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Exploring entomopathogenic fungi from South Sumatra (Indonesia) soil and their pathogenicity against a new invasive maize pest, *Spodoptera frugiperda*

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Abstract. *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*. Fall armyworm (*Spodoptera frugiperda*) is a new invasive maize pest in Indonesia that can cause maize yield losses of 18 million tons/year. To overcome the pest, local-specific entomopathogenic fungi are needed. This study aimed to explore entomopathogenic fungi from soil in South Sumatra and to determine their pathogenicity against *S. frugiperda* larvae. The fungi exploration was carried out in the lowlands and highlands of South Sumatra and the pathogenicity of obtained isolates were tested against the third instar larvae. The entomopathogenic fungi found were *Metarhizium* spp. and were successfully isolated as many as 14 isolates. All of the isolates were pathogenic to *S. frugiperda* larvae (70.67−78.67% mortality), the most pathogenic caused 78.67% mortality and significantly suppressed the emergence of adults up to 81.2%. Unhealthy larvae had a dry, shrunken, shrinking, odorless body, and its integument was covered in mycelia and conidia like yellowish-white powdery mixed with dark green. The unhealthy pupae and adults were in the abnormal and malformation shape. The abnormal pupae were shorter in size, bent, the to-be wings got wrinkled, and darker color, while the unhealthy adults had folded wings and were unable to fly. The two most pathogenic isolates were found from the lowland (PirOI) and highland (CasPsPGA) soil of South Sumatra. In conclusion, both of these isolates had the potential to be developed into local-specific mycoinsecticides to control pest insects in the highlands and/or lowlands in Indonesia.

Keywords: Fall armyworm, isolate, larvae, *Metarhizium*, pupae

INTRODUCTION

Currently, the maize (*Zea may*) in Indonesia is under severe invasion by a new invasive pest called the fall armyworm (FAW) with the scientific name *Spodoptera frugiperda* (Lepidoptera: Noctuidae). The *S. frugiperda* originally comes from South America (Nagoshi et al. 2017; Otim et al. 2018) and now this pest has spread to various continents. In 2016, the FAW was reported to have entered Africa (Goergen et al. 2016). In 2017, the FAW spread into Europe (Early et al. 2018). The pest began to enter Asia in 2018 and was first discovered in India (Ganiger et al. 2018). In April 2019, the FAW reportedly began entering Indonesia and was first discovered in West Sumatra, after which it spread throughout Sumatra, including Lampung (Lestari et al. 2020) and Palembang. Then, the pest crossed to Java and Kalimantan (IPPC 2019).

Maize attacked by *S. frugiperda* larvae generally suffered very heavy damage. In 2016, the maize yield loss in 12 African countries reached 18 million tons/year and the value of losses reached US$ 13 millions (Harrison et al. 2019). In 2018, the FAW caused losses of about a third of the annual production maize in Kenya, estimated at about 1 million tons/year (De Groote et al. 2020). The pest was also reported to attack rice, sugar cane, cotton, and ornamental plants (IPPC 2019). In Brazil, around 76 plant families were reported to be attacked by the pest (Montezano et al. 2018). The outbreaks of FAW moth in maize was influenced by the growth stage of the crop, rainfall, and relative humidity (Nboyine et al. 2020). From the initial survey in South Sumatra, it was found that the *S. frugiperda* larvae attacked leaves, stems, flowers, fruits, and growth points to the whole maize stalk. The *S. frugiperda* becomes a new invasive pest in Indonesia because of the ideal ecosystem for its growth and development.

In Indonesia, *S. frugiperda* is controlled by synthetic insecticides and farmers in various countries in the world rely heavily on synthetic insecticides (Kumela et al. 2018). However, the more often sprayed with the synthetic insecticides, the FAW invasion is increasingly widespread and severe and until March 2020 we have been getting the sweet maize in various agriculture centers in South Sumatra destroyed by the pest. This is because the pest has been resistant to various active ingredients of synthetic insecticides (Wu et al. 2016; Yang et al. 2017). For this reason, a better strategy for controlling *S. frugiperda* by utilizing local natural enemies which are explored from the

ecosystems in Indonesia, for example, entomopathogenic fungi from soil.

The soil which is a fungal habitat during the saprophytic phase has high entomopathogenic fungi inoculum potential, especially those close to plant roots (Safitri et al. 2018) and is effective in killing the insect pests (Ayudya et al. 2019; Sumikarsih et al. 2019; Gustianingtyas et al. 2020). Research results from other countries show the high pathogenicity of entomopathogenic fungi from soil in controlling *S. frugiperda* population. In Mexico, *Beauveria bassiana* and *Metarhizium anisopliae* are reported to be effective in killing *S. frugiperda* larvae (Rivero-Borja et al. 2018) and in Tanzania, the *B. bassiana* and *M. anisopliae* can suppress the adult population of *S. frugiperda* (Ngangambe and Mwatawala 2020). In India, the *S. frugiperda* were attacked by *Nomuraea rileyi* (Shylesha et al. 2018). In addition to killing the larvae, the *B. bassiana* and *M. anisopliae* can kill the *S. frugiperda* eggs (Akutse et al. 2019) and adults (Gutiérrez-Cárdenas et al. 2019). In Cuba, endophytic *B. bassiana* and *M. anisopliae* were found to be pathogenic to *S. frugiperda* larvae (Ramos et al. 2020). However, the entomopathogenic fungi do not harm the egg and larval parasitoids of *S. frugiperda* (Ngangambe and Mwatawala 2020) and the predatory arthropods (Prabawati et al. 2019). The entomopathogenic fungi can be integrated with botanical insecticides (Hernandez-Trejo et al. 2019) and the entomopathogenic virus, such as nucleopolyhedroviruses (Souza et al. 2019). The entomopathogenic fungi from the South Sumatra soil need to be explored and tested for their pathogenicity against the *S. frugiperda*. The location-specific fungi are needed because they tend to be more effective than exogenous ones (Thaochan and Sausa-Ard 2017). This study aimed to explore entomopathogenic fungi from soil in South Sumatra and to determine their pathogenicity against *S. frugiperda* larvae.

MATERIALS AND METHODS

Study area

The exploration was carried out in soils near the roots of rice, maize, and vegetables following the method Safitri et al. (2018). The explored survey sites were from the lowlands to the highlands of South Sumatra, Indonesia, namely the Districts/Cities of Ogan Komering Ilir, Ogan Ilir, Prabumulih, Muara Enim, Lahat, Pagar Alam, and Banyuasin (Figure 1).

Exploring entomopathogenic fungi

The collection of entomopathogenic fungi was carried out by modifying the method of Anwar et al. (2015) and Safitri et al. (2018), using a bait method. The bait method used larvae of *Tenebrio molitor* as insect bait fed in soil samples containing the fungus conidia. The method of soil sampling used the method of Anwar et al. (2015) by digging the soil using a surface soil sampler (Ø 20 mm) as deep as 10-15 cm around the plant rhizosphere according to the method of Thaochan and Sausa-Ard (2017) and by taking 5 points to collect 1000 g of soil. Then, the soil sample was put into a plastic bag and labeled with information about the height of the location, type of commodity, soil pH, and date of collection.

Figure 1. Locations of exploration for entomopathogenic fungi from South Sumatra, Indonesia: 1. Ogan Komering Ilir, 2. Ogan Ilir, 3. Prabumulih, 4. Muara Enim, 5. Lahat, 6. Pagar Alam, and 7. Banyuasin

The soil sample was first cleaned from plant roots and sieved with 10 mesh sieves. Then, it was put into a plastic tray (32 x 25 x 5 cm^3) each containing as much as 1000 g. It was then moistened with sterile distilled water with soil moisture exceeding 20% according to the method of Chen et al. (2014). After that, the 30 newly molted larvae of the third instar *T. molitor* were placed at the bottom of the tray and the bodies of the larva were sprinkled with a layer of sample soil whose thickness was 20-30 mm. Then the tray containing the sample soil covered with a black cloth and sprayed with sterile aqua dest to maintain soil moisture. The larvae were infested in soil samples for 7 days to allow time for entomopathogenic fungal conidia to infect them, after which the dead larvae infected with entomopathogenic fungi were grown in Sabouraud Dextrose Agar (SDA, Merck) media.

Isolation and identification of the entomopathogenic fungi

The infected *Tenebrio* bait was then isolated and purified using the method of Safitri et al. (2018). The entomopathogenic fungi infecting the *Tenebrio* bait and growing on the surface of the integument were isolated and cultured in the SDA media. The surface of the larvae infected with the entomopathogenic fungi was first sterilized by modifying the method of Sharma et al. (2018) by rinsing with 1% NaOCl for 1 minute, then rinsing with 100 mL of distilled water for 3 times. The surface larvae sterilization was carried out to obtain the fungus that was in the hemocoel or the one that already penetrated the larvae cuticle and prevented the presence of the air fungus. The sterilized larvae were grown in SDA media and incubated for 2 days. Then, the growing fungi were purified in the SDA media to get pure isolates. The entomopathogenic fungi already isolated from *T. molitor* larvae were identified based on the morphological characteristics, for example, the colony colors and shapes of culture on SDA, conidia shape and color using a taxonomic book of Humber (2005) and El-Ghany (2015). The conidia density was calculated using the method of Sumikarsih et al. (2019), while the viability was observed by growing 10 µL of fungal conidia suspension (1 x 10^6 conidia mL^{-1}) on 2% of agar-water medium, containing 2 g of agar which was given 100 mL distilled water (w/v), then the culture was incubated for 2 x 24 hours.

Pathogenicity test of the entomopathogenic fungi

Pathogenic test has been carried out in Laboratory of Entomology, Department of Plant Pest and Disease, Faculty of Agriculture, Universitas Sriwijaya, Indralaya from January to March 2020. The room temperature and relative humidity during the experiment were 29.30ºC and 89%. This experiment used the randomized block design with isolates as treatments and was replicated three times.

Preparation of test insect

S. frugiperda larvae were collected from maize fields in Indralaya Village which were not applied with synthetic insecticides. These larvae were brought to the laboratory and kept in plastic cups (Ø 6.5 cm, height 4.6 cm) porous separately between individuals because of their cannibal nature at room temperature. Into the containers were put the maize leaves to feed *S. frugiperda* and the leaves were replaced daily with fresh new leaves. The last stage larvae entering the pupae stage were transferred into a plastic container (Ø15 cm, height 25 cm) that was already provided with the soil (5 cm thickness) sterilized in the oven for 2 hours at 100ºC. The containers containing pupae were placed in gauze cages (30 x 30 x 30 cm^3), and the gauze cages were also provided with 10 pieces of water spinach (*Ipomoea aquatica*) for egg placement for female adults. The water spinach was placed in a plastic bottle (Ø 5.5 cm, height 17 cm) containing tap water height of 10 cm to maintain its freshness. The egg clutch placed by the adults on the surface of the water spinach leaves were moved into the container containing the water spinach leaves. The feed for the first instar larvae was water spinach leaves. After molting the skin, the second-instar larvae until the last instar were fed young maize leaves. The larvae of the second instar to the last one were kept separately in a porous plastic cup (Ø 6.5 cm, height 4.6 cm) because the second instar and so on were cannibalistic. Mass-rearing was carried out to obtain the third generation of culture. The second stage larvae aged 1 day were used for test insects in this study.

Fungus application

The suspension of each isolate culture of the fungus aged 7 x 24 hours with a concentration of 1 x 10^6 conidia mL^{-1} of 1 mL was dripped on maize leaves and topically also applied to the larvae, then the maize leaf was forced to be eaten by the second instar larvae of *S. frugiperda* as much as 25 larvae per isolate for 6 hours and it was repeated three times. The larvae before being treated were first fasted for 2 hours and weighed using a Portable Jewelry Scale (capacity of 30 g x 0.01 g). After 6 hours of infestation and confirmed that all larvae already ate the leaves moistened with the fungus suspension, then they were transferred into a porous plastic cup (Ø 6.5 cm, height 4.6 cm) and each cup contained only 1 larvae and provided 2 x 5 cm^2 maize leaves per day per larvae. To measure the percentage of leaf area eaten (foliar damage caused) by the larvae of *S. frugiperda* used bioleaf application by Machado et al. (2016). Every 1 x 24 hours the dead test larvae were recorded and it was carried out for 12 days based on the previous research by Ayudya et al. (2019) and the number of larvae becoming pupae and the pupae becoming adults were also counted. The number of dead larvae was used to calculate mortality, the Median Lethal Time (LT_{50}), and the 90% of Lethal Time (LT_{95}). The area of the eaten maize leaves, the weight of the feces, and the weight of the larvae body were measured every day from the first day to the 12th.

Data analysis

The data differences among the larval weight data, eaten leaf area, and feces produced each day among the isolate treatments, and mortality and time of death (the LT_{50} and LT_{95}) larvae of *S. frugiperda*, the percentage of larvae into pupae and pupae into adults were analyzed using analysis of variance (ANOVA). The Tukey's

Honestly Significant Difference (HSD) test was employed to test the significant differences among the treatments (isolates) at P = 0.05. The LT_{50} and LT_{95} values were calculated by using probit analysis. All data were calculated using SAS University Edition software 2.7 9.4 M5.

RESULTS AND DISCUSSION

The entomopathogenic fungi found in the survey

The entomopathogenic fungi that were found could only be identified up to Genus. All isolates (14 isolates) found belong to one genus, all *Metarhizium* (Table 1). These isolates were found from 14 locations spreading from the lowlands to the highlands of South Sumatra. The isolates of entomopathogenic fungi were isolated from the soil near the roots of rice, maize, and vegetables depending on the presence of the plants during the survey. The soil pH of the survey site varied between 5 and 6.8 and the low pH was generally found in the lowlands, while in the highlands the soil pH was relatively higher.

Macroscopically, all isolates of *Metarhizium* sp. growing on SDA media had a colony that was initially clear, then developed to a yellowish-white color, then white hyphae formed mycelia and continued to grow and spread evenly, the older they were (5-7 days) the more evenly they spread and produced dark green conidia shaped like flour (powdery) (Figure 2). Microscopically, conidia *Metarhizium* sp. was cylindrical, single, one-celled, hyaline, and its length ranged from 8 to 10 μm, while the mycelium was insulated.

Conidia density of 14 isolates of *Metarhizium* sp. found in this study did not show significant differences among the isolates (Table 2). The viability of conidia both incubated 1 x 24 hours and 2 x 24 hours also did not show significant differences among the isolates. The viability of conidia increased after being incubated for 2 x 24 hours.

Fungal pathogenicity against the *Spodoptera frugiperda* larvae

The measurement of leaf area eaten by the treated larvae with entomopathogenic fungi 1 x 10^6 conidia mL^{-1} from the first to the fourth day showed that the consumed leaf area was not significantly different from those eaten by the untreated (control) larvae. However, starting on the fifth day, the treated *S. frugiperda* larvae began to significantly decrease its appetite when compared to the untreated larvae (Table 3). On the following day, the leaf area eaten by the treated larvae with the fungus remained lower than that of the control. The symptoms of the leaves eaten by the treated and untreated larvae also showed differences (Figure 3). The decreased appetite in the treated larvae with the fungus did not show consistency in the larvae weight loss, yet on the third day of the observation, the treated larvae body weight was significantly lower than that of the untreated larvae weight (Table 4). From the 4th to the 6th days of the observations, all treatments of the larvae body weight showed no significant difference, but on the seventh, to the twelfth days, there was one isolate (PdmOI) which consistently reduced the weight of *S. frugiperda* larvae. The weight of feces produced by the treated and control larvae tended to show a significant difference, i.e. the feces weight produced by the treated larvae tended to be lighter than that of the untreated (control) larvae (Table 5).

All *Metahrizium* isolates tested against the *S. frugiperda* larvae were pathogenic (70.67−78.67%); the most pathogenic caused 78.67% of the mortality and significantly suppressed the emergence of adults up to 81.2% (Table 6 and Figure 4) and they were significantly higher than the untreated larvae (control) (13.33%). Although the mortality among the isolates did not show any significant difference, LT_{50} (6.43 days) and LT_{95} (12.57 days) were found most briefly in PirOI isolates originating from the soil near the rice roots in the lowlands of Pemulutan Ilir, Ogan Ilir District (Table 6). The treated larvae that were still alive and turned into pupae were only around 21.3-29.3% and the larvae that successfully became into adults were only around 18.8-28%, while the untreated larvae managed to become adults as many as 86.67% (Table 7). The lowest emergence of adults (18.8%) was found in CasPsPGA isolates originating from the soil near the roots of chili in the highlands of Pasai, Kota Pagar Alam with LT_{50} for only 6.68 days. Consequently, the fungi in this study could reduce the emergence of *S. frugiperda* adults up to 81.2%.

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

District/City	Village	Crop plants	Species of fungi	Isolate codes	Soil pH	Altitude (m)
Muara Enim	Talang Taling	Pumpkin	*Metarhizium* sp.	LpTtME	5.5	67.2
Ogan Ilir	Pemulutan Ilir	Paddy	*Metarhizium* sp.	PirOI	5.2	11.5
Pagar Alam	Pasai	Eggplant	*Metarhizium* sp.	TePsPGA	6.0	870.0
Ogan Ilir	Bakung	Tomato and maize	*Metarhizium* sp.	ToBkOI	6.0	11.5
Prabumulih	Gunung Ibul	Chili	*Metarhizium* sp.	CaGiPR	5.6	64.3
Prabumulih	Sindur	Chili	*Metarhizium* sp.	CaSnPR	5.7	27.6
Muara Enim	Talang Taling	Chili	*Metarhizium* sp.	CaTtME	5.5	67.2
Ogan Komering Ilir	Lempuing	Cucumber	*Metarhizium* sp.	TiCmLpOKI	5.6	19
Ogan Ilir	Bakung	Chili and maize	*Metarhizium* sp.	CaBkOI	6.0	11.5
Pagar Alam	Pasai	Chili	*Metarhizium* sp.	CasPsPGA	5.9	870.0
Ogan Ilir	Semambu Seteko	Bitter melon	*Metarhizium* sp.	PrSestOI	5.6	22.7
Muara Enim	Skarda	Eggplant	*Metarhizium* sp.	TeSkME	5.1	60.7
Ogan Ilir	Pelabuhan Dalam	Paddy	*Metarhizium* sp.	PdmOI	5.0	15.8
Lahat	Merapi Timur	Peanuts	*Metarhizium* sp.	KtMtLH	6.8	112.0

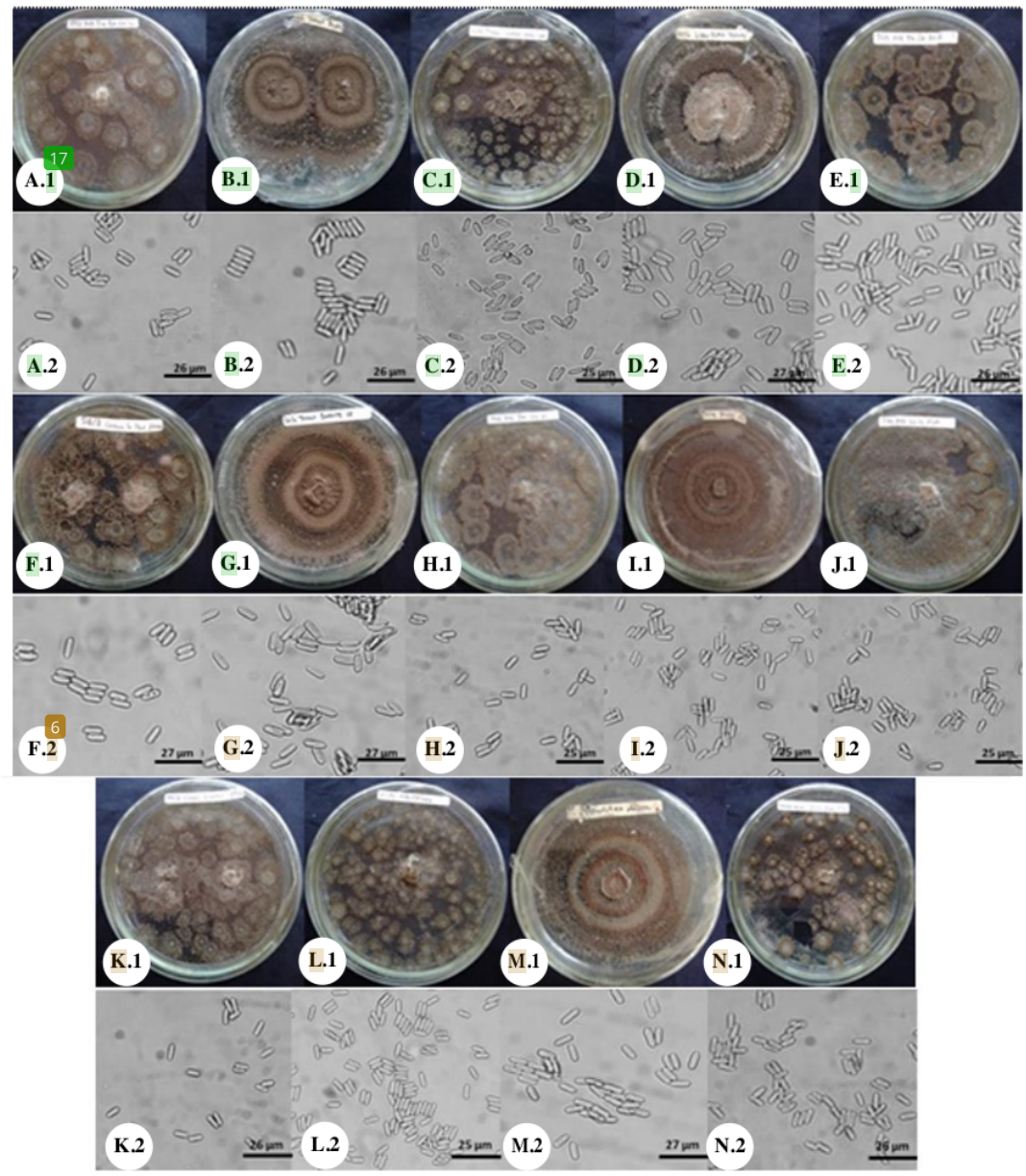

Figure 2. Colonial (*above*) and conidial (*below*) morphology of *Metarhizium* spp. on culture SDA: CaBkOI (A), CaTtME (B), TiCmLpOKI (C), LpTtME (D), TeSkME (E), CaGiPR (F), ToBkOI (G), PrSeStOI (H), TePsPGA (I), CasPsPGA (J), CaSnPR (K), PirOI (L), PdmOI (M), dan KtMtLH (N)

The treated dead larvae showed typical symptoms that distinguished them from healthy larvae. The treated larvae's body was dry, contracted, shrinking, odorless, and the integument was covered by mycelia and conidia like yellowish-white flour mixed with dark green (Figure 5). The treated larvae could produce abnormal and malformation pupae, while the untreated larvae could produce healthy normal pupae (Figure 6). The abnormal pupae were shorter in size, bent, wrinkled wings to-be, darker color, not moving when touched with a brush, and unable to become adults. The adults formed from the treated larvae had folded wings and were smaller than the

untreated larvae (Figure 7). However, this study did not measure the length of pupae and adults formed but only documented in the form of photographs. From the data, the entomopathogenic fungi could kill *S. frugiperda* larvae and pupae, produce abnormal pupae and adults, and reduce the percentage of their adult emergence.

The entomopathogenic fungi can be found from the lowlands to the highlands of South Sumatra because the source of the fungus inoculum in the soil near the roots in this study has a pH range of 5-6.8. The Soil pH determines the presence of the fungus inoculums; the fungi can survive as saprophytes in soil whose pH ranges from 4 to 6.7 (Safitri et al. 2018). In the low acidity and moisture, soil is more easily found fungi in the saprophytic phase (Thaochan and Sausa-Ard 2017). On in vitro media, the entomopathogenic fungi can still grow at a pH ranging from 3 to 9, while the ideal pH for fungal growth ranges from 6 to 7 (Karthikeyan et al. 2008). In addition to pH, soil moisture content also influences the presence of entomopathogenic fungi, and in soil moisture 6-21%, *Beauveria bassiana* conidia are still found to be pathogenic and capable of killing up to 80% of *Anastrepha ludens* adults (Wilson et al. 2020). The ideal in vitro medium moisture for the entomopathogenic fungi to survive ranges from 15% to 35% and the optimum moisture is 35% (Chen et al. 2014). In this study, the soil moisture in the survey location ranged from 13% to 20.9% which is the range of moisture suitable for saprophytic fungal habitat.

The fungi found in this study have morphological characteristics in accordance with the characteristics of the Genus, *Metarhizium* which has been described by Thaochan and Sausa-Ard (2017), Lopes et al. (2018), and Chen et al. (2019). The fungus colony form in the agar medium is initially colorless, then becomes light yellow after the colony is more than 5 days old, the conidia turns green which indicates that the conidia are matured with the conidial mass bluish olive (Thaochan and Sausa-Ard 2017; Lopes et al. 2018) The color of conidia of *Metarhizium* varies from yellow to green (Chen et al. 2019). The *Metarhizium* has cylindrical, single and one-celled, and hyaline conidia while the hyphae septate, smooth-walled, and hyaline (Lopes et al. 2018; Chen et al. 2019), the length of the *Metarhizium* conidia ranges from 7.3 to 14.4 µm (Chen et al. 2019) and this measure of the fungal conidia in this study (8-10 µm) falls into the above range.

Table 2. Mean of conidial density and viability of entomopathogenic fungal isolates

Isolates	Conidial density 1x10^8 (conidia mL^{-1})	Conidial viability (%)	
		24-hour culture	48-hour culture
LpTtME	5.11±0.80	58.33±5.42	68.00±2.49
PirOI	4.63±0.11	53.33±1.52	62.67±4.91
TePsPGA	3.69±0.28	53.67±4.25	62.67±7.09
ToBkOI	5.13±0.10	58.67±6.28	67.33±5.66
CaGiPR	4.24±0.08	49.67±4.06	59.67±3.34
CaSnPR	4.60±0.59	51.33±3.54	57.67±2.88
CaTtME	5.69±0.83	50.33±3.31	65.67±5.04
TiCmLpOKI	5.06±0.14	56.33±1.78	61.33±4.84
CaBkOI	4.52±0.59	59.33±1.91	61.33±5.19
CasPsPGA	5.16±0.25	67.00±2.05	62.33±6.28
PrSeStOI	5.08±0.27	56.67±3.93	63.00±4.78
TeSkME	4.68±0.22	57.67±4.23	64.67±5.86
PdmOI	5.29±0.32	53.67±5.19	68.67±4.38
KtMtLH	4.79±0.11	48.67±4.23	69.33±3.03
F-value	1.37^{ns}	1.01^{ns}	0.35^{ns}
P value	0.23	0.47	0.98
HSD value	-	-	-

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

Table 3. Mean of leaf area eaten by *Spodoptera frugiperda* larvae treated with entomopathogenic fungal isolates with 1 x 10^6 conidia mL^{-1}

Isolates	Leaf area eaten by larvae (cm^2 larvae^{-1} day^{-1}) on observation for 12 days											
	1	2	3	4	5	6	7	8	9	10	11	12
Control	3.91	4.02	8.96	8.64	9.67 b	8.85 b	9.09 b	9.37 b	9.41 b	9.46 b	9.46 b	9.28 b
LpTtME	3.89	4.17	8.01	9.44	8.82 ab	7.48 ab	7.04 a	7.00 a	7.06 a	7.11 ab	7.35 a	6.24 a
PirOI	3.98	3.94	7.98	10.43	8.26 ab	6.80 a	6.81 a	6.65 a	6.79 a	6.72 a	7.23 a	6.03 a
TePsPGA	4.03	4.02	7.54	8.83	7.53 a	6.81 a	7.45 ab	6.87 a	6.95 a	6.67 a	7.10 a	6.12 a
ToBkOI	3.91	3.88	7.64	10.08	8.25 ab	7.22 a	6.86 a	7.11 a	7.38 a	6.41 a	7.02 a	5.80 a
CaGiPR	4.13	4.07	7.73	9.88	7.75 a	7.06 a	7.02 a	7.01 a	6.95 a	6.97 a	7.29 a	7.16 a
CaSnPR	4.08	4.14	7.78	9.99	8.06 ab	6.89 a	7.30 ab	6.71 a	6.59 a	7.14 a	7.76 a	6.54 a
CaTtME	4.00	4.01	8.15	9.11	8.07 ab	7.11 a	6.73 a	7.07 a	6.90 a	6.86 a	7.21 a	7.13 a
TiCmLpOKI	4.09	4.23	8.06	9.50	8.21 ab	6.66 a	7.99 ab	6.89 a	7.57 a	7.27 a	6.67 a	7.17 a
CaBkOI	4.07	4.16	8.13	10.28	8.01 ab	7.28 a	7.20 ab	6.89 a	6.90 a	6.70 a	6.96 a	6.68 a
CasPsPGA	4.03	4.05	7.68	9.73	7.56 a	6.76 a	6.41 a	6.92 a	7.38 a	7.78 a	7.16 a	7.00 a
PrSeStOI	4.12	4.07	8.17	9.77	8.11 ab	6.85 a	7.43 ab	7.05 a	6.96 a	7.25 a	7.40 a	6.62 a
TeSkME	4.12	3.96	8.01	10.27	7.94 a	7.00 a	6.94 a	7.34 a	6.97 a	7.08 a	7.07 a	6.45 a
PdmOI	4.09	4.05	8.38	10.07	7.46 a	7.31 a	6.33 a	6.93 a	7.29 a	7.31 a	7.07 a	6.92 a
KtMtLH	4.04	3.98	8.17	9.33	7.69 a	6.54 a	6.50 a	7.02 a	6.59 a	6.97 a	7.44 a	6.91 a
F-value	1.07^{ns}	0.36^{ns}	1.02^{ns}	1.90^{ns}	2.81*	3.24*	3.32*	2.70*	4.78*	6.18*	3.34*	5.73*
P value	0.42	0.98	0.46	0.07	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00
HSD value	-	-	-	-	1.72	1.57	1.96	2.02	1.60	1.48	1.79	1.74

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

Table 4. Mean of weight of *Spodoptera frugiperda* larvae treated with entomopathogenic fungal isolates with 1 x 10^6 conidia mL^{-1}

Isolates	Larval weight (mg $larvae^{-1}$) on observation for 12 days											
	1	3	3	4	5	6	7	8	9	10	11	12
Control	3.90 b	6.93	17.93 b	23.68	26.85	36.72	42.94 ab	52.99 ab	64.40 ab	81.33 abc	88.10 ab	112.73 ab
LpTtME	3.51 a	5.68	11.23 a	17.34	24.14	38.53	49.08 ab	56.94 ab	74.63 ab	84.17 abc	98.83 ab	120.38 ab
PirOI	3.47 a	6.14	10.92 a	21.35	27.84	41.72	54.95 b	71.65 b	95.01 b	117.68 c	132.59 b	159.13 b
TePsPGA	3.80 a	5.93	10.00 a	17.84	28.69	36.41	51.26 ab	61.08 ab	74.54 ab	90.64 abc	102.06 ab	117.57 ab
ToBkOI	2.49 a	5.04	9.35 a	18.16	26.40	35.81	46.20 ab	61.45 ab	67.81 ab	91.95 abc	103.89 ab	120.03 ab
CaGiPR	2.29 a	6.02	11.36 a	17.22	25.00	32.79	47.19 ab	60.45 ab	72.42 ab	86.91 abc	111.62 ab	149.44 ab
CaSnPR	3.33 a	5.89	10.47 a	20.48	27.30	36.76	50.67 ab	63.37 ab	80.36 ab	97.81 abc	117.69 ab	138.70 ab
CaTtME	2.69 a	8.23	11.50 a	17.91	23.22	32.69	46.86 ab	62.24 ab	76.13 ab	88.41 abc	105.22 ab	125.29 ab
TiCmLpOKI	1.77 a	5.29	12.61 a	19.41	26.09	34.93	44.34 ab	55.16 ab	61.31 ab	70.96 ab	86.59 ab	97.44 ab
CaBkOI	3.09 a	6.30	12.49 a	18.14	25.09	33.12	45.94 ab	57.69 ab	77.05 ab	88.90 abc	104.85 ab	119.39 ab
CasPsPGA	1.59 a	6.08	11.56 a	17.45	26.11	34.02	46.88 ab	63.22 ab	85.10 ab	97.56 abc	122.99 ab	129.71 ab
PrSeStOI	3.96 b	6.07	12.35 a	17.24	23.75	33.08	43.89 ab	61.63 ab	76.11 ab	83.97 abc	97.10 ab	120.61 ab
TeSkME	3.04 a	7.07	11.50 a	17.67	25.34	33.78	40.15 ab	54.92 ab	64.13 ab	73.64 ab	91.49 ab	101.15 ab
PdmOI	2.48 a	4.86	9.69 a	15.47	22.23	29.91	35.64 a	42.26 a	52.69 a	58.46 a	76.93 a	94.76 a
KtMtLH	2.59 a	6.99	11.60 a	20.93	27.86	39.60	54.88 b	65.89 ab	83.29 ab	107.28 bc	118.55 ab	142.51 ab
F-value	7.54*	1.41^{ns}	4.32*	1.82^{ns}	0.78^{ns}	1.55^{ns}	2.69*	2.07*	2.28*	3.31*	2.50*	2.27*
P value	0.00	0.21	0.00	0.08	0.68	0.15	0.01	0.05	0.03	0.00	0.02	0.03
HSD value	2.73	-	4.94	-	-	-	16.16	23.96	35.98	40.82	38.91	63.10

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 5. Mean of feces weight produced by *Spodoptera frugiperda* larvae treated with entomopathogenic fungal isolates with 1 x 10^6 conidia mL^{-1}

Isolates	Larval feces weight (mg $larvae^{-1}$ day^{-1}) on observation for 12 days											
	1	3	3	4	5	6	7	8	9	10	11	12
Control	1.97	5.05	7.23 b	11.15 b	16.14 c	20.56 d	23.50 c	27.23 c	28.32 c	30.60 b	34.39 b	36.40 b
LpTtME	1.78	3.63	6.70 b	6.31 a	8.06 ab	7.69 ab	14.18 ab	13.70 ab	14.37 ab	12.38 a	16.52 a	17.18 a
PirOI	1.59	2.71	6.08 ab	7.41 ab	9.59 ab	9.37 abc	11.92 ab	13.72 ab	24.78 bc	18.57 a	24.70ab	22.07 a
TePsPGA	1.02	2.38	4.80 ab	7.07 ab	6.49 ab	6.66 ab	7.83 a	10.29 a	16.44 ab	16.21 a	16.76 a	17.50 a
ToBkOI	1.03	2.40	5.28 ab	6.95 a	5.67 a	8.79 abc	14.55 ab	10.78 a	15.07 ab	15.17 a	19.41 a	20.61 a
CaGiPR	1.02	2.65	3.89 ab	8.01 ab	5.77 ab	10.88 bc	16.39 bc	11.53 ab	13.53 ab	16.24 a	20.07 a	22.96 a
CaSnPR	1.10	2.67	7.07 b	8.02 ab	9.50 ab	8.53 abc	13.07 ab	9.97 a	16.93 abc	16.38 a	21.31 a	23.49 ab
CaTtME	1.39	3.44	4.11 ab	6.15 a	8.62 ab	10.58 bc	11.95 ab	17.16 ab	17.31 abc	19.87 ab	22.17 a	21.52 a
TiCmLpOKI	0.99	3.04	5.87 ab	8.69 ab	10.23 abc	12.90 c	11.29 ab	17.48 abc	10.88 a	14.38 a	15.43 a	17.31 a
CaBkOI	1.68	3.27	4.30 ab	8.19 ab	9.78 ab	8.25 ab	9.48 ab	18.24 abc	21.18 abc	20.91 ab	20.19 a	21.89 a
CasPsPGA	1.88	2.74	4.19 ab	7.33 ab	10.83 abc	9.10 abc	8.78 a	21.52 bc	20.82 abc	19.77 ab	18.90 a	21.62 a
PrSeStOI	1.77	3.14	4.09 ab	6.75 a	11.69 abc	8.82 abc	15.04 ab	17.83 abc	21.41 abc	18.28 a	21.59 a	23.01 a
TeSkME	1.58	2.72	4.54 ab	5.73 a	10.45 abc	5.91 a	11.92 ab	14.48 ab	15.48 ab	17.51 a	17.58 a	18.80 a
PdmOI	0.72	3.07	2.28 a	5.70 a	11.08 abc	9.43 abc	9.33 ab	11.62 ab	12.96 ab	15.00 a	14.69 a	16.07 a
KtMtLH	1.61	2.75	6.10 ab	7.39 ab	11.42 bc	10.84 bc	12.04 ab	17.28 abc	19.87 abc	18.91 a	22.29 a	23.64 ab
F-value	1.24^{ns}	2.01^{ns}	2.62*	2.76*	4.95*	14.09*	7.28*	5.85*	3.79*	3.60*	4.34*	3.57*
P value	0.30	0.05	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
HSD value	-	-	4.40	4.24	6.13	4.70	7.33	10.10	12.53	11.32	11.82	13.14

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

In this study, the two most pathogenic isolates of *S. frugiperda* larvae and pupae characterized by the highest mortality of larvae and pupae and able to kill them in the shortest amount of time were PirOI and CasPsPGA isolates. The PirOI isolates originate from the soil near rice roots in the lowlands, while CasPsPGA isolates originate from the soil near chilli roots in the highlands. The soil origin of the isolate did not determine the virulence of the fungus, in line with the results of the study (Thaochan and Sausa-Ard 2017) stating that the original host or the geographic origin of the isolate has no association with the virulence of the fungus. The virulence of this fungus is more determined by the ability of germ tubes of conidia to penetrate the insect cuticle when it infects its host insect (El-Ghany 2015). The discovery of the two most pathogenic isolates originating from the lowlands and highlands of South Sumatra is a useful finding to develop local-specific mycoinsecticides to control pest insects in the highlands and/or lowlands. Because *S. frugiperda* is a maize insect pest spreading from the lowlands to highlands in South Sumatra in particular and Indonesia in general, the location-specific entomopathogenic fungi are needed.

The data of area of the leaves eaten by the treated larvae showed the entomopathogenic fungus, *Metarhizium* sp. can reduce larvae appetite and reduce larvae weight. On the fifth day of observation, the treated *S. frugiperda* larvae began to significantly decrease their appetite because this is the period of which the process of infection by the fungus was taking place. Initially, the conidia sticking on its cuticle or entering through the mouth of the larvae began to show its effect on the 5th day. The process of infection by the conidia through an insect integument usually begins with the conidia sticking to the cuticle, then the conidia ruptures to form germ tubes which grow toward the soft integument to penetrate the cuticle (Fernando et al. 2007). Infection begins when germ tubes are able to penetrate the insect cuticle and the ability to infect is a determining factor for the fungus virulence (Altre and Vandenberg 2001). After the germ tubes penetrate the cuticle and reach hemocoel, they produce specific infection hyphae originating at appressoria (El-Ghany 2015).

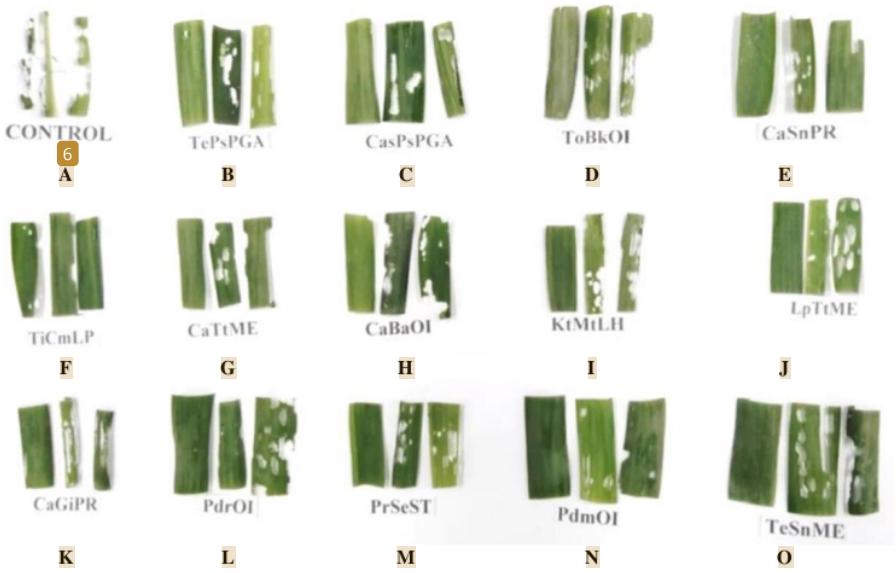

Figure 3. The symptoms on maize leaves eaten by *Spodoptera frugiperda* larvae treated with entomopathogenic fungal isolates with 1 x 10^6 conidia mL^{-1}: Control (A), TePsPGA (B), CasPsPGA (C), ToBkOI (D), CaSnPR (E), TiCmLpOKI (F), CaTtME (G), CaBkOI (H), KtMtLH (I), LpTtME (J), CaGiPR (K), PirOI (L), PrSeStOI (M), PdmOI (N), dan TeSkME (O)

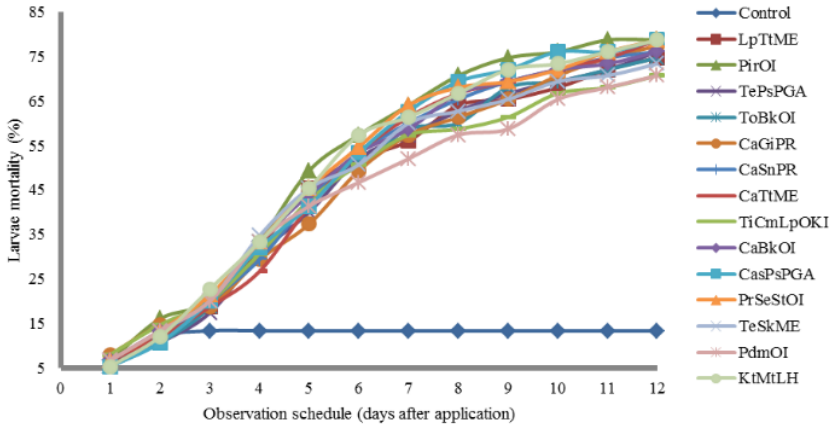

Figure 4. Mortality of *Spodoptera frugiperda* larvae treated with entomopathogenic fungal isolates with 1 x 10^6 conidia mL^{-1} on observation for 12 days

Table 6. Mean of larvae mortality, LT_{50}, and LT_{95} of *Spodoptera frugiperda* larvae treated with entomopathogenic fungal isolates with 1 x 10^6 conidia mL^{-1}

Isolates	Mortality ± SE (%)	LT_{50} ± SE (days)	LT_{95} ± SE (days)
Control	13.33±2.17 a	-	-
LpTtME	74.67±1.09 b	7.23±0.15	15.12±0.04
PirOI	78.67±1.09 b	6.43±0.20	14.31±0.13
TePsPGA	76.00±0.00 b	7.14±0.08	15.01±0.18
ToBkOI	74.67±2.18 b	7.01±0.30	14.89±0.41
CaGiPR	77.33±2.88 b	7.09±0.43	14.97±0.41
CaSnPR	76.00±1.89 b	6.98±0.12	14.86±0.05
CaTtME	78.67±1.09 b	7.10±0.19	14.98±0.27
TiCmLpOKI	70.67±2.18 b	7.18±0.16	15.06±0.24
CaBkOI	76.00±1.89 b	6.82±0.18	14.69±0.31
CasPsPGA	78.67±1.09 b	6.68±0.32	14.55±0.44
PrSeStOI	78.67±1.09 b	6.53±0.23	14.40±0.20
TeSkME	73.33±1.09 b	6.93±0.25	14.81±0.37
PdmOI	70.67±2.18 b	7.54±0.20	15.41±0.24
KtMtLH	78.67±2.18 b	6.74±0.40	14.61±0.38
F-value	58.09*	0.96^{ns}	0.70^{ns}
P-value	0.00	0.51	0.74
HSD value	11.07	-	-

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 7. Mean of percentage of *Spodoptera frugiperda* pupae formation and adults emerged after their larvae treated with entomopathogenic fungi 1 x 10^6 conidia mL^{-1}

Isolates	Pupae formation (%)	Adults emerged (%)
Control	86.7 b	86.7 b
LpTtME	25.3 a	24.0 a
PirOI	21.3 a	21.3 a
TePsPGA	24.0 a	21.3 a
ToBkOI	25.3 a	22.7 a
CaGiPR	22.7 a	21.3 a
CaSnPR	24.0 a	21.3 a
CaTtME	21.3 a	18.7 a
TiCmLpOKI	29.3 a	28.0 a
CaBkOI	24.0 a	22.7 a
CasPsPGA	21.3 a	18.7 a
PrSeStOI	21.3 a	21.3 a
TeSkME	26.7 a	18.7 a
PdmOI	29.3 a	28.0 a
KtMtLH	21.3 a	20.0 a
F-value	58.09*	69.21*
P-value	0.00	0.00
HSD value	11.07	10.49

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.

A B

Figure 5. Morphology of *Spodoptera frugiperda* larvae: healthy larvae of control (A) and dead larvae infected by entomopathogenic fungi (B)

A B C

Figure 6. Morphology of *Spodoptera frugiperda* pupae: healthy pupae of control (A) and unhealthy with malformation pupae infected by entomopathogenic fungi (B and C)

A B C

Figure 7. Morphology of *Spodoptera frugiperda* adults: healthy adults of control (A) and unhealthy with malformation adults infected by entomopathogenic fungi (B and C)

Then, the hyphae spread to the hemolymph and develop to produce blastospores. After that, the blastospores produce secondary metabolites and enzymes, for example, *B. bassiana* produces enzymes of proteolytic and chitinolytic which can disrupt normal cell metabolism (Mancillas-Paredes et al. 2019) which is seen from the decreased appetite that ultimately reduces the weight of host insects. The next process, toxins from secondary metabolites begin to kill host insects, but the death of these host insects is not only by the toxin but also due to the

mechanical damage by fungal penetration into the body of an insect (El-Ghany 2015).

The treated larvae were 78.67% dead and the surviving larvae were only able to become abnormal pupae and adults and malformations. The larvae that were infected and death caused by the fungi in this study showed the symptoms of his body got dried, shriveled, smaller, and odorless, while the integument was covered by mycelia and conidia resembling yellowish-white flour mixed with dark green simila[41] the symptoms described by Thaochan and Sausa-Ard (Thaochan and Sausa-Ard 2017). The symptoms of the infected insects due to the entomopathogenic fungal infections vary depending on favorable environmental conditions, for example, relative humidity higher than 62% (Gutiérrez-Cárdenas et al. 2019), then the mycelia and mature conidia can grow well and cover the entire body of the insect, and the condition of unfavorable mycelia and conidia does not appear (El-Ghany 2015). *Metarhizium* had been reported to be effective against *Aphis craccivora* (Mweke et al. 2019), *Spodoptera litura* (Gustianingtyas et al. 2020), and *Sitophilus oryzae* (Kavallieratos et al. 2015).

Pupae getting unhealthy due to the treatment with *Metarhizium* sp. are abnormal and malformation and unable to become adults, while those that can become adults generally have folded wings and a smaller body than those of the healthy ones. Abnormal and malformation of insects because of the infectious entomopathogenic fungi result from the activity of proteases and chitinases that dissolve protein and chitin in the body of the insects (Mancillas-Paredes et al. 2019). The abnormal adults with folded wings could not continue their offspring because they were not able to fly for having the mating which automatically can reduce the population of the offspring. In line with the research of Kalvnadi et al. (2018), the entomopathogenic fungi can also disrupt insect normal growth, reduc[25]cundity, and generate fitness.

From the results of this study, it can be concluded that the found entomopathogenic fungi all generate from Genus, *Metarhizium* and all isolates are pathogenic to *S. frugiperda* larvae (70.67−78.67%), the two most pathogenic isolates (PirOI and CasPsPGA) caused 78.67% of the mortality and significantly suppressed the emergence of adults up to 81.2%. The most pathogenic isolates found [4]m the lowlands (PirOI) and highlands (CasPsPGA) of South Sumatra both have the pot[14]al to be developed into local-specific mycoinsecticides to control pest insects in the highlands and/or lowlands in Indonesia.

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