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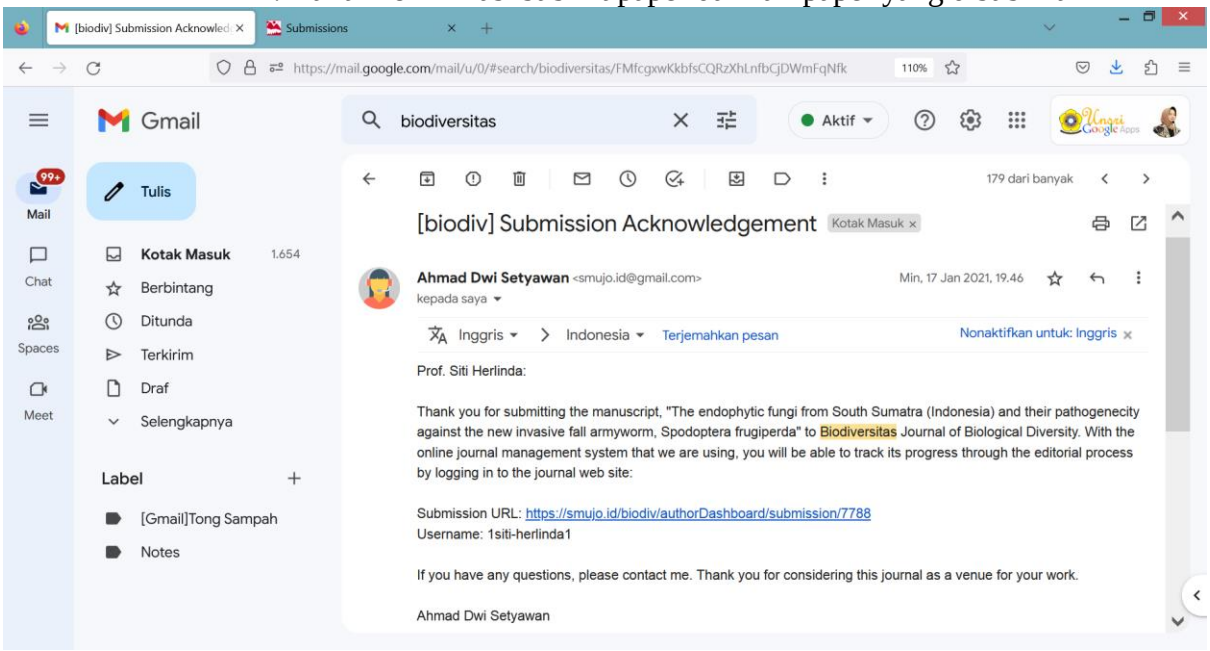

COVERING LETTER

Dear **Editor-in-Chief,**

I herewith enclosed a research article,

Title:

The endophytic fungi from South Sumatra (Indonesia) and their pathogenecity against the new invasive fall armyworm, *Spodoptera frugiperda*

Author(s) name:

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This study highlights several findings, such as we found four new emerging endophytic fungi, *Aspergillus* sp., *Beauveria* sp., *Chaetomium* sp., and *Curvularia* sp. The fungi isolated from the roots of maize, banana, and chili from the lowlands to highlands of South Sumatra are pathogenic against the new invasive fall armyworm, *Spodoptera frugiperda.* First report of *Aspergillus* sp., *Chaetomium* sp., and *Curvularia* sp. have insecticidal activity against *S. frugiperda.*

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The endophytic fungi from South Sumatra (Indonesia) and their pathogenecity against the new invasive fall armyworm, *Spodoptera frugiperda*

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Abstract. Maize in Indonesia is currently experiencing attacks and outbreaks of the new invasive fall armyworm, *Spodoptera frugiperda*. The *S. frugiperda* larvae emerge from the leaf midrib when eating, after hiding in the maize stalk so that it is difficult to control by contact. This study aimed to find out the endophytic fungi from the roots of maize, banana and chili in South Sumatra and to determine their pathogenicity against *S. frugiperda* larvae. The endophytic fungi were isolated from the plant roots. Fungal isolates proven to be endophytic were dropped (1×10^6 conidia mL^{-1}) on the second instar larvae. The result showed that the endophytic fungi found were 8 isolates consisting of the genus, *Aspergillus* sp., *Beauveria* sp., *Chaetomium* sp., and *Curvularia* sp. First report of *Aspergillus* sp., *Chaetomium* sp., and *Curvularia* sp. have insecticidal activity against *S. frugiperda* larvae. However, the two most pathogenic isolates were JgCrJr and JgSPK isolates of *Beauveria* sp. with a larval mortality of 29.33% and 26.67%, respectively and could reduce the emergence of *S. frugiperda* adults up to 44%. So, the two isolates of *Beauveria* sp. have a high potential to be developed to control *S. frugiperda* larvae in maize both in the lowlands and the highlands.

Key words: *Aspergillus* sp., *Beauveria* sp., *Chaetomium* sp., *Curvularia* sp., insecticidal activity

Running title: The endophytic fungi against *Spodoptera frugiperda*

INTRODUCTION

Maize in Indonesia is currently facing a big problem in invasion and outbreaks of newcomer insect pests, namely the fall armyworm (*Spodoptera frugiperda*) (Lepidoptera: Noctuidae). *S. frugiperda* comes from South America (Nagoshi et al. 2017; Otim et al. 2018) and entered Indonesia for the first time on March 26, 2019 in West Sumatra, then in June 2019 it was found in Banten and West Java (Sartiami et al. 2020) and now it has spread rapidly to various provinces in Indonesia, such as South Sumatra (Herlinda et al. 2020b; Hutasoit et al. 2020), Lampung (Lestari et al. 2020), and Bengkulu (Ginting et al. 2020). The fall armyworm has caused maize yield losses in Africa of 250–630 million US dollars per year (Bateman et al. 2018). Kenya lost maize production of up to 1 million tons per year (De Groote et al. 2020). In Indonesia the pest was reported to attack both hybrid maize and local maize varieties (Ginting et al.2020). The pest are polyphagous because they are able to attack and to damage various species of plants from various families, for example maize, rice, sugar cane, cotton, and ornamental plants (Montezano et al. 2018). The *S. frugiperda* larvae can eat greedily on leaves, stems, flowers, fruit, growing points, fruit, and the whole maize until it is bare (Ginting et al. 2020).

To overcome the invasion and outbreaks of *S. frugiperda*, the synthetic insecticides are generally used in the world (Tambo et al. 2020). The synthetic insecticides of organophosphates and carbamates (Boaventura et al. 2020) and other synthetic insecticides have been shown to be resistant to the fall armyworm (Gutiérrez-moreno et al. 2018) and even the entomopathogenic bacterium, *Bacillus thuringiensis* (Bt) can be broken by *S. frugiperda* (Flagel et al. 2018). Another control method that has not shown resistance is the use of the entomopathogenic fungi (fungi causing disease in insects). The entomopathogenic fungi that have been shown to be effective at killing the insect pests of the genus *Spodoptera* are *Beauveria bassiana*, *Metarhizium anisopliae* (Ayudya et al. 2019; Gustianingtyas et al. 2020), *Penicillium citrinum*, and *Talaromyces diversus* (Herlinda et al. 2020a). *S. frugiperda* was also killed by *B. bassiana*, *M. anisopliae*, *Metarhizium rileyi* (Ramanujam et al. 2020), and *Metarhizium* spp. (Herlinda et al. 2020b). The entomopathogenic fungus species effectively killed *S. frugiperda* larvae by contact (Herlinda et al. 2020b). If the mode of action of the fungus is contact only, the fungus is not very effective in controlling *S. frugiperda* larvae hidden in maize leaf midribs because the larvae

only appear when eating leaves in the morning (Bentivenha et al. 2017). In the field, the *S. frugiperda* larvae were found appearing on leaf surfaces from 6.30 a.m. to 8.00 a.m. To control the larvae of *S. frugiperda*, it is more effective to use an endophytic entomopathogenic fungus because the endophytic fungi are those that systemically colonize host plant tissues, associating mutually, and without being pathogenic to the host plants (Lira et al. 2020; Kasambala et al. 2018). The endophytic fungus has many advantages, apart from having a mode of action through stomach poison (Russo et al. 2020), it can also kill by contact (Ramirez-Rodriguez and Sánchez-Peña 2016), and it can also stimulate plant growth (Jaber and Ownley 2018; Ahmad et al. 2020; Bamisile et al. 2020; Barra-Bucarei et al. 2020). The endophytic fungi pathogenic to *S. frugiperda* larvae need to be found from maize and other plant tissues in Indonesia, especially in South Sumatra and are expected to be potential alternatives to the use of synthetic insecticides. The objectives of this research were to find out the endophytic fungi from maize, banana and chili roots around the maize ecosystem in South Sumatra and to determine their pathogenicity against *S. frugiperda* larvae.

MATERIALS AND METHODS

This study has been conducted at the Entomology Laboratory, Department of Pests and Plant Diseases, Faculty of Agriculture, Sriwijaya University from February to December 2020. The maize cultivation for *S. frugiperda* mass rearing has been conducted from February to December 2020 and the mass rearing from April to November 2020. The fungi exploration and identification have been performed since April 2020. The fungi were identified at the Laboratory of Agricutural Biotecnology (accredited according to the ISO/IEC 17025 standard), Department of Plant Protection, Faculty of Agriculture, Universitas Lampung, Indonesia. The bioassay was conducted from October to December 2020. It was carried out in an incubator at a constant temperature and relative humidity (RH), namely 30 °C and 93%, respectively. All endophytic fungal isolates used in this experiment were explored from the lowlands to highlands of South Sumatra, Indonesia.

Exploration, isolation, and purification of endophytic fungus

The exploration of endophytic fungi was carried out by taking the roots of maize, bananas and vegetables (chili) around the maize ecosystem. The survey locations for sampling the fungi were carried out in maize production centers in South Sumatra from lowlands to highlands (Table 1). The individual plants selected following the method of Kasambala et al. (2018) that had the most healthy characteristics, and were not attacked by pests or diseases. Parts of plant tissues taken were the roots of maize, bananas and vegetables (chili) around the maize ecosystem. Furthermore, the root samples were wrapped in sterile straw paper and given the code name of the plant, location, date of sampling, and soil pH then put into a plastic zipper and placed in an ice box, then taken to the laboratory.

In the laboratory the plant root samples were washed using aseptically under running tap water. The surface sterilization and sample isolation were carried out to avoid unwanted airborne microspore contamination. In the laminar air flow cabinet the plant roots were cut to a size of 0.5 cm x 0.5 cm, then the surface was sterilized, modifying the method of Elfita et al. (2019) by immersing plant tissue in 70% EtOH (Ethyl alcohol) for 2 minutes, then dipping it in 1% NaOCl (Sodium hypochlorite) for 1 minute, then rinsed three times in the sterile distilled water for 1 minute. To determine the success of this surface sterilization, the last rinse was grown onto Potato Dextrose Agar (PDA) which modified the method of Russo et al. (2020). If the PDA media did not grow the microorganisms, it meant that the surface sterilization was successful (Ramirez-Rodriguez and Sánchez-Peña 2016).

The surface of the sterile roots was isolated following the method of Elfita et al. (2019) in the laminar air flow cabinet by growing onto the malt extract agar (MEA) media. The MEA media was the specific selected media for growing fungi isolated from the root tissue (Silva *et al.*, 2018). The roots grown on the MEA media were as many as five pieces (5 mm in length and 1-5 mm in diameter) and incubated for 7 days at a room temperature. The fungus growing from the root was then purified to get an isolate. After the isolates were isolated, the fungal isolates aged 7 days were observed for their colony color and shape, hyphae and conidial shape, and continued with an assessment of their colonization ability to enter plant tissue.

Inoculation of endophytic fungi into plant tissue

The isolated fungi were then inoculated into the maize tissue to ensure that the fungus was endophytic. The maize seeds already sterilized using the Elfita et al. (2019) method were then soaked as many as 15 seeds in 10 mL of the fungi suspension with a concentration of 1 x 10^6 conidia mL^{-1} for 6 hours. The control seeds were not soaked with the fungal suspension but soaked in 10 mL of distilled water. All treatments (isolates and controls) in this experiment were repeated three times. Then, the seeds were grown in a sterile glass bottle (volume 250 mL), which is based on a sterile filter paper (whatman no. 42) moistened with 1 mL distilled water and incubated for 10 days in the sterile laminar flow cabinet. In the 10-day-old plants, the stem tissue was sliced crosswise and longitudinally with a thickness of 0.02 mm each and stained with 0.05% lactophenol trypan blue dye to be observed with a light microscope at 40 x magnification to detect the presence of penetrating endophytic fungal mycelium in the plant tissue. The plant tissue colonized by the endophytic fungi was evidenced by the presence of the fungal tissue in the form of mycelia which grew to fill the plant tissues. The fungi

proven to be endophytic were then observed for color and colony shape, hyphae and conidial shape, and the conidial size measure to obtain distinctive features used for species identification. The fungi were identified based on their morphological characteristics using the taxonomic books of Humber (2005) and El-Ghany (2015).

Calculation of conidial density and viability

Only the endophytic fungal isolates were used for bioassays against *S. frugiperda* larvae. Before the bioassay was carried out, first the density and viability of each isolate were calculated. The conidial density calculations were carried out on the endophytic fungi aged 7 days. The conidial density were enumerated following the method of Sumikarsih et al. (2019) using a haemocytometer and observed with a light microscope at 40 x magnification. The viability was observed by growing 1 mL fungal suspension (1 x 10^6 conidia mL^{-1}) in 2% agar-water medium, containing 2 g agar given 100 mL distilled water (w/v), then the culture was incubated for 1 x 24 hours and 2 x 24 hours. The culture was observed with a light microscope at 40 x magnification to determine the number of germinated and non-germinated spores/conidia.

Mass rearing of *Spodoptera frugiperda*

Before the bioassay of endophytic fungi against larvae of *spodoptera frugiperda* was conducted, the mass rearing of the test insects was carried out first. The insect used in this study was *S. frugiperda* taken from the farmers' maize farms. *S. frugiperda* was then taken to the laboratory to be maintained and mass reared. The insect mass-rearing modified the method of Herlinda et al. (2020b). In the laboratory, the *S. frugiperda* larvae were reared individually in porous plastic cups (Ø 6.5 cm, height 4.6 cm). In the cup, the maize leaves (2 cm x 5 cm) were added to feed *S. frugiperda* and the leaves were replaced every day with the fresh new ones. When the final instar larvae got into the pupae stage, they were transferred to a plastic container (Ø15 cm, height 25 cm) whose bottom was given sterile soil (5 cm in thickness). The container containing the pupae was placed in a gauze cage (30 x 30 x 30 cm^3), and in the gauze cage there were 10 maize leaves provided for laying eggs and replaced every day. The egg clutch that the female adults laid on the surface of the maize leaves was moved into the container containing kale leaves (*Ipomoea aquatica*) used to feed the first instar larvae. After the first instar molting, the second instar larvae up to the last instar were fed with young maize leaves and maintained individually in a porous plastic cup (Ø 6.5 cm, height 4.6 cm) because the second instar and so on were cannibalistic. The mass rearing was carried out until getting the third-generation culture. The second instar larvae aged 1 day were used for the bioassay.

The bioassay of endophytic fungi against larvae of *Spodoptera frugiperda*

Only the fungal isolates proven to be endophytic were tested for their pathogenicity against the second instar larvae of *S. frugiperda*. The bioassay of endophytic fungi against *S. frugiperda* larvae followed the method of Ramirez-Rodriguez and Sánchez-Peña (2016). The endophytic fungi were first propagated in PDA media. The endophytic fungi aged 7 days were made suspension with a density of 1 x 10^6 conidia mL^{-1}. Before dropping the fungi suspension, the larvae were fasted for 2 hours and weighed using the portable jewelry scale (capacity 30 g x 0.01 g). Then, 1 mL^{-1} of the fungus suspension was dripped topically to wet 25 *S. frugiperda* larvae, while the control ones were only dropped 1 mL^{-1} of the distilled water. This experiment was designed using completely randomized designs with treatments of isolates, three replications per treatment, and 25 larvae per replication. Furthermore, the larvae were put individually into porous plastic cups (Ø 6.5 cm, height 4.6 cm) and fed with maize leaves measuring 2 x 5 cm^2 per day per larvae. To measure the percentage of foliar damage caused by the larvae of *S. frugiperda*, the bioleaf application by Machado et al. (2016) was used. Each day the dead larvae were recorded and carried out for 12 days based on the previous studies by Herlinda et al. (2020b) and the dead larvae were grown in the agar-water medium to prove infection by the endophytic fungus. The number of larvae becoming pupae and adults that emerged was also counted. The number of dead larvae was used to calculate mortality, the Median Lethal Time (LT_{50}), and the 95% of Lethal Time (LT_{95}). The maize leaf area eaten, faecal weight and body weight of the larvae were measured daily from the first to the 12th day.

Data analysis

The difference in larvae weight data and area of the leaves eaten and feces produced every day among the treatments (isolates), as well as mortality and time of death (the LT_{50} and LT_{95}) larvae of *S. frugiperda*, the percentage of larvae becoming pupae and adults emerged were analyzed using analysis of variance (ANOVA). The Tukey's Honestly Significant Difference (HSD) test (Tukey's test) was employed to test for a significant difference among the treatments at P = 0.05. The LT_{50} and LT_{95} values were calculated using the probit analysis. The data were all calculated using software of SAS University Edition 2.7 9.4 M5.

RESULTS AND DISCUSSION

Endophytic fungi and their colonization on maize tissues

Of the 52 isolates of fungi obtained from the roots of maize, banana and vegetables (chili) around the maize ecosystem, there were only eight isolates confirmed as the endophytic fungi (Table 1). The endophytic fungi were evidenced by the

entry of the fungal tissue in the form of mycelia which grew to fill the plant tissue. The results of detection of fungal colonization in maize tissues showed differences from the controls (Figure 1). There was no colonization of endophytic fungi found in the untreated control plants. The plant tissue colonized by the endophytic fungi showed that mycelia grew to fill the plant tissue, while the control plant tissue was clean and no mycelium. The plants colonized by the endophytic fungi also showed a difference compared to the control plants (Figure 2), the inoculated plants tended to be taller with more roots and longer than the control plants.

Table 1. Species and isolates of entomopathogenic fungi found from soil in South Sumatra, Indonesia

District/City	Village	Crop plants	Fungal species	Isolate codes	Soil pH	Altitude (m)
Pagar Alam	Curup Jare	Maize	*Beauveria* sp.	JgCrJr	6.2	806.7
Pagar Alam	Simpang Padang Karet	Maize	*Beauveria* sp.	JgSPK	6.4	797.7
Ogan Ilir	Tanjung Pering	Banana	*Aspergillus* sp.	PsgTjPr	7.0	36.00
Banyuasin	Banyu Urip	Maize	*Aspergillus* sp.	JgByU	6.8	13.00
Banyuasin	Purwosari	Maize	*Aspergillus* sp.	JgPwSr	5.5	15.00
Banyuasin	Telang Sari	Maize	*Curvularia* sp.	JgTgSr	6.2	15.00
Pagar Alam	Tanjung Payang	Chili	*Curvularia* sp.	CMTjP	6.0	689.6
Ogan Ilir	Tanjung Pering	Maize	*Chaetomium* sp.	JgTjPr	6.4	36.00

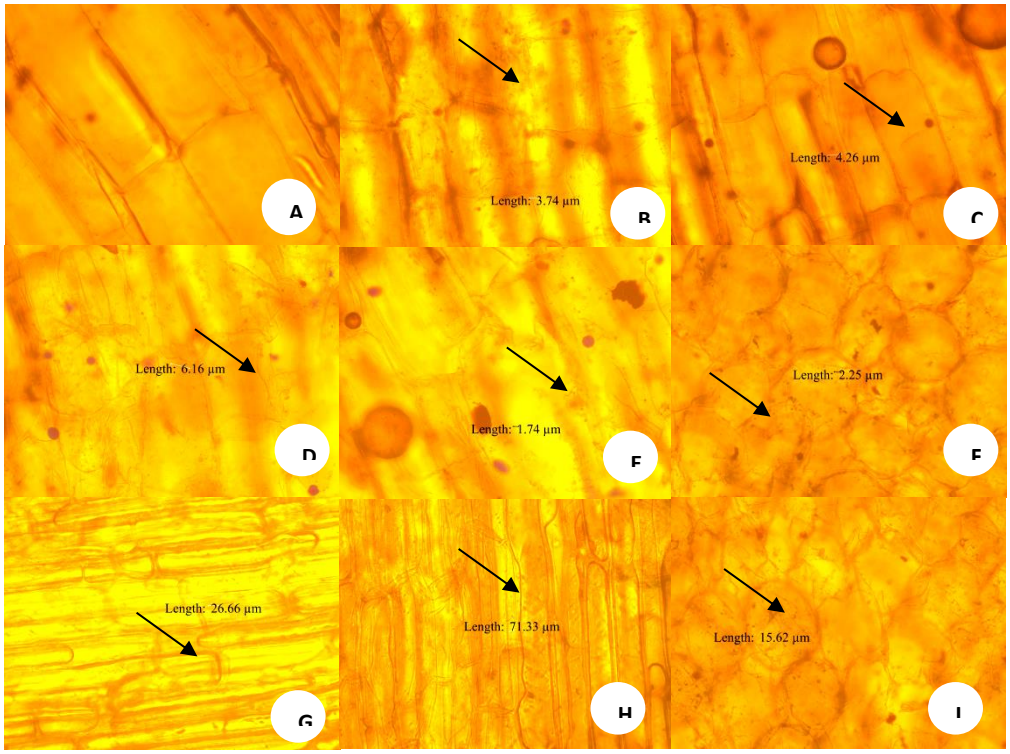

Figure 1. Ten-day maize tissues colonized by endophytic fungi: Control (A), and isolate of JgCrJr (B), JgSPK (C), PsgTjPr (D), JgByU (E), JgPwSr (F), JgTgSr (G), CMTjP (H), and JgTjPr (I)

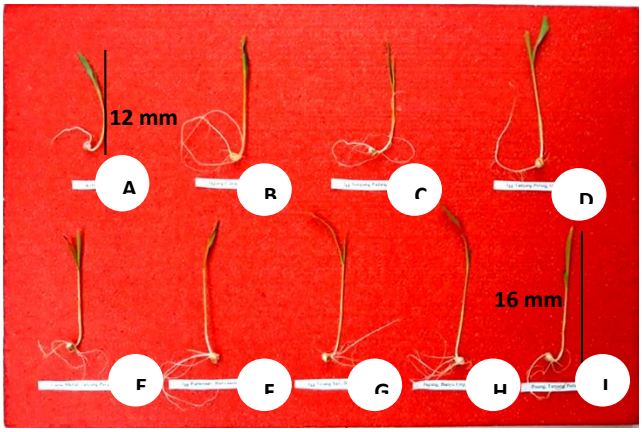

Figure 2. Ten-day maize plants treated with endophytic fungi (1 x 10^6 conidia mL^{-1}): Control (A), and isolate of JgCrJr (B), JgSPK (C), PsgTjPr (D), JgByU (E), JgPwSr (F), JgTgSr (G), CMTjP (H), and JgTjPr (I)

The colony morphology of the eight isolates of the endophytic fungi showed different colors (Figure 3) and so did the morphology of hyphae and conidia, each isolate showing its own characteristics (Figure 4). The morphology of the JgCrJr and JgSPK isolates showed similarities, namely their colony was white, white hyphae and mycelia, and the conidia was globose and non-septation. However, the conidia of JgCrJr isolate was 2.21 x 2.80 µm diameter and 3.07 µm long, whereas the conidia of JgSPK isolate was 2.41 x 2.97 µm diameter and 3.07 µm long. The genus of JgCrJr and JgSPK isolates was *Beauveria* sp. The PsgTjPr and JgByU isolates had black colony, black hyphae and mycelia, and the non-septate globose-shaped conidia was 2.27 µm long. So, the PsgTjPr and JgByU isolates were *Aspergillus* sp. The colony of JgPwSr isolates were green, and had green hyphae and mycelia. The JgPwSr conidia were non-septate globose with a length of 2.49 µm and attached to phialides and the phialides adhered to vesicles. The genus of JgPwSr isolate was also *Aspergillus* sp. The JgTgSr and CMTjP isolates had a black colony, black hyphae and mycelia, and two septated boomerang-shaped conidia. Yet, the length of the JgTgSr conidia (6.23 µm) was smaller than that of the CMTjP conidia (10.51 µm). On the basis of the isolate morphological characters, the genus of the JgTgSr and CMTjP isolates was *Curvularia* sp. The JgTjPr isolate had purple colony, purple hyphae and mycelia, and the conidia had D-shape (asymmetric/elliptical), non-septate with a length of 3.96 µm. The genus of JgTjPr isolate was *Chaetomium* sp. So, the genus of the eight isolates of the endophytic fungi were *Aspergillus* sp., *Beauveria* sp., *Chaetomium* sp., and *Curvularia* sp.

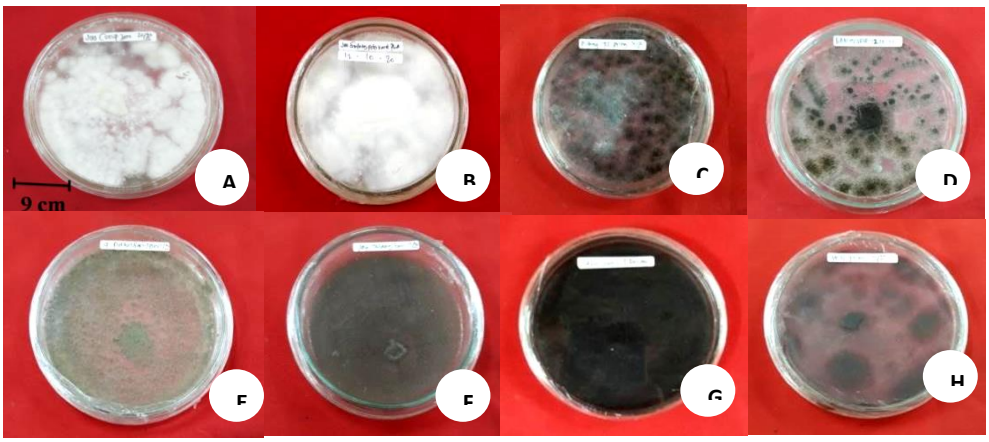

Figure 3. Colony morphology of of endophytic fungi cultured on PDA media: JgCrJr (A), JgSPK (B), PsgTjPr (C), JgByU (D), JgPwSr (E), JgTgSr (F), CMTjP (G), and JgTjPr (H)

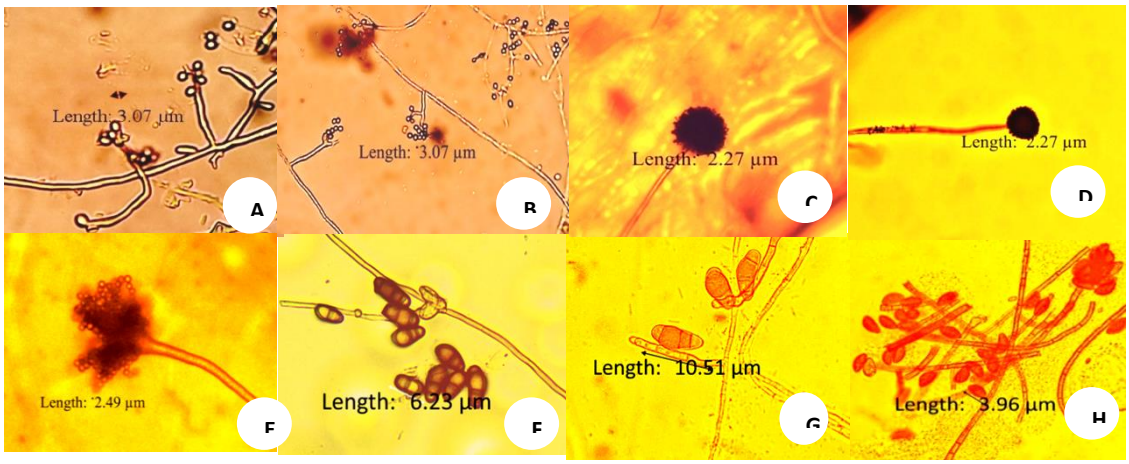

Figure 4. Conidial and hyphal morphology of endophytic fungi: JgCrJr (A), JgSPK (B), PsgTjPr (C), JgByU (D), JgPwSr (E), JgTgSr (F), CMTjP (G), and JgTjPr (H)

The conidia density of the eight isolates of the endophytic fungus did not show a significant difference among the isolates (Table 2). Nevertheless, the viability of conidia incubated either 1 x 24 hours or 2 x 24 hours showed a significant difference among the isolates. The conidial viability increased after the incubation of 2 x 24 hours. The highest conidia viability was found in JgSPK isolate (*Beauveria* sp.), while the lowest was in JgTgSr isolate (*Curvularia* sp.).

Table 2. Mean of conidial density and viability of endophytic fungi

Fungal species	Isolate codes	Conidial density ($1x10^8$ conidia mL^{-1})	Conidial viability (%)	
			24-hour culture	48-hour culture
Beauveria sp.	JgCrJr	4.17±0.24	$55.17±4.93^{bc}$	$55.66±5.05^{bc}$
Beauveria sp.	JgSPK	3.19±0.58	$58.69±0.89^{c}$	$61.87±0.98^{c}$
Aspergillus sp.	PsgTjPr	1.57±0.10	$46.58±2.15^{abc}$	$48.84±2.88^{ab}$
Aspergillus sp.	JgByU	3.36±0.18	$45.04±2.73^{ab}$	$50.95±2.77^{abc}$
Aspergillus sp.	JgPwSr	1.69±0.30	$41.36±3.85^{ab}$	$47.71±0.21^{ab}$
Curvularia sp.	JgTgSr	3.74±0.38	$38.98±3.25^{a}$	$42.73±2.53^{a}$
Curvularia sp.	CMTjP	3.94±0.42	$42.03±3.14^{ab}$	$51.18±1.85^{ab}$
Chaetomium sp.	JgTjPr	3.22±0.30	$39.50±0.15^{a}$	$43.14±7.53^{a}$
F-value		0.06^{ns}	4.91^{*}	5.90^{*}
P value		1.00	0.00	0.00
HSD value		3.06	9.02	7.12

Note: ns= not 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 3. Leaf area eaten by *Spodoptera frugiperda* larvae treated with of endophytic fungi (1 x 10^6 conidia mL^{-1})

Isolates	Mean of leaf area eaten by larvae (cm^2 $larvae^{-1}$ day^{-1}) during 12 days of observation											
	1	2	3	4	5	6	7	8	9	10	11	12
Control	4.60^{b}	4.53	7.73	8.29	8.58	8.83^{c}	8.74^{b}	8.83^{b}	8.85^{b}	8.41	8.86	8.99^{b}
JgCrJr	3.62^{ab}	4.18	5.30	6.11	7.42	8.12^{bc}	7.97^{ab}	7.67^{ab}	7.07^{a}	6.64	6.32	5.77^{a}
JgSPK	3.36^{a}	4.12	5.11	6.34	6.91	7.31^{abc}	7.36^{ab}	7.14^{ab}	7.13^{a}	7.57	7.11	6.00^{a}
PsgTjPr	3.78^{ab}	4.35	6.21	7.98	7.67	7.36^{abc}	7.47^{ab}	7.38^{ab}	7.36^{ab}	7.36	6.73	6.21^{a}
JgByU	3.78^{ab}	4.15	5.25	6.27	7.58	7.53^{abc}	7.80^{ab}	7.72^{ab}	7.67^{ab}	7.86	7.56	7.05^{ab}
JgPwSr	3.76^{ab}	4.43	6.17	6.88	7.79	8.12^{bc}	8.07^{ab}	7.49^{ab}	7.27^{ab}	7.14	6.95	6.45^{a}
JgTgSr	3.52^{a}	3.88	7.13	7.72	6.86	6.52^{ab}	7.96^{ab}	7.03^{ab}	6.67^{a}	6.69	7.44	6.53^{a}
CMTjP	3.62^{ab}	3.98	6.40	7.89	6.88	6.33^{a}	6.73^{a}	6.73^{a}	6.48^{a}	6.87	7.42	6.80^{ab}
JgTjPr	3.66^{ab}	4.24	5.82	6.53	6.78	7.21^{abc}	6.78^{ab}	7.31^{ab}	7.92^{ab}	7.50	7.52	7.28^{ab}
F-value	2.96^{*}	1.69^{ns}	1.14^{ns}	0.67^{ns}	0.63^{ns}	5.01^{*}	3.08^{*}	2.59^{*}	5.25^{ns}	1.79^{ns}	2.30^{ns}	4.94^{*}
P value	0.03	0.17	0.39	0.71	0.74	0.00	0.02	0.04	0.00	0.14	0.07	0.00

HSD value	0.23	0.18	0.77	0.90	0.67	0.31	0.32	0.32	0.27	0.38	0.41	0.38

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.

The endophytic fungi pathogenecity against *Spodoptera frugiperda* larvae

The results of the measurement of leaf area eaten by the larvae dripped with the endophytic fungi suspension (1 x 10^6 conidia mL^{-1}) and the control (untreated) on the first day showed significant differences. In the control, the larvae ate the most maize leaves (Table 3). On the second to the fifth days, the leaf area eaten by the larvae from all treatments was not significantly different, while on the sixth to 12th days, the leaf area eaten by the control larvae was wider and tended to be significantly different from that eaten by the larvae being already treated with the endophytic fungi. Consequently, the treated larvae experienced a significant decrease in appetite compared to that of the control. The symptoms of leaves eaten by the larvae treated with the fungus and those eaten by the control also showed significant differences (Figure 5).

The decrease in appetite in the larvae treated with the endophytic fungi was followed by a decrease in their body weight. On the second day, the weight loss of the treated larvae was significant compared to the that of the control, while on the next day, the weight of the larvae among the treatments was not significantly different (Table 4). The feacal weight produced by the treated and untreated larvae tended to show a significant difference. The stool weight produced by the treated larvae tended to be heavier than that produced by the untreated larvae (control) (Table 5). This phenomenon is interesting because generally the normal larvae, which eat a lot, produce a lot of faeces, but in this experiment the result showed the opposite.

Of the eight endophytic fungal isolates found, the most pathogenic JgCrJr isolate (*Beauveria* sp.) resulted in 29.33% larval mortality with LT_{50} for 17.40 days, followed by JgSPK isolate (*Beauveria* sp.) (26.67% mortality) with LT_{50} for 15 days (Table 6). The mortality caused by these two isolates from the beginning of observation to the last day was always higher; the isolate with the lowest ability to cause mortality was JgTgSr (*Curvularia* sp.) (Figure 6). Besides *Beauveria* sp., *Aspergillus* sp., *Chaetomium* sp., and *Curvularia* sp. were also able to cause mortality of *S. frugiperda* larvae. In Indonesia, first report of *Aspergillus* sp., *Chaetomium* sp., and *Curvularia* sp. have insecticidal activity against *S. frugiperda* larvae. The isolate that had the highest reduction in the emergence of adults occurred in JgCrJr isolate (*Beauveria* sp.), causing only 56% of *S. frugiperda* adults to emerge (Table 7). Therefore, the isolate JgCrJr (*Beauveria* sp.) could reduce the adult emergence of *S. frugiperda* by 44%.

The treated larvae exhibited distinctive symptoms that distinguished them from the healthy larvae (Figure 7). The healthy larvae were longer and bigger, and had flexible movements and a tight body, while the larvae that were sick due to being infected with the endophytic fungi were stiff, its body was smaller, shrivels, hardens like a mummy, and over time the body changes color to black but did not smell. The dead larvae were grown in the agar-water medium and their integument grew mycelia and conidia that covered the cadaver. Apart from the larval mortality, the endophytic fungus caused the pupae and adults to be abnormal and malformed (Figures 8 and 9). The abnormal pupae were thinner, bent, shriveled wings and darker in color, and when their body was touched they did not move. The abnormal adults had folded and smaller wings than those of the normal adults.

Table 4. Weight of *Spodoptera frugiperda* larvae treated with endophytic fungi (1 x 10^6 conidia mL^{-1})

Isolates	Mean of larvae weight (mg $larvae^{-1}$) during 12 days observation											
	1	3	3	4	5	6	7	8	9	10	11	12
Control	22.99	74.45^{b}	49.16	69.65	97.89	124.53	129.27	136.17	173.73	190.91	207.07	217.27
JgCrJr	23.72	38.67^{ab}	61.24	66.11	83.40	102.58	125.08	137.88	161.84	168.24	181.25	171.60
JgSPK	22.09	25.57^{a}	42.17	74.95	87.53	110.08	115.04	134.66	173.90	197.97	195.24	183.52
PsgTjPr	28.92	45.49^{ab}	44.87	53.95	81.57	90.41	104.52	127.67	160.22	192.28	179.91	180.66
JgByU	26.63	45.45^{ab}	57.12	68.35	74.09	88.80	115.26	132.24	144.85	157.14	161.54	168.66
JgPwSr	15.13	53.59^{ab}	65.47	67.29	93.61	106.29	124.06	145.27	176.09	192.37	184.76	183.23
JgTgSr	19.29	34.12^{a}	52.73	56.47	65.89	75.23	103.57	126.66	146.83	162.20	177.46	172.63
CMTjP	25.31	32.15^{a}	39.76	56.37	65.97	87.10	119.70	137.81	176.33	195.52	188.19	176.03
JgTjPr	24.85	35.51^{a}	48.48	60.21	80.51	95.32	126.71	139.42	197.67	179.91	186.21	185.67
F-value	0.79^{ns}	4.33^{*}	1.61^{ns}	0.41^{ns}	1.28^{ns}	2.18^{ns}	0.69^{ns}	0.18^{ns}	1.33^{ns}	1.64^{ns}	0.75^{ns}	0.99^{ns}
P value	0.62	0.00	0.19	0.90	0.31	0.08	0.70	0.99	0.29	0.18	0.65	0.47
HSD value	2.59	2.54	2.34	3.61	2.73	2.49	2.56	2.86	2.72	2.30	2.67	2.61

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

Table 5. Feacal weight produced by *Spodoptera frugiperda* larvae treated with of endophytic fungi (1 x 10^6 conidia mL^{-1})

Isolates	Mean of larvae feacal weight (mg $larvae^{-1}$ day^{-1}) during 12 days of observation											
	1	3	3	4	5	6	7	8	9	10	11	12
Control	11.67^{ab}	15.67^{ab}	17.67	20.67^{a}	25.33^{a}	28.00	31.33	35.00^{a}	38.00^{a}	41.33	47.80	53.33^{c}
JgCrJr	4.22^{a}	7.21^{a}	25.59	55.35^{c}	71.60^{c}	72.14	73.47	64.62^{ab}	57.24^{b}	40.87	47.45	30.98^{ab}
JgSPK	8.98^{ab}	13.58^{ab}	32.12	39.37^{bc}	47.73^{abc}	49.06	59.15	71.79^{b}	62.52^{b}	60.49	44.05	36.99^{abc}
PsgTjPr	20.28^{b}	25.05^{bcd}	28.26	31.21^{ab}	43.59^{abc}	45.75	49.96	45.65^{ab}	41.91^{b}	41.63	38.34	35.09^{abc}
JgByU	16.56^{ab}	21.39^{bcd}	31.55	37.65^{abc}	48.19^{abc}	51.45	55.68	63.66^{ab}	47.76^{b}	46.29	41.93	36.27^{abc}

JgPwSr	21.13^{b}	32.60^{d}	43.62	46.10^{bc}	55.19^{bc}	55.89	54.37	57.60^{ab}	58.10^{b}	53.67	44.08	41.12^{bc}
JgTgSr	13.10^{ab}	17.60^{bc}	25.75	31.53^{ab}	35.00^{ab}	40.41	45.86	47.81^{ab}	40.65^{b}	36.83	33.79	21.57^{a}
CMTjP	19.28^{ab}	34.39^{d}	41.87	40.89^{bc}	43.51^{abc}	57.52	60.45	63.46^{ab}	60.74^{b}	54.64	46.32	38.63^{bc}
JgTjPr	24.80^{b}	29.39^{cd}	36.25	39.22^{bc}	47.26^{abc}	55.54	57.04	61.80^{ab}	60.66^{b}	55.00	45.02	40.77^{bc}
F-value	3.74*	14.29*	2.50^{ns}	6.42*	4.16*	2.26^{ns}	1.72^{ns}	2.77*	3.86*	1.68^{ns}	1.41^{ns}	5.18*
P value	0.01	0.00	0.05	0.00	0.01	0.07	0.16	0.03	0.01	0.17	0.26	0.00
HSD value	2.28	1.35	2.39	1.57	2.22	2.83	2.92	2.35	1.74	2.21	1.50	1.55

Note: ns = not significantly different; * = significantly different; values within a column (the data of each isolate) followed by the same letter were not significantly different at P < 0.05 according to Tukey's HSD test.

Table 6. Mean of larvae mortality, LT_{50}, and LT_{95} of *Spodoptera frugiperda* larvae treated with of endophytic fungi (1 x 10^{6} conidia mL^{-1})

Isolates	Mortality ± SE (%)	LT_{50} ± SE (days)	LT_{95} ± SE (days)
Control	0.00±0.00^{a}	-	-
JgCrJr	29.33±3.53^{d}	17.40±1.37	30.08±2.51
JgSPK	26.67±3.53^{cd}	15.00±1.06	27.69±2.20
PsgTjPr	18.67±3.53^{bcd}	17.94±0.68	30.62±1.68
JgByU	9.33±3.53^{b}	23.66±3.01	36.35±4.14
JgPwSr	17.33±3.53^{bcd}	18.89±1.72	31.58±2.84
JgTgSr	9.33±1.33^{b}	22.12±2.15	34.81±3.30
CMTjP	12.00±2.31^{bc}	20.37±1.64	33.06±2.75
JgTjPr	14.67±3.53^{bcd}	20.14±2.28	32.82±3.14
F-value	15.51*	2.13^{ns}	0.88^{ns}
P value	0.00	0.09	0.55
HSD value	11.88	8.74	13.58

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

Table 7. Percentage of *Spodoptera frugiperda* pupae formation and adults emerged after their larvae treated with endophytic fungi (1 x 10^{6} conidia mL^{-1})

Isolates	Mean of pupae formation (%)	Mean of adults emerged (%)
Control	100.00^{d}	100.00^{d}
JgCrJr	70.67^{a}	56.00^{a}
JgSPK	73.33^{ab}	60.00^{ab}
PsgTjPr	81.33^{abc}	70.67^{abc}
JgByU	90.67^{c}	80.00^{bc}
JgPwSr	82.67^{abc}	74.67^{abc}
JgTgSr	90.67^{c}	84.00^{c}
CMTjP	88.00^{bc}	80.00^{bc}
JgTjPr	85.33^{abc}	80.00^{bc}
F-value	16.03*	17.24*
P value	0.00	0.00
HSD value	11.88	14.29

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

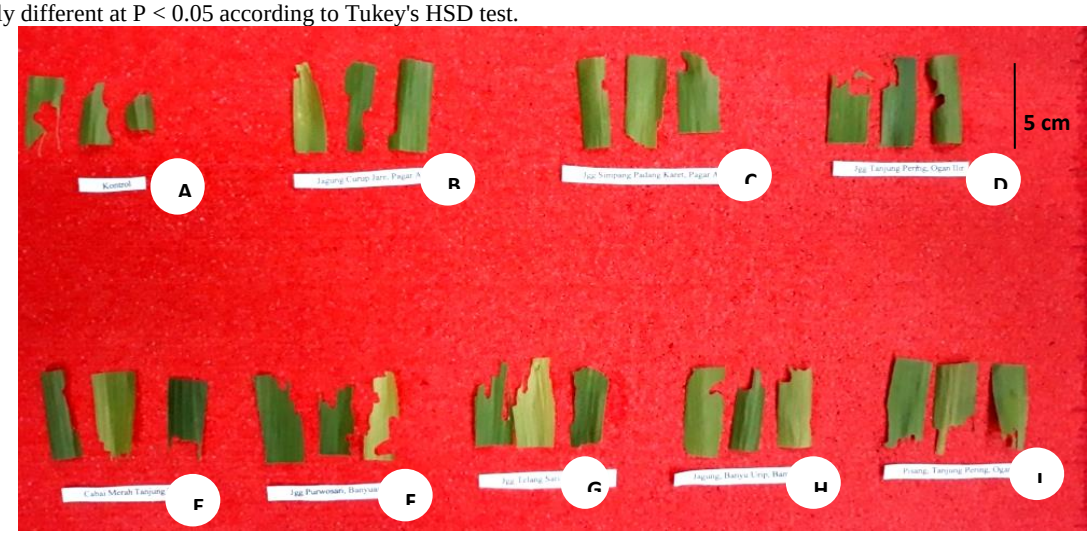

Figure 5. The symptoms on maize leaves eaten by *Spodoptera frugiperda* larvae treated with endophytic fungi (1 x 10^6 conidia mL^{-1}): Control (A), JgCrJr (B), JgSPK (C), PsgTjPr (D), JgByU (E), JgPwSr (F), JgTgSr (G), CMTjP (H), and JgTjPr (I).

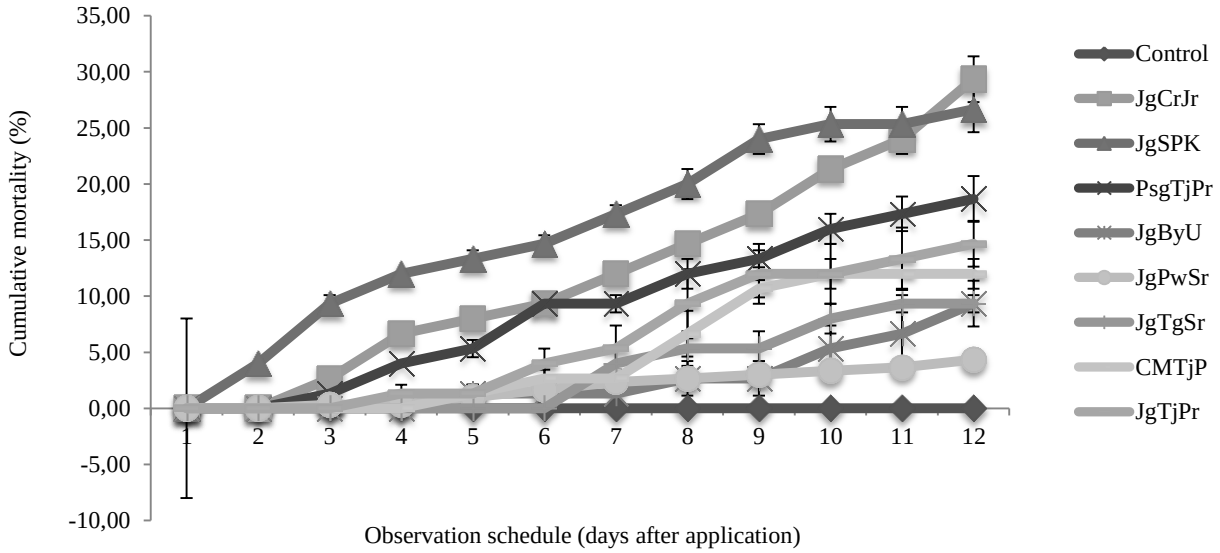

Figure 6. Cumulative mortality of *Spodoptera frugiperda* larvae treated with endophytic fungi (1 x 10^6 conidia mL^{-1}) during 12 days observation

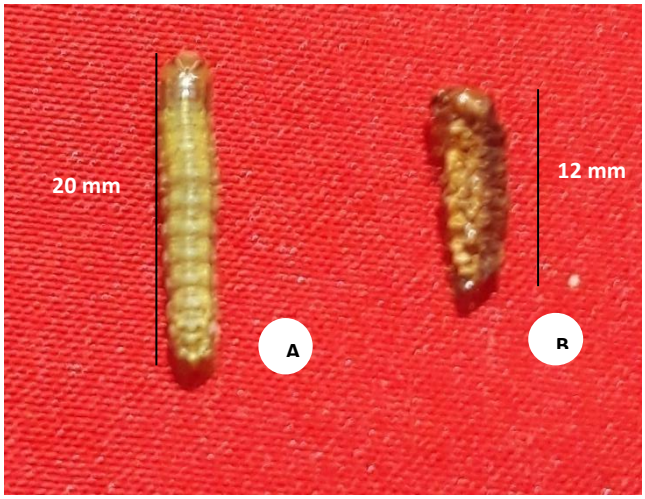

Figure 7. Morphology of *Spodoptera frugiperda* larvae: healthy larvae of control (A) and dead larvae infected by endophytic fungi (B)

Figure 8. Morphology of *Spodoptera frugiperda* pupae: healthy pupae of control (A) and unhealthy with malformation pupae infected by endophytic fungi (B)

Figure 9. Morphology of *Spodoptera frugiperda* adults: healthy adults of control (A) and unhealthy with malformation adults infected by endophytic fungi (B)

Discussion

Based on the morphological characteristics, the JgCrJr and JgSPK isolates belong to the genus of *Beauveria* sp. The PsgTjPr, JgByU, and JgPwSr isolates belong to the genus of *Aspergillus* sp. The JgTgSr and CMTjP isolates include in *Curvularia* sp. The genus of JgTjPr isolate is *Chaetomium* sp. The morphological characteristics of the four fungal genus match to description by Humber (2005) and El-Ghany (2015). All genus of the endophytic fungi found in this study have insecticidal activity against the *S. frugiperda.* . First report of *Aspergillus* sp., *Chaetomium* sp., and *Curvularia* sp. are pathogenic against *S. frugiperda* larvae. The endophytic *Beauveria* spp. have been shown to kill various species of the insect pests, such as *Diaphorina citri* (Bamisile et al. 2019), *Trialeurodes vaporariorum* (Barra-Bucarei et al. 2020) Wicklow et al. (2000) reported that *Chaetomium* sp is pathogenic against *Helicoverpa zea*. *Chaetomium globosum* significantly inhibit the growth and reproduction of *Myzus persicae* (Qi et al. 2011). *Aspergillus* sp. and *Curvularia* sp. are opportunistic fungi that probably they display an important role in regulating insect populations (Assaf et al. 2011).

In this study, the endophytic fungi isolated from maize, banana and chili roots were able to colonize the plant tissue, both the stems and leaves of maize. The congruent results were also found by Renuka et al. (2016) stating that the endophytic *B. bassiana* colonized the maize leaf and stem tissue. The colonized maize tissues had a characteristic of mycelia fungal color varies depending on the species of fungus and this characteristic is in line with the results of the study by (Jones et al. 2018).

The existence of endophytic fungi in the plant tissues are an association that is mutual, mutually beneficial; the fungi get a habitat and niche, while the plants get protection from pests (Jones et al. 2018) and promote growth due to the presence of the endophytic fungi (Barra-Bucarei et al. 2020; Jaber and Ownley 2018). The data in this study prove that the plants inoculated with the endophytic fungi tended to be taller with more and longer roots than the untreated plants. In addition, the treated plants looked healthy and showed no symptoms of illness. These are the preliminary data to be the basis for the future studies on the effect of the endophytic fungus on plant growth.

The endophytic fungal isolates in this study isolated from the root tissues of maize, banana and chili, and the isolates were then re-inoculated through the roots again and proved to enter the maize stalks and leaves systemically as seen from the presence mycelia in the entire plant stems and leaf tissues. Barra-Bucarei et al. (2020) state that endophytic fungal isolates have the ability to have a systemic mode of action. The results of detection by Carolina et al. (2020) show that the endophytic fungi can still be found in the roots, stems and leaves up to 30 days after inoculation. However, according to Shikano (2018) the endophytic fungi are able to colonize plant parts for several months and the duration of their persistence in the plant tissue varies depending on the age of the plant (high persistence in young tissues). The high fungal persistence in the plant tissues has the potential to develop seed treatment for maize seeds. The seed treatment through seeds allows the endophytic to colonize the plant and prevents *S. frugiperda* larvae from attacking the leaves, stems, and shoots.

In this study, the mortality of larvae treated with the endophytic fungal suspension (1 x 10^6 conidia mL^{-1}) was 29.33%. This result is similar to the study results of Akutse et al. (2019) on the endophytic *B. bassiana* which caused the mortality of *S. frugiperda* larvae for only 30%. According to Resquín-Romero et al. (2016) the mortality by the endophytic fungi can increase if the spore concentration is increased to 1 x 10^8 conidia mL^{-1} and the mortality can range from 41.70–50.00%

and it is higher when the application of the combination of the insects eats the part of the colonized tissue by the fungus and in contact. Ramos et al. (2020) stated that the mortality caused by the endophytic *B. bassiana* reached 87% while that caused by the endophytic *M. anisopliae* reached 75%. The variations in the mortality data indicate that the pathogenecity of the fungus depends on the strain of the fungus. In addition, variations in the application method of the fungus also affect mortality. The combination of the fungal treatment in contact with insects and fungi entering through the eaten inoculated leaves can increase the effectiveness of the fungus. In this study, there were two isolates that caused higher mortality of *S. frugiperda* larvae, namely JgCrJr isolate of *Beauveria* sp. (29.33%) and JgSPK isolate of *Beauveria* sp. (26.67%). The two isolates were isolated from the maize root tissue and this finding is interesting because of the high potential to successfully kill *S. frugiperda* larvae hidden in leaf midribs due to the systemic nature of fungi able to colonize maize leaves and stalks. The potential for fungus to be developed as a seed treatment for maize seeds is also high because of the high ability of fungi to colonize roots.

The endophytic fungi in this experiment also decreased the appetite of *S. frugiperda* larvae. The decreased appetite resulted in weight loss. The decrease in appetite was significant on the sixth day after the spray of the fungal conidia. According to El-Ghany (2015) this decreased appetite of the larvae was due to an ongoing fungal infection. The infection occurs when the fungal conidia germinates and can penetrate the host insect's integument (Fernandes et al. 2007). Then, the germ tubes produce specific infection hyphae (El-Ghany 2015). The hyphae spreads to the haemolymph and develops to produce blastospores capable of producing proteolytic or chitinolytic enzymes which can disrupt normal cell metabolism (Mancillas-Paredes et al. 2019) whose symptoms can be seen from the decreased appetite of host larvae. Then, the toxins from secondary metabolites begin to kill the host insect (El-Ghany 2015).

The larvae and pupae that got sick or die after being inoculated with the conidia of endophytic fungi were generally stiff, and the body was smaller, shriveled, hardens like a mummy, and over time the body changed color to black but did not smell. The mycelia and the fungal conidia enveloped the cadaver. In addition, the morphology of pupae and adults becomes abnormal and malformed. The symptoms of these sick larvae and pupae are similar to those found by Herlinda et al. (2020b). The folded wings of adults can cause them to be unable to copulate and thus indirectly lead to a decrease in the population density of the next generation.

Finally, this study found that the endophytic fungi were isolated from the root tissue of maize, banana, and chili from the lowlands to highlands of South Sumatra as many as eight isolates consisting of the genus, *Aspergillus* sp., *Beauveria* sp., *Chaetomium* sp., and *Curvularia* sp. The two most pathogenic isolates against *S. frugiperda* larvae were found from the roots of maize, namely JgCrJr isolate (*Beauveria* sp.) and JgSPK isolate (*Beauveria* sp.) with a mortality of 29.33% and 26.67%, respectively. The isolate JgCrJr (*Beauveria* sp.) can reduce the emergence of *S. frugiperda* adults up to 44%. Consequently, The two endophytic fungal isolates of *Beauveria* sp. have a high potential to be developed to control *S. frugiperda* larvae in maize in both the lowlands and the highlands.

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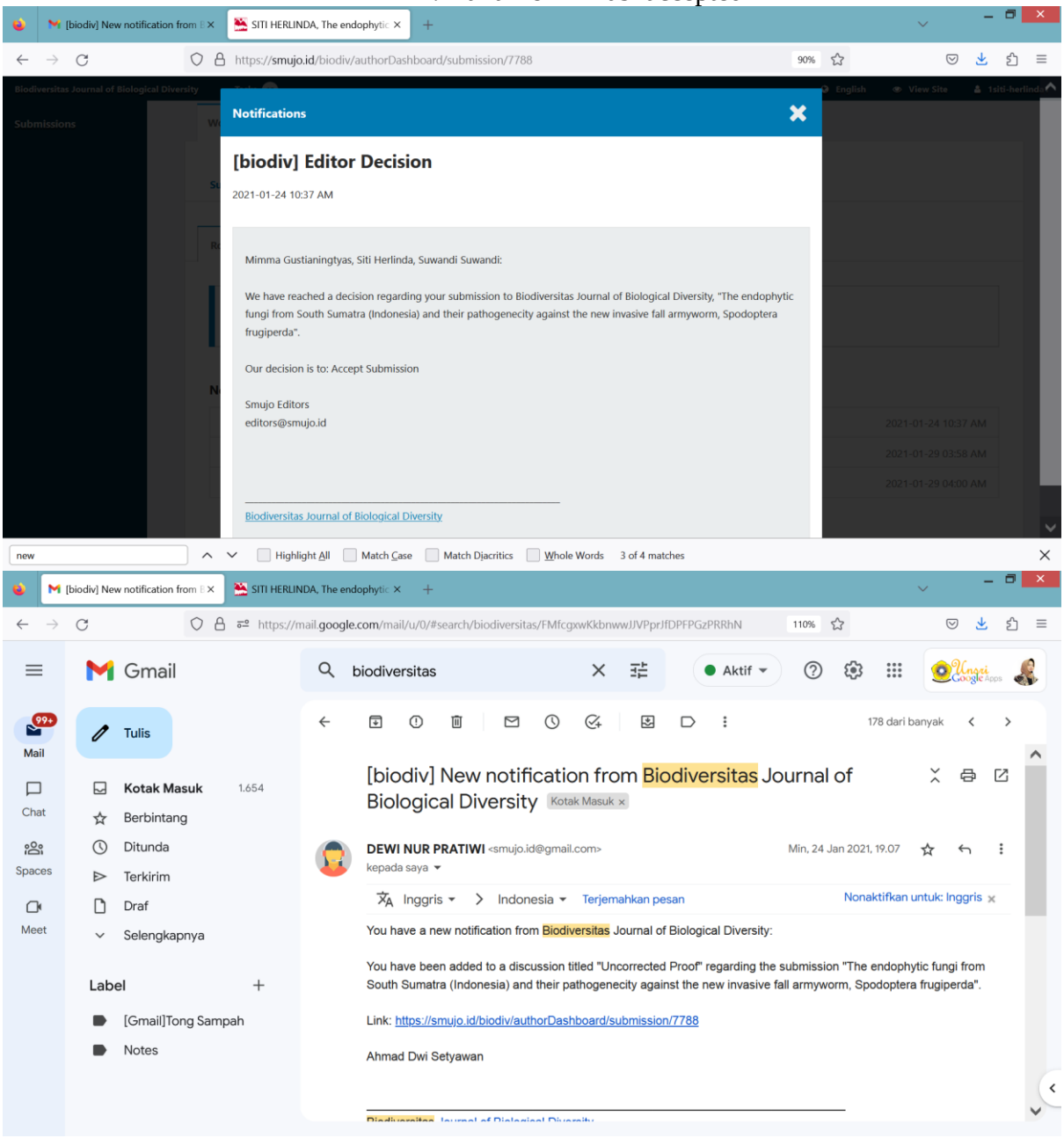

The endophytic fungi from South Sumatra (Indonesia) and their pathogenecity against the new invasive fall armyworm, *Spodoptera frugiperda*

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Abstract. *Gustianingtyas M, Herlinda S, Suwandi. 2021. The endophytic fungi from South Sumatra (Indonesia) and their pathogenecity against the new invasive fall armyworm,* Spodoptera frugiperda. *Biodiversitas 22: xxxx.* Maize in Indonesia is currently experiencing attacks and outbreaks of the new invasive fall armyworm, *Spodoptera frugiperda*. The *S. frugiperda* larvae emerge from the leaf midrib when eating, after hiding in the maize stalk so that it is difficult to control by contact. This study aimed to find out the endophytic fungi from the roots of maize, banana and chili in South Sumatra and to determine their pathogenicity against *S. frugiperda* larvae. The endophytic fungi were isolated from the plant roots. Fungal isolates proven to be endophytic were dropped (1×10^6 conidia mL^{-1}) on the second instar larvae. The result showed that the endophytic fungi found were 8 isolates consisting of the genus, *Aspergillus* sp., *Beauveria* sp., *Chaetomium* sp., and *Curvularia* sp. First report of *Aspergillus* sp., *Chaetomium* sp., and *Curvularia* sp. have insecticidal activity against *S. frugiperda* larvae. However, the two most pathogenic isolates were JgCrJr and JgSPK isolates of *Beauveria* sp. with a larval mortality of 29.33% and 26.67%, respectively and could reduce the emergence of *S. frugiperda* adults up to 44%. So, the two isolates of *Beauveria* sp. have a high potential to be developed to control *S. frugiperda* larvae in maize both in the lowlands and the highlands.

Keywords: *Aspergillus* sp., *Beauveria* sp., *Chaetomium* sp., *Curvularia* sp., insecticidal activity

INTRODUCTION

Maize in Indonesia is currently facing a big problem in invasion and outbreaks of newcomer insect pests, namely the fall armyworm (*Spodoptera frugiperda*) (Lepidoptera: Noctuidae). *S. frugiperda* comes from South America (Nagoshi et al. 2017; Otim et al. 2018) and entered Indonesia for the first time on March 26, 2019 in West Sumatra, then in June 2019 it was found in Banten and West Java (Sartiami et al. 2020) and now it has spread rapidly to various provinces in Indonesia, such as South Sumatra (Herlinda et al. 2020b; Hutasoit et al. 2020), Lampung (Lestari et al. 2020), and Bengkulu (Ginting et al. 2020). The fall armyworm has caused maize yield losses in Africa of 250–630 million US dollars per year (Bateman et al. 2018). Kenya lost maize production of up to 1 million tons per year (De Groote et al. 2020). In Indonesia the pest was reported to attack both hybrid maize and local maize varieties (Ginting et al.2020). The pest are polyphagous because they are able to attack and to damage various species of plants from various families, for example maize, rice, sugar cane, cotton, and ornamental plants (Montezano et al. 2018). The *S. frugiperda* larvae can eat greedily on leaves, stems, flowers, fruit, growing points, fruit, and the whole maize until it is bare (Ginting et al. 2020).

To overcome the invasion and outbreaks of *S. frugiperda*, the synthetic insecticides are generally used in the world (Tambo et al. 2020). The synthetic insecticides of organophosphates and carbamates (Boaventura et al. 2020) and other synthetic insecticides have been shown to be resistant to the fall armyworm (Gutiérrez-moreno et al. 2018) and even the entomopathogenic bacterium, *Bacillus thuringiensis* (Bt) can be broken by *S. frugiperda* (Flagel et al. 2018). Another control method that has not shown resistance is the use of the entomopathogenic fungi (fungi causing disease in insects). The entomopathogenic fungi that have been shown to be effective at killing the insect pests of the genus *Spodoptera* are *Beauveria bassiana*, *Metarhizium anisopliae* (Ayudya et al. 2019; Gustianingtyas et al. 2020), *Penicillium citrinum*, and *Talaromyces diversus* (Herlinda et al. 2020a). *S. frugiperda* was also killed by *B. bassiana, M. anisopliae, Metarhizium rileyi* (Ramanujam et al. 2020), and *Metarhizium* spp. (Herlinda et al. 2020b). The entomopathogenic fungus species effectively killed *S. frugiperda* larvae by contact (Herlinda et al. 2020b). If the mode of action of the fungus is contact only, the fungus is not very effective in controlling *S. frugiperda* larvae hidden in maize leaf midribs because the larvae only appear when eating leaves in the morning (Bentivenha et al. 2017). In the field, the *S. frugiperda* larvae were found appearing on leaf surfaces from 6.30 a.m. to 8.00 a.m. To control the larvae of *S. frugiperda*, it is more effective to use an endophytic entomopathogenic fungus because the endophytic fungi are those that systemically colonize host plant tissues, associating mutually, and without being pathogenic to the host plants (Lira et al. 2020; Kasambala et al. 2018). The endophytic fungus has many advantages, apart from having a mode of action through stomach poison (Russo et al. 2020), it can also kill by contact (Ramirez-Rodriguez and Sánchez-Peña 2016), and it can also stimulate plant growth (Jaber and Ownley 2018; Ahmad et al. 2020; Bamisile et al. 2020; Barra-Bucarei et al. 2020). The endophytic fungi pathogenic to *S. frugiperda* larvae need to be found from maize and other plant tissues in Indonesia, especially in South Sumatra and are expected to be potential alternatives to the use of synthetic insecticides. The objectives of this research were to find out the endophytic fungi from maize, banana and chili roots around the maize ecosystem in South Sumatra and to determine their pathogenicity against *S. frugiperda* larvae.

MATERIALS AND METHODS

This study has been conducted at the Entomology Laboratory, Department of Pests and Plant Diseases, Faculty of Agriculture, Sriwijaya University from February to December 2020. The maize cultivation for *S. frugiperda* mass rearing has been conducted from February to December 2020 and the mass rearing from April to November 2020. The fungi exploration and identification have been performed since April 2020. The fungi were identified at the Laboratory of Agricutural Biotecnology (accredited according to the ISO/IEC 17025 standard), Department of Plant Protection, Faculty of Agriculture, Universitas Lampung, Indonesia. The bioassay was conducted from October to December 2020. It was carried out in an incubator at a constant temperature and relative humidity (RH), namely 30 °C and 93%, respectively. All

endophytic fungal isolates used in this experiment were explored from the lowlands to highlands of South Sumatra, Indonesia.

Exploration, isolation, and purification of endophytic fungus

The exploration of endophytic fungi was carried out by taking the roots of maize, bananas and vegetables (chili) around the maize ecosystem. The survey locations for sampling the fungi were carried out in maize production centers in South Sumatra from lowlands to highlands (Table 1). The individual plants selected following the method of Kasambala et al. (2018) that had the most healthy characteristics, and were not attacked by pests or diseases. Parts of plant tissues taken were the roots of maize, bananas and vegetables (chili) around the maize ecosystem. Furthermore, the root samples were wrapped in sterile straw paper and given the code name of the plant, location, date of sampling, and soil pH then put into a plastic zipper and placed in an ice box, then taken to the laboratory.

In the laboratory the plant root samples were washed using aseptically under running tap water. The surface sterilization and sample isolation were carried out to avoid unwanted airborne microspore contamination. In the laminar air flow cabinet the plant roots were cut to a size of 0.5 cm x 0.5 cm, then the surface was sterilized, modifying the method of Elfita et al. (2019) by immersing plant tissue in 70% EtOH (Ethyl alcohol) for 2 minutes, then dipping it in 1% NaOCl (Sodium hypochlorite) for 1 minute, then rinsed three times in the sterile distilled water for 1 minute. To determine the success of this surface sterilization, the last rinse was grown onto Potato Dextrose Agar (PDA) which modified the method of Russo et al. (2020). If the PDA media did not grow the microorganisms, it meant that the surface sterilization was successful (Ramirez-Rodriguez and Sánchez-Peña 2016).

The surface of the sterile roots was isolated following the method of Elfita et al. (2019) in the laminar air flow cabinet by growing onto the malt extract agar (MEA) media. The MEA media was the specific selected media for growing fungi isolated from the root tissue (Silva *et al.*, 2018). The roots grown on the MEA media were as many as five pieces (5 mm in length and 1-5 mm in diameter) and incubated for 7 days at a room temperature. The fungus growing from the root was then purified to get an isolate. After the isolates were isolated, the fungal isolates aged 7 days were observed for their colony color and shape, hyphae and conidial shape, and continued with an assessment of their colonization ability to enter plant tissue.

Inoculation of endophytic fungi into plant tissue

The isolated fungi were then inoculated into the maize tissue to ensure that the fungus was endophytic. The maize seeds already sterilized using the Elfita et al. (2019) method were then soaked as many as 15 seeds in 10 mL of the fungi suspension with a concentration of 1 x 10^6 conidia mL^{-1} for 6 hours. The control seeds were not soaked with the fungal suspension but soaked in 10 mL of distilled water. All treatments (isolates and controls) in this experiment were repeated three times. Then, the seeds were grown in a sterile glass bottle (volume 250 mL), which is based on a sterile filter paper (whatman no. 42) moistened with 1 mL distilled water and incubated for 10 days in the sterile laminar flow cabinet. In the 10-day-old plants, the stem tissue was sliced crosswise and longitudinally with a thickness of 0.02 mm each and stained with 0.05% lactophenol trypan blue dye to be observed with a light microscope at 40 x magnification to detect the presence of penetrating endophytic fungal mycelium in the plant tissue. The plant tissue colonized by the endophytic fungi was evidenced by the presence of the fungal tissue in the form of mycelia which grew to fill the plant tissues. The fungi proven to be endophytic were then observed for color and colony shape, hyphae and conidial shape, and the conidial size measure to obtain distinctive features used for species identification. The fungi were identified based on their morphological characteristics using the taxonomic books of Humber (2005) and El-Ghany (2015).

Calculation of conidial density and viability

Only the endophytic fungal isolates were used for bioassays against *S. frugiperda* larvae. Before the bioassay was carried out, first the density and viability of each isolate were calculated. The conidial density calculations were carried out on the endophytic fungi aged 7 days. The conidial density were enumerated following the method of Sumikarsih et al. (2019) using a haemocytometer and observed with a light microscope at 40 x magnification. The viability was observed by growing 1 mL fungal suspension (1 x 10^6 conidia mL^{-1}) in 2% agar-water medium, containing 2 g agar given 100 mL distilled water (w/v), then the culture was incubated for 1 x 24 hours and 2 x 24 hours. The culture was observed with a light microscope at 40 x magnification to determine the number of germinated and non-germinated spores/conidia.

Mass rearing of *Spodoptera frugiperda*

Before the bioassay of endophytic fungi against larvae of *spodoptera frugiperda* was conducted, the mass rearing of the test insects was carried out first. The insect used in this study was *S. frugiperda* taken from the farmers' maize farms. *S. frugiperda* was then taken to the laboratory to be maintained and mass reared. The insect mass-rearing modified the method of Herlinda et al. (2020b). In the laboratory, the *S. frugiperda* larvae were reared individually in porous plastic cups (Ø 6.5 cm, height 4.6 cm). In the cup, the maize leaves (2 cm x 5 cm) were added to feed *S. frugiperda* and the leaves were replaced every day with the fresh new ones. When the final instar larvae got into the pupae stage, they were transferred to a plastic container (Ø15 cm, height 25 cm) whose bottom was given sterile soil (5 cm in thickness). The container containing the pupae was placed in a gauze cage (30 x 30 x 30 cm^3), and in the gauze cage there were 10 maize leaves provided for laying eggs and replaced every day. The egg clutch that the female adults laid on the surface of the maize leaves was moved into the container containing kale leaves (*Ipomoea aquatica*) used to feed the first instar larvae. After the first instar molting, the second instar larvae up to

the last instar were fed with young maize leaves and maintained individually in a porous plastic cup (Ø 6.5 cm, height 4.6 cm) because the second instar and so on were cannibalistic. The mass rearing was carried out until getting the third-generation culture. The second instar larvae aged 1 day were used for the bioassay.

The bioassay of endophytic fungi against larvae of *Spodoptera frugiperda*

Only the fungal isolates proven to be endophytic were tested for their pathogenicity against the second instar larvae of *S. frugiperda*. The bioassay of endophytic fungi against *S. frugiperda* larvae followed the method of Ramirez-Rodriguez and Sánchez-Peña (2016). The endophytic fungi were first propagated in PDA media. The endophytic fungi aged 7 days were made suspension with a density of 1 x 10^6 conidia mL^{-1}. Before dropping the fungi suspension, the larvae were fasted for 2 hours and weighed using the portable jewelry scale (capacity 30 g x 0.01 g). Then, 1 mL^{-1} of the fungus suspension was dripped topically to wet 25 *S. frugiperda* larvae, while the control ones were only dropped 1 mL^{-1} of the distilled water. This experiment was designed using completely randomized designs with treatments of isolates, three replications per treatment, and 25 larvae per replication. Furthermore, the larvae were put individually into porous plastic cups (Ø 6.5 cm, height 4.6 cm) and fed with maize leaves measuring 2 x 5 cm^2 per day per larvae. To measure the percentage of foliar damage caused by the larvae of *S. frugiperda*, the bioleaf application by Machado et al. (2016) was used. Each day the dead larvae were recorded and carried out for 12 days based on the previous studies by Herlinda et al. (2020b) and the dead larvae were grown in the agar-water medium to prove infection by the endophytic fungus. The number of larvae becoming pupae and adults that emerged was also counted. The number of dead larvae was used to calculate mortality, the Median Lethal Time (LT_{50}), and the 95% of Lethal Time (LT_{95}). The maize leaf area eaten, faecal weight and body weight of the larvae were measured daily from the first to the 12th day.

Data analysis

The difference in larvae weight data and area of the leaves eaten and feces produced every day among the treatments (isolates), as well as mortality and time of death (the LT_{50} and LT_{95}) larvae of *S. frugiperda*, the percentage of larvae becoming pupae and adults emerged were analyzed using analysis of variance (ANOVA). The Tukey's Honestly Significant Difference (HSD) test (Tukey's test) was employed to test for a significant difference among the treatments at P = 0.05. The LT_{50} and LT_{95} values were calculated using the probit analysis. The data were all calculated using software of SAS University Edition 2.7 9.4 M5.

RESULTS AND DISCUSSION

Endophytic fungi and their colonization on maize tissues

Of the 52 isolates of fungi obtained from the roots of maize, banana and vegetables (chili) around the maize ecosystem, there were only eight isolates confirmed as the endophytic fungi (Table 1). The endophytic fungi were evidenced by the entry of the fungal tissue in the form of mycelia which grew to fill the plant tissue. The results of detection of fungal colonization in maize tissues showed differences from the controls (Figure 1). There was no colonization of endophytic fungi found in the untreated control plants. The plant tissue colonized by the endophytic fungi showed that mycelia grew to fill the plant tissue, while the control plant tissue was clean and no mycelium. The plants colonized by the endophytic fungi also showed a difference compared to the control plants (Figure 2), the inoculated plants tended to be taller with more roots and longer than the control plants.

Table 1. Species and isolates of entomopathogenic fungi found from soil in South Sumatra, Indonesia

District/City	Village	Crop plants	Fungal species	Isolate codes	Soil pH	Altitude (m)
Pagar Alam	Curup Jare	Maize	*Beauveria* sp.	JgCrJr	6.2	806.7
Pagar Alam	Simpang Padang Karet	Maize	*Beauveria* sp.	JgSPK	6.4	797.7
Ogan Ilir	Tanjung Pering	Banana	*Aspergillus* sp.	PsgTjPr	7.0	36.00
Banyuasin	Banyu Urip	Maize	*Aspergillus* sp.	JgByU	6.8	13.00
Banyuasin	Purwosari	Maize	*Aspergillus* sp.	JgPwSr	5.5	15.00
Banyuasin	Telang Sari	Maize	*Curvularia* sp.	JgTgSr	6.2	15.00
Pagar Alam	Tanjung Payang	Chili	*Curvularia* sp.	CMTjP	6.0	689.6
Ogan Ilir	Tanjung Pering	Maize	*Chaetomium* sp.	JgTjPr	6.4	36.00

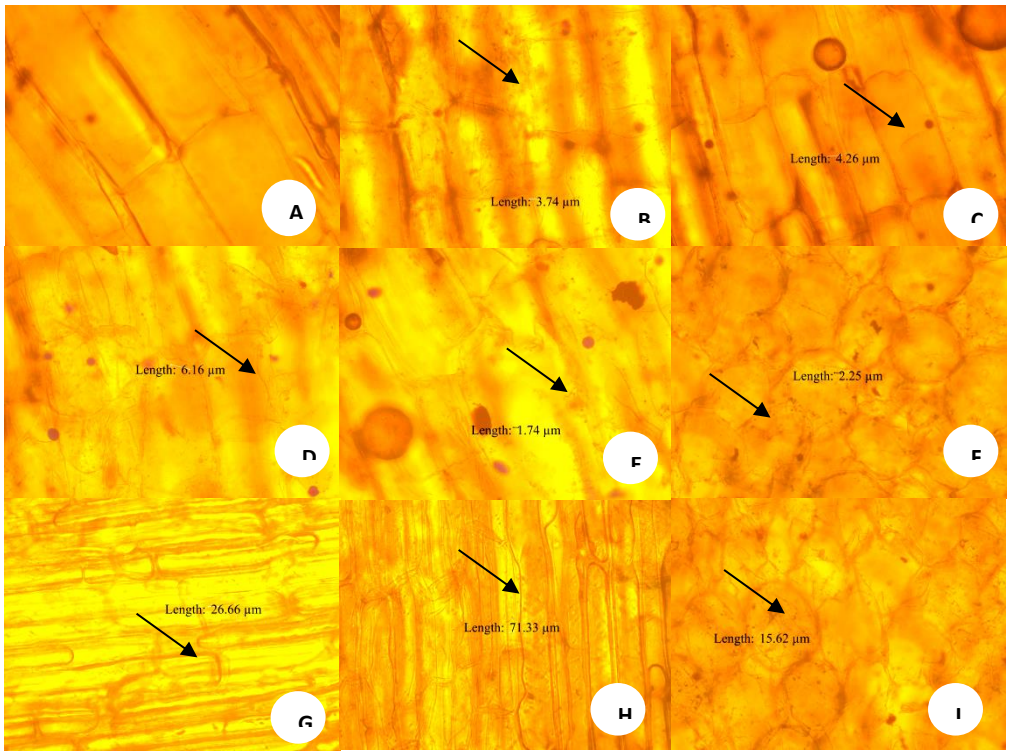

Figure 1. Ten-day maize tissues colonized by endophytic fungi: Control (A), and isolate of JgCrJr (B), JgSPK (C), PsgTjPr (D), JgByU (E), JgPwSr (F), JgTgSr (G), CMTjP (H), and JgTjPr (I)

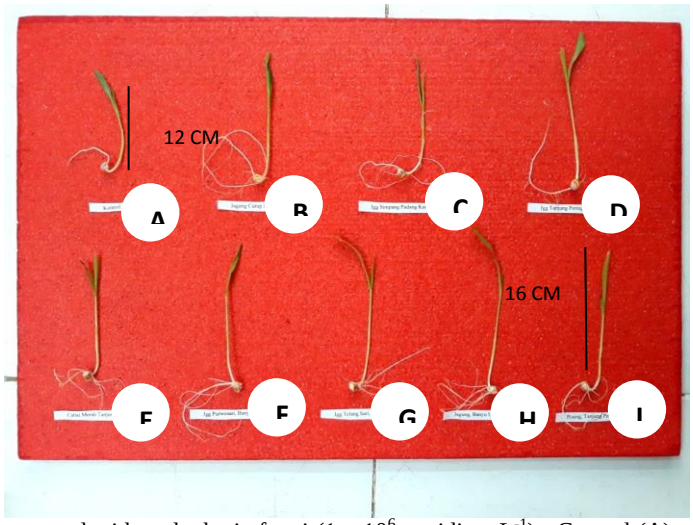

Figure 2. Ten-day maize plants treated with endophytic fungi (1 x 10^6 conidia mL^{-1}): Control (A), and isolate of JgCrJr (B), JgSPK (C), PsgTjPr (D), JgByU (E), JgPwSr (F), JgTgSr (G), CMTjP (H), and JgTjPr (I).

The colony morphology of the eight isolates of the endophytic fungi showed different colors (Figure 3) and so did the morphology of hyphae and conidia, each isolate showing its own characteristics (Figure 4). The morphology of the JgCrJr and JgSPK isolates showed similarities, namely their colony was white, white hyphae and mycelia, and the conidia was globose and non-septation. However, the conidia of JgCrJr isolate was 2.21 x 2.80 µm diameter and 3.07 µm long, whereas the conidia of JgSPK isolate was 2.41 x 2.97 µm diameter and 3.07 µm long. The genus of JgCrJr and JgSPK isolates was *Beauveria* sp. The PsgTjPr and JgByU isolates had black colony, black hyphae and mycelia, and the non-septate globose-shaped conidia was 2.27 µm long. So, the PsgTjPr and JgByU isolates were *Aspergillus* sp. The colony of JgPwSr isolates were green, and had green hyphae and mycelia. The JgPwSr conidia were non-septate globose with a length of 2.49 µm and attached to phialides and the phialides adhered to vesicles. The genus of JgPwSr isolate was also *Aspergillus* sp. The JgTgSr and CMTjP isolates had a black colony, black hyphae and mycelia, and two septated boomerang-shaped conidia. Yet,

the length of the JgTgSr conidia (6.23 µm) was smaller than that of the CMTjP conidia (10.51 µm). On the basis of the isolate morphological characters, the genus of the JgTgSr and CMTjP isolates was *Curvularia* sp. The JgTjPr isolate had purple colony, purple hyphae and mycelia, and the conidia had D-shape (asymmetric/elliptical), non-septate with a length of 3.96 µm. The genus of JgTjPr isolate was *Chaetomium* sp. So, the genus of the eight isolates of the endophytic fungi were *Aspergillus* sp., *Beauveria* sp., *Chaetomium* sp., and *Curvularia* sp.

The conidia density of the eight isolates of the endophytic fungus did not show a significant difference among the isolates (Table 2). Nevertheless, the viability of conidia incubated either 1 x 24 hours or 2 x 24 hours showed a significant difference among the isolates. The conidial viability increased after the incubation of 2 x 24 hours. The highest conidia viability was found in JgSPK isolate (*Beauveria* sp.), while the lowest was in JgTgSr isolate (*Curvularia* sp.).

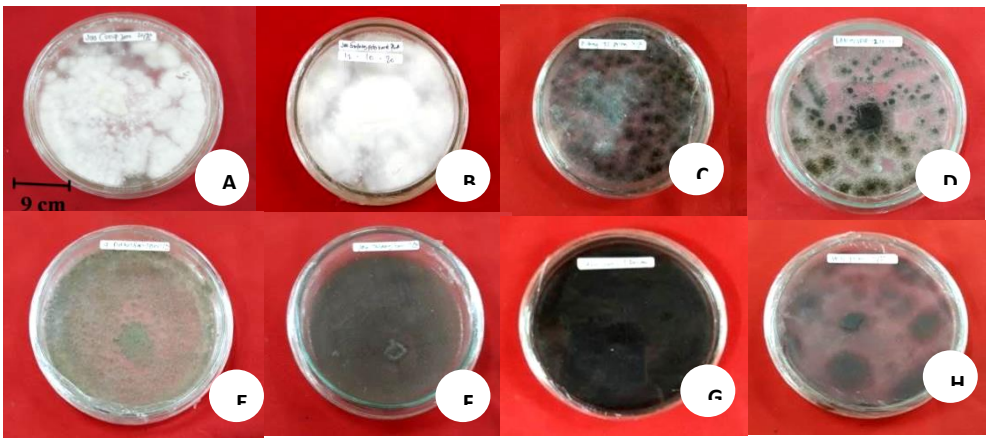

Figure 3. Colony morphology of of endophytic fungi cultured on PDA media: JgCrJr (A), JgSPK (B), PsgTjPr (C), JgByU (D), JgPwSr (E), JgTgSr (F), CMTjP (G), and JgTjPr (H)

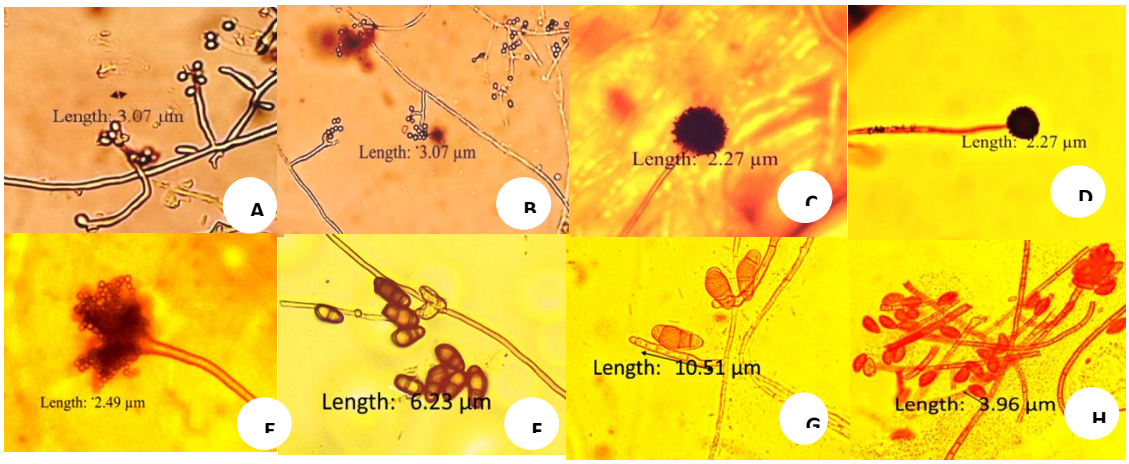

Figure 4. Conidial and hyphal morphology of endophytic fungi: JgCrJr (A), JgSPK (B), PsgTjPr (C), JgByU (D), JgPwSr (E), JgTgSr (F), CMTjP (G), and JgTjPr (H)

Table 2. Mean of conidial density and viability of endophytic fungi

Fungal species	Isolate codes	Conidial density ($1x10^8$ conidia mL^{-1})	Conidial viability (%)	
			24-hour culture	48-hour culture
Beauveria sp.	JgCrJr	4.17±0.24	$55.17±4.93^{bc}$	$55.66±5.05^{bc}$
Beauveria sp.	JgSPK	3.19±0.58	$58.69±0.89^{c}$	$61.87±0.98^{c}$
Aspergillus sp.	PsgTjPr	1.57±0.10	$46.58±2.15^{abc}$	$48.84±2.88^{ab}$
Aspergillus sp.	JgByU	3.36±0.18	$45.04±2.73^{ab}$	$50.95±2.77^{abc}$
Aspergillus sp.	JgPwSr	1.69±0.30	$41.36±3.85^{ab}$	$47.71±0.21^{ab}$
Curvularia sp.	JgTgSr	3.74±0.38	$38.98±3.25^{a}$	$42.73±2.53^{a}$
Curvularia sp.	CMTjP	3.94±0.42	$42.03±3.14^{ab}$	$51.18±1.85^{ab}$
Chaetomium sp.	JgTjPr	3.22±0.30	$39.50±0.15^{a}$	$43.14±7.53^{a}$

F-value	0.06^{ns}	4.91^{*}	5.90^{*}
P value	1.00	0.00	0.00
HSD value	3.06	9.02	7.12

Note: ns= not 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 3. Leaf area eaten by *Spodoptera frugiperda* larvae treated with of endophytic fungi (1 x 10^6 conidia mL^{-1})

Isolates	Mean of leaf area eaten by larvae (cm^2 $larvae^{-1}$ day^{-1}) during 12 days of observation											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Control	4.60^{b}	4.53	7.73	8.29	8.58	8.83^{c}	8.74^{b}	8.83^{b}	8.85^{b}	8.41	8.86	8.99^{b}
JgCrJr	3.62^{ab}	4.18	5.30	6.11	7.42	8.12^{bc}	7.97^{ab}	7.67^{ab}	7.07^{a}	6.64	6.32	5.77^{a}
JgSPK	3.36^{a}	4.12	5.11	6.34	6.91	7.31^{abc}	7.36^{ab}	7.14^{ab}	7.13^{a}	7.57	7.11	6.00^{a}
PsgTjPr	3.78^{ab}	4.35	6.21	7.98	7.67	7.36^{abc}	7.47^{ab}	7.38^{ab}	7.36^{ab}	7.36	6.73	6.21^{a}
JgByU	3.78^{ab}	4.15	5.25	6.27	7.58	7.53^{abc}	7.80^{ab}	7.72^{ab}	7.67^{ab}	7.86	7.56	7.05^{ab}
JgPwSr	3.76^{ab}	4.43	6.17	6.88	7.79	8.12^{bc}	8.07^{ab}	7.49^{ab}	7.27^{ab}	7.14	6.95	6.45^{a}
JgTgSr	3.52^{a}	3.88	7.13	7.72	6.86	6.52^{ab}	7.96^{ab}	7.03^{ab}	6.67^{a}	6.69	7.44	6.53^{a}
CMTjP	3.62^{ab}	3.98	6.40	7.89	6.88	6.33^{a}	6.73^{a}	6.73^{a}	6.48^{a}	6.87	7.42	6.80^{ab}
JgTjPr	3.66^{ab}	4.24	5.82	6.53	6.78	7.21^{abc}	6.78^{ab}	7.31^{ab}	7.92^{ab}	7.50	7.52	7.28^{ab}
F-value	2.96^{*}	1.69^{ns}	1.14^{ns}	0.67^{ns}	0.63^{ns}	5.01^{*}	3.08^{*}	2.59^{*}	5.25^{ns}	1.79^{ns}	2.30^{ns}	4.94^{*}
P value	0.03	0.17	0.39	0.71	0.74	0.00	0.02	0.04	0.00	0.14	0.07	0.00
HSD value	0.23	0.18	0.77	0.90	0.67	0.31	0.32	0.32	0.27	0.38	0.41	0.38

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.

The endophytic fungi pathogenecity against *Spodoptera frugiperda* larvae

The results of the measurement of leaf area eaten by the larvae dripped with the endophytic fungi suspension (1 x 10^6 conidia mL^{-1}) and the control (untreated) on the first day showed significant differences. In the control, the larvae ate the most maize leaves (Table 3). On the second to the fifth days, the leaf area eaten by the larvae from all treatments was not significantly different, while on the sixth to 12th days, the leaf area eaten by the control larvae was wider and tended to be significantly different from that eaten by the larvae being already treated with the endophytic fungi. Consequently, the treated larvae experienced a significant decrease in appetite compared to that of the control. The symptoms of leaves eaten by the larvae treated with the fungus and those eaten by the control also showed significant differences (Figure 5).

The decrease in appetite in the larvae treated with the endophytic fungi was followed by a decrease in their body weight. On the second day, the weight loss of the treated larvae was significant compared to the that of the control, while on the next day, the weight of the larvae among the treatments was not significantly different (Table 4). The feacal weight produced by the treated and untreated larvae tended to show a significant difference. The stool weight produced by the treated larvae tended to be heavier than that produced by the untreated larvae (control) (Table 5). This phenomenon is interesting because generally the normal larvae, which eat a lot, produce a lot of faeces, but in this experiment the result showed the opposite.

Of the eight endophytic fungal isolates found, the most pathogenic JgCrJr isolate (*Beauveria* sp.) resulted in 29.33% larval mortality with LT_{50} for 17.40 days, followed by JgSPK isolate (*Beauveria* sp.) (26.67% mortality) with LT_{50} for 15 days (Table 6). The mortality caused by these two isolates from the beginning of observation to the last day was always higher; the isolate with the lowest ability to cause mortality was JgTgSr (*Curvularia* sp.) (Figure 6). Besides *Beauveria* sp., *Aspergillus* sp., *Chaetomium* sp., and *Curvularia* sp. were also able to cause mortality of *S. frugiperda* larvae. In Indonesia, first report of *Aspergillus* sp., *Chaetomium* sp., and *Curvularia* sp. have insecticidal activity against *S. frugiperda* larvae. The isolate that had the highest reduction in the emergence of adults occurred in JgCrJr isolate (*Beauveria* sp.), causing only 56% of *S. frugiperda* adults to emerge (Table 7). Therefore, the isolate JgCrJr (*Beauveria* sp.) could reduce the adult emergence of *S. frugiperda* by 44%.

The treated larvae exhibited distinctive symptoms that distinguished them from the healthy larvae (Figure 7). The healthy larvae were longer and bigger, and had flexible movements and a tight body, while the larvae that were sick due to being infected with the endophytic fungi were stiff, its body was smaller, shrivels, hardens like a mummy, and over time the body changes color to black but did not smell. The dead larvae were grown in the agar-water medium and their integument grew mycelia and conidia that covered the cadaver. Apart from the larval mortality, the endophytic fungus caused the pupae and adults to be abnormal and malformed (Figures 8 and 9). The abnormal pupae were thinner, bent, shriveled wings and darker in color, and when their body was touched they did not move. The abnormal adults had folded and smaller wings than those of the normal adults.

Table 4. Weight of *Spodoptera frugiperda* larvae treated with endophytic fungi (1 x 10^6 conidia mL^{-1})

Isolates	Mean of larvae weight (mg $larvae^{-1}$) during 12 days observation											
	1	**3**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**

Control	22.99	74.45^{b}	49.16	69.65	97.89	124.53	129.27	136.17	173.73	190.91	207.07	217.27
JgCrJr	23.72	38.67^{ab}	61.24	66.11	83.40	102.58	125.08	137.88	161.84	168.24	181.25	171.60
JgSPK	22.09	25.57^{a}	42.17	74.95	87.53	110.08	115.04	134.66	173.90	197.97	195.24	183.52
PsgTjPr	28.92	45.49^{ab}	44.87	53.95	81.57	90.41	104.52	127.67	160.22	192.28	179.91	180.66
JgByU	26.63	45.45^{ab}	57.12	68.35	74.09	88.80	115.26	132.24	144.85	157.14	161.54	168.66
JgPwSr	15.13	53.59^{ab}	65.47	67.29	93.61	106.29	124.06	145.27	176.09	192.37	184.76	183.23
JgTgSr	19.29	34.12^{a}	52.73	56.47	65.89	75.23	103.57	126.66	146.83	162.20	177.46	172.63
CMTjP	25.31	32.15^{a}	39.76	56.37	65.97	87.10	119.70	137.81	176.33	195.52	188.19	176.03
JgTjPr	24.85	35.51^{a}	48.48	60.21	80.51	95.32	126.71	139.42	197.67	179.91	186.21	185.67
F-value	0.79^{ns}	4.33*	1.61^{ns}	0.41^{ns}	1.28^{ns}	2.18^{ns}	0.69^{ns}	0.18^{ns}	1.33^{ns}	1.64^{ns}	0.75^{ns}	0.99^{ns}
P value	0.62	0.00	0.19	0.90	0.31	0.08	0.70	0.99	0.29	0.18	0.65	0.47
HSD value	2.59	2.54	2.34	3.61	2.73	2.49	2.56	2.86	2.72	2.30	2.67	2.61

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

Table 5. Feacal weight produced by *Spodoptera frugiperda* larvae treated with of endophytic fungi (1 x 10^6 conidia mL^{-1})

Isolates	**Mean of larvae feacal weight (mg larvae^{-1} day^{-1}) during 12 days of observation**											
	1	**3**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Control	11.67^{ab}	15.67^{ab}	17.67	20.67^{a}	25.33^{a}	28.00	31.33	35.00^{a}	38.00^{a}	41.33	47.80	53.33^{c}
JgCrJr	4.22^{a}	7.21^{a}	25.59	55.35^{c}	71.60^{c}	72.14	73.47	64.62^{ab}	57.24^{b}	40.87	47.45	30.98^{ab}
JgSPK	8.98^{ab}	13.58^{ab}	32.12	39.37^{bc}	47.73^{abc}	49.06	59.15	71.79^{b}	62.52^{b}	60.49	44.05	36.99^{abc}
PsgTjPr	20.28^{b}	25.05^{bcd}	28.26	31.21^{ab}	43.59^{abc}	45.75	49.96	45.65^{ab}	41.91^{b}	41.63	38.34	35.09^{abc}
JgByU	16.56^{ab}	21.39^{bcd}	31.55	37.65^{abc}	48.19^{abc}	51.45	55.68	63.66^{ab}	47.76^{b}	46.29	41.93	36.27^{abc}
JgPwSr	21.13^{b}	32.60^{d}	43.62	46.10^{bc}	55.19^{bc}	55.89	54.37	57.60^{ab}	58.10^{b}	53.67	44.08	41.12^{bc}
JgTgSr	13.10^{ab}	17.60^{bc}	25.75	31.53^{ab}	35.00^{ab}	40.41	45.86	47.81^{ab}	40.65^{b}	36.83	33.79	21.57^{a}
CMTjP	19.28^{ab}	34.39^{d}	41.87	40.89^{bc}	43.51^{abc}	57.52	60.45	63.46^{ab}	60.74^{b}	54.64	46.32	38.63^{bc}
JgTjPr	24.80^{b}	29.39^{cd}	36.25	39.22^{bc}	47.26^{abc}	55.54	57.04	61.80^{ab}	60.66^{b}	55.00	45.02	40.77^{bc}
F-value	3.74*	14.29*	2.50^{ns}	6.42*	4.16*	2.26^{ns}	1.72^{ns}	2.77*	3.86*	1.68^{ns}	1.41^{ns}	5.18*
P value	0.01	0.00	0.05	0.00	0.01	0.07	0.16	0.03	0.01	0.17	0.26	0.00
HSD value	2.28	1.35	2.39	1.57	2.22	2.83	2.92	2.35	1.74	2.21	1.50	1.55

Note: ns = not significantly different; * = significantly different; values within a column (the data of each isolate) followed by the same letter were not significantly different at P < 0.05 according to Tukey's HSD test.

Table 6. Mean of larvae mortality, LT_{50}, and LT_{95} of *Spodoptera frugiperda* larvae treated with of endophytic fungi (1 x 10^6 conidia mL^{-1})

Isolates	**Mortality ± SE (%)**	**LT_{50} ± SE (days)**	**LT_{95} ± SE (days)**
Control	0.00±0.00^{a}	-	-
JgCrJr	29.33±3.53^{d}	17.40±1.37	30.08±2.51
JgSPK	26.67±3.53^{cd}	15.00±1.06	27.69±2.20
PsgTjPr	18.67±3.53^{bcd}	17.94±0.68	30.62±1.68
JgByU	9.33±3.53^{b}	23.66±3.01	36.35±4.14
JgPwSr	17.33±3.53^{bcd}	18.89±1.72	31.58±2.84
JgTgSr	9.33±1.33^{b}	22.12±2.15	34.81±3.30
CMTjP	12.00±2.31^{bc}	20.37±1.64	33.06±2.75
JgTjPr	14.67±3.53^{bcd}	20.14±2.28	32.82±3.14
F-value	15.51*	2.13^{ns}	0.88^{ns}
P value	0.00	0.09	0.55
HSD value	11.88	8.74	13.58

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

Table 7. Percentage of *Spodoptera frugiperda* pupae formation and adults emerged after their larvae treated with endophytic fungi (1 x 10^6 conidia mL^{-1})

Isolates	**Mean of pupae formation (%)**	**Mean of adults emerged (%)**
Control	100.00^{d}	100.00^{d}
JgCrJr	70.67^{a}	56.00^{a}
JgSPK	73.33^{ab}	60.00^{ab}
PsgTjPr	81.33^{abc}	70.67^{abc}
JgByU	90.67^{c}	80.00^{bc}
JgPwSr	82.67^{abc}	74.67^{abc}

JgTgSr	90.67^{c}	84.00^{c}
CMTjP	88.00^{bc}	80.00^{bc}
JgTjPr	85.33^{abc}	80.00^{bc}
F-value	16.03*	17.24*
P value	0.00	0.00
HSD value	11.88	14.29

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

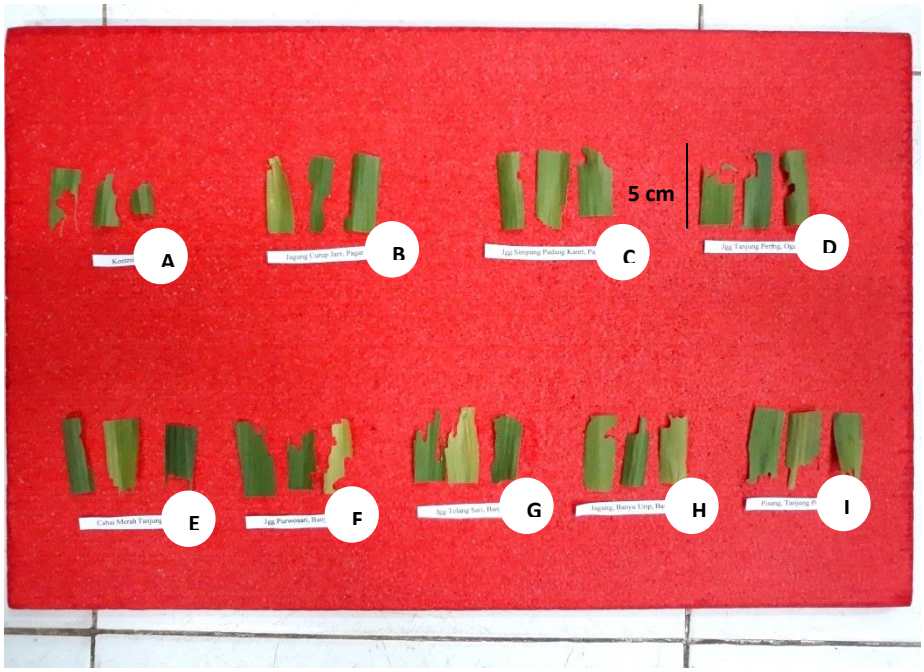

Figure 5. The symptoms on maize leaves eaten by *Spodoptera frugiperda* larvae treated with endophytic fungi (1 x 10^{6} conidia mL^{-1}): Control (A), JgCrJr (B), JgSPK (C), PsgTjPr (D), JgByU (E), JgPwSr (F), JgTgSr (G), CMTjP (H), and JgTjPr (I).

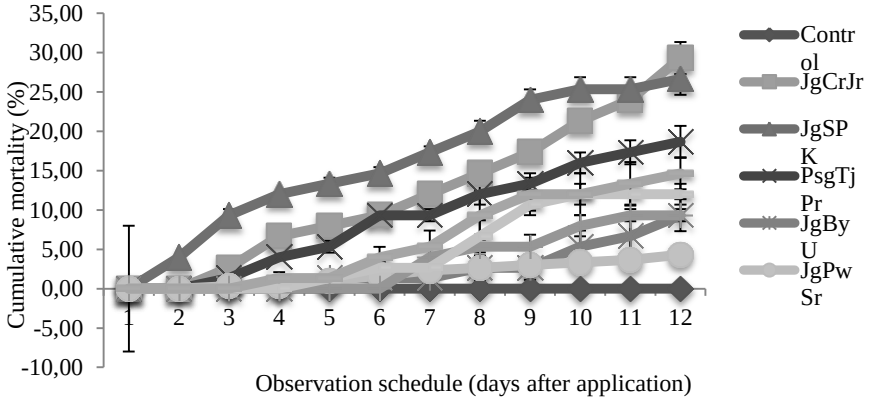

Figure 6. Cumulative mortality of *Spodoptera frugiperda* larvae treated with endophytic fungi (1 x 10^{6} conidia mL^{-1}) during 12 days observation

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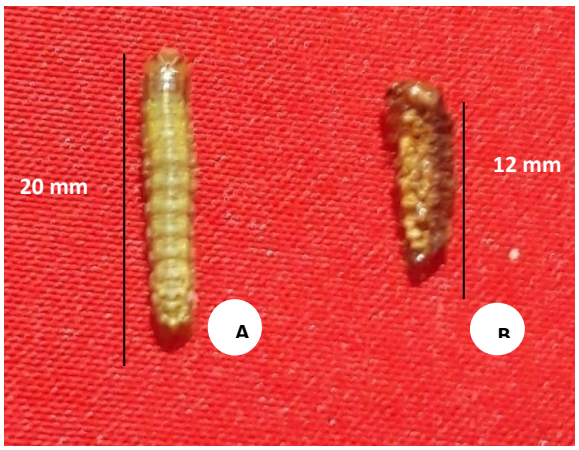

Figure 7. Morphology of *Spodoptera frugiperda* larvae: healthy larvae of control (A) and dead larvae infected by endophytic fungi (B)

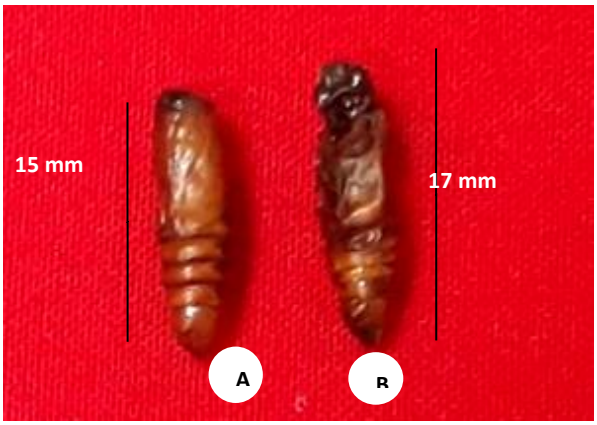

Figure 8. Morphology of *Spodoptera frugiperda* pupae: healthy pupae of control (A) and unhealthy with malformation pupae infected by endophytic fungi (B)

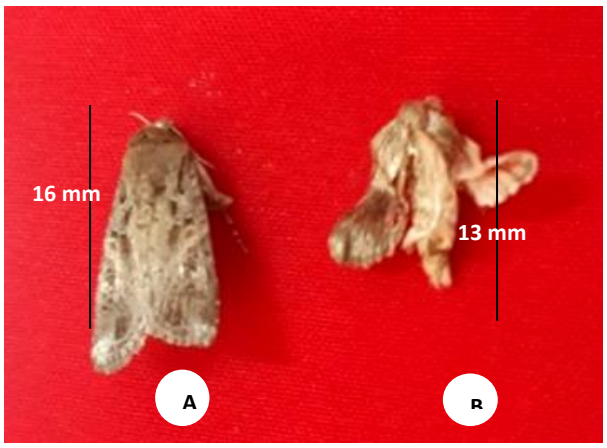

Figure 9. Morphology of *Spodoptera frugiperda* adults: healthy adults of control (A) and unhealthy with malformation adults infected by endophytic fungi (B)

Discussion

Based on the morphological characteristics, the JgCrJr and JgSPK isolates belong to the genus of *Beauveria* sp. The PsgTjPr, JgByU, and JgPwSr isolates belong to the genus of *Aspergillus* sp. The JgTgSr and CMTjP isolates include in *Curvularia* sp. The genus of JgTjPr isolate is *Chaetomium* sp. The morphological characteristics of the four fungal genus match to description by Humber (2005) and El-Ghany (2015). All genus of the endophytic fungi found in this study have insecticidal activity against the *S. frugiperda*. . First report of *Aspergillus* sp., *Chaetomium* sp., and *Curvularia* sp. are pathogenic against *S. frugiperda* larvae. The endophytic *Beauveria* spp. have been shown to kill various species of the insect pests, such as *Diaphorina citri* (Bamisile et al. 2019), *Trialeurodes vaporariorum* (Barra-Bucarei et al. 2020) Wicklow et al. (2000) reported that *Chaetomium* sp is pathogenic against *Helicoverpa zea*. *Chaetomium globosum* significantly inhibit the growth and reproduction of *Myzus persicae* (Qi et al. 2011). *Aspergillus* sp. and *Curvularia* sp. are opportunistic fungi that probably they display an important role in regulating insect populations (Assaf et al. 2011).

In this study, the endophytic fungi isolated from maize, banana and chili roots were able to colonize the plant tissue, both the stems and leaves of maize. The congruent results were also found by Renuka et al. (2016) stating that the endophytic *B. bassiana* colonized the maize leaf and stem tissue. The colonized maize tissues had a characteristic of mycelia fungal color varies depending on the species of fungus and this characteristic is in line with the results of the study by (Jones et al. 2018).

The existence of endophytic fungi in the plant tissues are association that is mutual, mutually beneficial; the fungi get a habitat and niche, while the plants get protection from pests (Jones et al. 2018) and promote growth due to the presence of the endophytic fungi (Barra-Bucarei et al. 2020; Jaber and Ownley 2018). The data in this study prove that the plants inoculated with the endophytic fungi tended to be taller with more and longer roots than the untreated plants. In addition, the treated plants looked healthy and showed no symptoms of illness. These are the preliminary data to be the basis for the future studies on the effect of the endophytic fungus on plant growth.

The endophytic fungal isolates in this study isolated from the root tissues of maize, banana and chili, and the isolates were then re-inoculated through the roots again and proved to enter the maize stalks and leaves systemically as seen from the presence mycelia in the entire plant stems and leaf tissues. Barra-Bucarei et al. (2020) state that endophytic fungal isolates have the ability to have a systemic mode of action. The results of detection by Carolina et al. (2020) show that the endophytic fungi can still be found in the roots, stems and leaves up to 30 days after inoculation. However, according to Shikano (2018) the endophytic fungi are able to colonize plant parts for several months and the duration of their persistence in the

plant tissue varies depending on the age of the plant (high persistence in young tissues). The high fungal persistence in the plant tissues has the potential to develop seed treatment for maize seeds. The seed treatment through seeds allows the endophytic to colonize the plant and prevents *S. frugiperda* larvae from attacking the leaves, stems, and shoots.

In this study, the mortality of larvae treated with the endophytic fungal suspension (1 x 10^6 conidia mL^{-1}) was 29.33%. This result is similar to the study results of Akutse et al. (2019) on the endophytic *B. bassiana* which caused the mortality of *S. frugiperda* larvae for only 30%. According to Resquín-Romero et al. (2016) the mortality by the endophytic fungi can increase if the spore concentration is increased to 1 x 10^8 conidia mL^{-1} and the mortality can range from 41.70–50.00% and it is higher when the application of the combination of the insects eats the part of the colonized tissue by the fungus and in contact. Ramos et al. (2020) stated that the mortality caused by the endophytic *B. bassiana* reached 87% while that caused by the endophytic *M. anisopliae* reached 75%. The variations in the mortality data indicate that the pathogenecity of the fungus depends on the strain of the fungus. In addition, variations in the application method of the fungus also affect mortality. The combination of the fungal treatment in contact with insects and fungi entering through the eaten inoculated leaves can increase the effectiveness of the fungus. In this study, there were two isolates that caused higher mortality of *S. frugiperda* larvae, namely JgCrJr isolate of *Beauveria* sp. (29.33%) and JgSPK isolate of *Beauveria* sp. (26.67%). The two isolates were isolated from the maize root tissue and this finding is interesting because of the high potential to successfully kill *S. frugiperda* larvae hidden in leaf midribs due to the systemic nature of fungi able to colonize maize leaves and stalks. The potential for fungus to be developed as a seed treatment for maize seeds is also high because of the high ability of fungi to colonize roots.

The endophytic fungi in this experiment also decreased the appetite of *S. frugiperda* larvae. The decreased appetite resulted in weight loss. The decrease in appetite was significant on the sixth day after the spray of the fungal conidia. According to El-Ghany (2015) this decreased appetite of the larvae was due to an ongoing fungal infection. The infection occurs when the fungal conidia germinates and can penetrate the host insect's integument (Fernandes et al. 2007). Then, the germ tubes produce specific infection hyphae (El-Ghany 2015). The hyphae spreads to the haemolymph and develops to produce blastospores capable of producing proteolytic or chitinolytic enzymes which can disrupt normal cell metabolism (Mancillas-Paredes et al. 2019) whose symptoms can be seen from the decreased appetite of host larvae. Then, the toxins from secondary metabolites begin to kill the host insect (El-Ghany 2015).

The larvae and pupae that got sick or die after being inoculated with the conidia of endophytic fungi were generally stiff, and the body was smaller, shriveled, hardens like a mummy, and over time the body changed color to black but did not smell. The mycelia and the fungal conidia enveloped the cadaver. In addition, the morphology of pupae and adults becomes abnormal and malformed. The symptoms of these sick larvae and pupae are similar to those found by Herlinda et al. (2020b). The folded wings of adults can cause them to be unable to copulate and thus indirectly lead to a decrease in the population density of the next generation.

Finally, this study found that the endophytic fungi were isolated from the root tissue of maize, banana, and chili from the lowlands to highlands of South Sumatra as many as eight isolates consisting of the genus, *Aspergillus* sp., *Beauveria* sp., *Chaetomium* sp., and *Curvularia* sp. The two most pathogenic isolates against *S. frugiperda* larvae were found from the roots of maize, namely JgCrJr isolate (*Beauveria* sp.) and JgSPK isolate (*Beauveria* sp.) with a mortality of 29.33% and 26.67%, respectively. The isolate JgCrJr (*Beauveria* sp.) can reduce the emergence of *S. frugiperda* adults up to 44%. Consequently, The two endophytic fungal isolates of *Beauveria* sp. have a high potential to be developed to control *S. frugiperda* larvae in maize in both the lowlands and the highlands.

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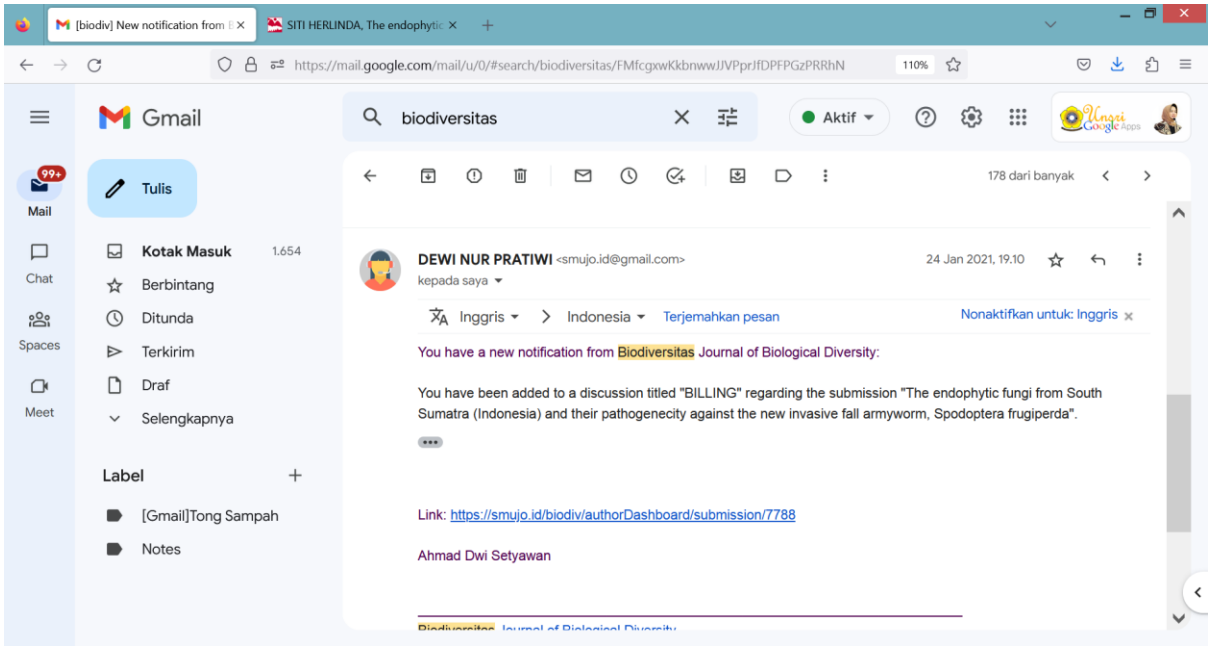

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