Octariati N, Suwandi S, Hasbi. 2020. Exploring entomopathogenic fungi from South Sumatra (Indonesia) soil and their pathogenicity against a new invasive maize pest, *Spodoptera frugiperda*. Biodiversitas 21: 2955-2965. DOI: 10.13057/biodiv/d210711.

Herlinda S, Suharjo R, Sinaga ME, Fawwazi F, Suwandi S. 2022. First report of occurrence of corn and rice strains of fall armyworm, *Spodoptera frugiperda* in South Sumatra, Indonesia and its damage in maize. J Saudi Soc Agric Sci 21: 412-419. DOI: 10.1016/j.jssas.2021.11.003.

Jaber LR, Ownley BH. 2018. Can we use entomopathogenic fungi as endophytes for dual biological control of insect pests and plant pathogens? Biol Control 116: 36-45. DOI: 10.1016/j.biocontrol.2017.01.018.

Kumela T, Simiyu J, Sisay B, Likhayo P, Gohole L, Tefera T. 2018. Farmers knowledge, perceptions, and management practices of the new invasive pest, fall armyworm (*Spodoptera frugiperda*) in Ethiopia and Kenya. Int J Pest Manag 65: 1-9. DOI: 10.1080/09670874.2017.1423129.

Lestari YA, Verawaty M, Herlinda S. 2022. Development of *Spodoptera frugiperda* fed on young maize plant's fresh leaves inoculated with endophytic fungi from South Sumatra, Indonesia. Biodiversitas 23: 5056-5063. DOI: 10.13057/biodiv/d231012.

Lira AC de, Mascarin GM, Júnior ID. 2020. Microsclerotia production of *Metarhizium* spp. for dual role as plant biostimulant and control of *Spodoptera frugiperda* through corn seed coating. Fungal Biol 124: 689-699. DOI: 10.1016/j.funbio.2020.03.011.

Maharani Y, Dewi VK, Puspasari LT, Rizkie L, Hidayat Y, Dono D. 2019. Cases of fall army worm *Spodoptera frugiperda* J. E. Smith (Lepidoptera: Noctuidae) attack on maize in Bandung, Garut and Sumedang District, West Java. Crop - J Plant Prot 2: 38. DOI: 10.24198/cropsaver.v2i1.23013.

Mancillas-Paredes JM, Hernández-Sánchez H, Jaramillo-Flores ME, García-Gutiérrez C. 2019. Proteases and chitinases induced in

Beauveria bassiana during infection by *Zabrotes subfasciatus*. Southwest Entomol 44: 125-137. DOI: 10.3958/059.044.0114.

Montezano DG, Specht A, Sosa-gómez DR, Brasília U De. 2018. Host plants of *Spodoptera frugiperda* (Lepidoptera : Noctuidae) in the Americas. African Entomol 26: 286-300. DOI: 10.4001/003.026.0286.

Mukkun L, Kleden YL, Simamora AV. 2021. Detection of *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) in maize field in East Flores District, East Nusa Tenggara Province, Indonesia. Intl J Trop Drylands 5: 20-26. DOI: 10.13057/tropdrylands/t050104.

Novianti V, Indradewa D, Maryani, Rachmawati D. 2020. Selection of local swamp rice cultivars from Kalimantan (Indonesia) tolerant to iron stress during vegetative stage. Biodiversitas 21: 5650-5661. DOI: 10.13057/biodiv/d211210.

Otim MH, Tay WT, Walsh TK, Kanyesigye D, Adumo S, Abongosi J, et al. 2018. Detection of sister-species in invasive populations of the fall armyworm *Spodoptera frugiperda* (Lepidoptera : Noctuidae) from Uganda. PLoS One 13: 1-18. DOI: 10.1371/journal.pone.0194571.

Quesada-moraga E, Vey A. 2004. Bassiacridin, a protein toxic for locusts secreted by the entomopathogenic fungus *Beauveria bassiana*. Mycol Res 108: 441-452. DOI: 10.1017/S0953756204009724.

Ramanujam B, Poornesha B, Shylesha AN. 2020. Effect of entomopathogenic fungi against invasive pest *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera : Noctuidae) in maize. Egypt J Biol Pest Control 30: 1-5. DOI: 10.1186/s41938-020-00291-4.

Ramos Y, Taibo AD, Jiménez JA, Portal O. 2020. Endophytic establishment of Beauveria bassiana and Metarhizium anisopliae in maize plants and its effect against *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) larvae. Egypt J Biol Pest Control 30: 1-6. DOI: 10.1186/s41938-020-00223-2.

Russo ML, Jaber LR, Scorsetti AC, Vianna F, Cabello MN, Pelizza SA. 2020. Effect of entomopathogenic fungi introduced as corn endophytes on the development, reproduction, and food preference of the invasive fall armyworm *Spodoptera frugiperda*. J Pest Sci 2004 93: 1-12. DOI: 10.1007/s10340-020-01302-x.

Safavi SA. 2012. In vitro and in vivo induction, and characterization of Beauvericin isolated from *Beauveria bassiana* and its bioassay on *Galleria mellonell*a larvae. J Agric Sci Technol 15: 1-10.

Sari JMP, Herlinda S, Suwandi S. 2022. Endophytic fungi from South Sumatra (Indonesia) in seed- treated corn seedlings Affecting development of the fall armyworm, *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae). Egypt J Biol Pest Control 32: 1-11. DOI: 10.1186/s41938-022-00605-8.

Sartiami D, Dadang, Harahap I, Kusumah Y, Anwar R. 2020. First record of fall armyworm (Spodoptera frugiperda) in Indonesia and its occurence in three provinces. In: IOP Conf. Ser.: Earth Environ. Sci. 468 012021. pp 1-8. DOI: 10.1088/1755-1315/468/1/012021.

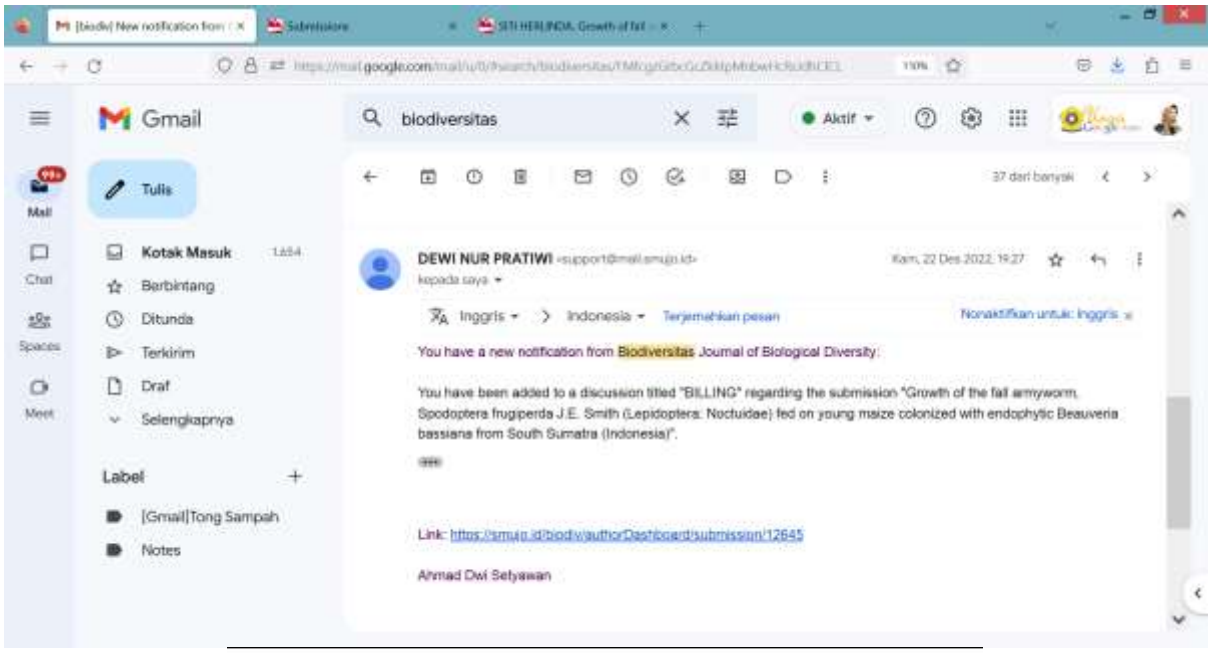
Sisay B, Simiyu J, Mendesil E, Likhayo P, Ayalew G, Mohamed S, et al. 2019. Fall armyworm, *Spodoptera frugiperda* infestations in East Africa: Assessment of damage and parasitism. Insects 10: 1-10. DOI: 10.3390/insects10070195.

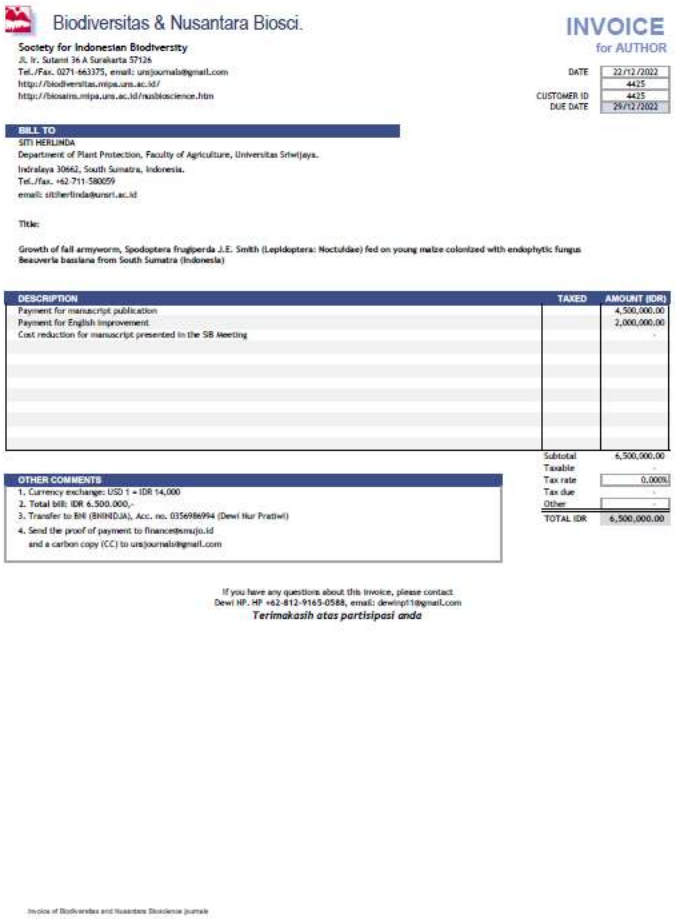
Supartha IW, Susila IW, Sunari AAAAS, Mahaputra IGF, Yudha IKW, Wiradana PA. 2021. Damage characteristics and distribution patterns of invasive pest, *Spodoptera frugiperda* (J.E Smith) (Lepidoptera: Noctuidae) on maize crop in Bali, Indonesia. Biodiversitas 22: 3378-3387. DOI: 10.13057/biodiv/d2206xx.

Vidal S, Jaber LR. 2015. Entomopathogenic fungi as endophytes: Plant-endophyte-herbivore interactions and prospects for use in biological control. Curr Sci 109: 46-54.

Zhang D, Xiao Y, Xu P, Yang X, Wu Q, Wu K. 2021. Insecticide resistance monitoring for the invasive populations of fall armyworm, *Spodoptera frugiperda* in China. J Integr Agric 20: 783-791. DOI: 10.1016/S2095-3119(20)63392-5.

4. Bukti tagihan untuk penerbitan artikel 22 Desember 2022




Biodiversitas & Nusantara Biosci.

Society for Indonesian Biodiversity
Jl. Ir. Sutami 36 A Surakarta 57126
Tel./Fax. 0271-663375, email: unsjournals@gmail.com
http://biodiversitas.mipa.uns.ac.id/
http://biosains.mipa.uns.ac.id/nusbioscience.htm

INVOICE
for AUTHOR

DATE	22/12/2022
	4425
CUSTOMER ID	4425
DUE DATE	29/12/2022

BILL TO
SITI HERLINDA
Department of Plant Protection, Faculty of Agriculture, Universitas Sriwijaya.
Indralaya 30662, South Sumatra, Indonesia.
Tel./fax. +62 711 580059
email: sitiherlinda@unsri.ac.id

Title:

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)

DESCRIPTION	TAXED	AMOUNT (IDR)
Payment for manuscript publication		4,500,000.00
Payment for English improvement		2,000,000.00
Cost reduction for manuscript presented in the SB Meeting		-

Subtotal	6,500,000.00
Taxable	-
Tax rate	0.000%
Tax due	-
Other	-
TOTAL IDR	6,500,000.00

OTHER COMMENTS
1. Currency exchange: USD 1 = IDR 14,000
2. Total bill: IDR 6,500,000,-
3. Transfer to BNI (BNINIDJA), Acc. no. 0356986994 (Dewi Nur Pratiwi)
4. Send the proof of payment to finance@smujo.id and a carbon copy (CC) to unsjournals@gmail.com

If you have any questions about this invoice, please contact
Dewi HP. HP +62-812-9165-0588, email: dewinp10@gmail.com
Terimakasih atas partisipasi anda

Invoice of Biodiversitas and Nusantara Biosciences journals