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COVERING LETTER

Dear Editor-in-Chief,

I herewith enclosed a research article,

Title:

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

Author(s) name:

Siti Herlinda^{1,2}, Noni Octariati¹, Suwandi^{1,2}, Hasbi^{2,3}

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This study highlights several findings, such as new information that an exogenous pest, *Spodoptera frugiperda* have been found in South Sumatra. We also have found 14 isolates of *Metarhizium* sp. that are pathogenic againts *S. frugiperda* larvae and the most two pathogenic isolates found were PirOI isolate from lowlands and CasPsPGA isolate from highland of South Sumatra.

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Place and date:

Palembang, 14 April 2020

Sincerely yours,

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Siti Herlinda

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

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10 Abstract. Fall armyworm (Spodoptera frugiperda) is a new invasive maize pest in Indonesia that can cause maize yield losses of 18 11 million tons/year. To overcome these pests, local-specific entomopathogenic fungi are needed. This study aimed to explore 12 entomopathogenic fungi from soil in South Sumatra and to determine their pathogenicity against S. frugiperda larvae. The fungi 13 exploration was carried out in the lowlands and highlands of South Sumatra and the pathogenicity of obtained isolates were tested 14 against the third instar larvae. The entomopathogenic fungi found were Metarhizium spp. and were successfully isolated as many as 14 15 isolates. All of the isolates were pathogenic to S. frugiperda larvae (70.67-78.67% mortality), the most pathogenic caused 78.67% 16 mortality and suppressed the emergence of adults up to 81.2%. Unhealthy larvae had a dry, shrunken, and shrinking, odorless body, and 17 its integument was covered in mycelia and conidia like yellowish white powdery mixed with dark green. The unhealthy pupae and 18 adults were in the abnormal and malformation shape. The abnormal pupae were shorter in size, bent, the to-be wings got wrinkled, and 19 darker color, while the unhealthy adults had folded wings and were unable to fly. The two most pathogenic isolates were found from the 20 lowland (PirOI) and highland (CasPsPGA) of South Sumatra. In conclusion, both of these isolates had the potential to be developed into 21 local-specific mycoinsecticides to control pest insects in the highlands and/or lowlands in Indonesia.

22 Key words: Fall armyworm, isolate, larvae, *Metarhizium* sp., pupae

23 Abbreviations (if any): -

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24 Running title: Entomopathogenic fungi againts Spodoptera frugiperda

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). *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, this FAW was reported to have entered Africa (Goergen et al. 2016). In 2017, FAW spread into Europe (Early et al. 2018). This pest began to enter Asia in 2018 and was first discovered in India (Ganiger et al. 2018). In April 2019, this 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, this pest crossed to Java and Kalimantan (IPPC 2019).

33 Maize attacked by S. frugiperda larvae generally suffered very heavy damage. In 2016, the maize yield loss in 12 34 African countries reached 18 million tons/year and the value of losses reached US \$ 13 million (Harrison et al. 2019). In 35 2018, FAW caused losses of about a third of the annual production maize in Kenya, estimated at about 1 million tons/year 36 (De Groote et al. 2020). This pest was also reported to attack rice, sugar cane, cotton, and ornamental plants (IPPC 2019). 37 In Brazil around 76 plant families were reported to be attacked by this pest (Montezano et al. 2018). The outbreaks of 38 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 S. frugiperda larvae attacked leaves, stems, flowers, fruits, and 39 40 growing points to the whole maize stalk. S. frugiperda becomes a new invasive pest in Indonesia because of the ideal 41 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 the synthetic insecticides (Kumela et al. 2018). However, the more often sprayed with synthetic insecticides, the FAW invasion is increasingly widespread and severe and until March 2020 we have been getting the sweet maize in various maize centers in South Sumatra destroyed by this pest. This is because these pests have been resistant to various active ingredients of synthetic insecticides (Wu et al. 2016; Yang et al. 2017). For this reason, a better strategy for 47 controlling *S. frugiperda* by utilizing local natural enemies which are explored from the ecosystems in Indonesia, for
 48 example entomopathogenic fungi.

49 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; 50 Sumikarsih et al. 2019; Gustianingtyas et al. 2020). Research results from other countries show the high pathogenicity of 51 entomopathogenic fungi from soil in controlling S. frugiperda population. In Mexico, Beauveria bassiana and 52 Metarhizium anisopliae are reported to be effective in killing S. frugiperda larvae (Rivero-Borja et al. 2018) and in 53 54 Tanzania, B. bassiana and M. anisopliae can suppress the adult population of S. frugiperda (Ngangambe and Mwatawala 55 2020). In India, the S. frugiperda were attacked by Nomuraea rileyi (Shylesha et al. 2018). In addition to killing the larvae, B. bassiana and M. anisopliae can kill the eggs of S. frugiperda eggs (Akutse et al. 2019) and adults (Gutiérrez-56 Cárdenas et al. 2019). In Cuba, endophytic B. bassiana and M. anisopliae were found to be pathogenic to S. frugiperda 57 58 larvae (Ramos et al. 2020). Entomopathogenic fungi do not harm the egg parasitoids and S. frugiperda larvae (Ngangambe 59 and Mwatawala 2020) and predatory arthropods (Prabawati et al. 2019). The entomopathogenic fungi can be integrated 60 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 61 62 pathogenicity against the newcomer pest, 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 63 64 from soil in South Sumatra and to determine their pathogenicity against *S. frugiperda* larvae.

MATERIALS AND METHODS

66 Study area

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67 The exploration was carried out in soils near the roots of rice, maize and vegetables following the method Safitri et al.
68 (2018). The explored survey sites were from the lowlands to the highlands of South Sumatra, namely the Districts/Cities
69 of Ogan Komering Ilir, Ogan Ilir, Prabumulih, Muara Enim, Lahat, Pagar Alam, Banyuasin, and East OKU (Figure 1).



Figure 1. Locations of exploration for entomopathogenic lungi: District/City of Ogan Komering Inr (1), Ogan Ilir (2), Prabumulih
 (C), Muara Enim (4), Lahat (5), Pagar Alam (6), and Banyuasin (7)

91 Exploring entomopathogenic fungi

The collection of entomopathogenic fungi was carried out by modifying the method of Anwar et al. (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.

The soil sample was first cleaned from plant roots and sieved with 10 mesh sieves. Then, it was put into a plastic tray ($32 \times 25 \times 5 \text{ cm}^3$) 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.

107 Isolation and identification of entomopathogenic fungi

108 The infected *Tenebrio* bait was then isolated and purified using the method of Safitri et al. (2018). 109 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 entomopathogenic fungi was first sterilized by 110 111 modifying the method of Sharma et al. (Sharma et al. 2018) by rinsing with 1% NaOCl for 1 minute, then rinsing with 112 100 mL of distilled water for 3 times. The surface larvae sterilization was carried out to obtain the fungus that was in the 113 haemocoel 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 114 pure isolates. The entomopathogenic fungi already isolated from T. molitor larvae were identified based on the 115 morphological characteristics, for example the colony colors and shapes of culture on SDA, conidia shape and color using 116 a taxonomic book of Humber (2005) and El-Ghany (2015). The conidia density was calculated using the method of 117 118 Sumikarsih et al. (2019), while the viability was observed by growing 10 μ L of fungal conidia suspension (1 x 10⁶ conidia 119 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 120 was incubated for 2 x 24 hours.

121 Pathogenicity test of entomopathogenic fungi

Pathogenicity 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 29.30°C and 89%.

125 *Preparation of test insect*

126 The S. frugiperda larvae were collected from Indralaya Village maize which was not applied with synthetic 127 insecticides. These larvae were brought to the laboratory and kept in plastic cups (Ø 6.5 cm, height 4.6 cm) porous 128 separately between individuals because of their cannibal nature at room temperature. Into the containers were put the 129 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 (5 130 cm thickness) and sterilized in the oven for 2 hours at 100°C. The containers containing pupae were placed in gauze cages 131 (30 x 30 x 30 cm³), and the gauze cages were also provided with 10 pieces of water spinach (*Ipomoea aquatica*) for egg 132 133 placement for female adults. The water spinach was placed in a plastic bottle (Ø 5.5 cm, height 17 cm) containing tap 134 water height of 10 cm to maintain its freshness. The egg clutch placed by the adults on the surface of the water spinach 135 leaves were moved into the container containing the water spinach leaves. The feed for the first instar larvae was water 136 spinach leaves. After molting the skin, the second-instar larvae until the last instar were fed young maize leaves. The 137 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 a third generation of culture. The 138 139 second stage larvae aged 1 day were used for test insects in this study.

140 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 141 142 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 animals per isolate for 6 hours and it was repeated three times. The 143 144 larvae before being treated were first fasted for 2 hours and weighed using a Portable Jewelry Scale (capacity of 30 g x 145 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 1 146 147 larvae and provided 2 x 5 cm² maize leaves per day per larva. To measure the percentage of leaf area eaten (foliar damage 148 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 149 150 number of larvae becoming pupae and the pupae becoming adults were also counted. The number of dead larvae was used 151 to calculate mortality, the Median Lethal Time (LT_{50}), and the 90% of Lethal Time (LT_{90}). The area of the eaten maize 152 leaves, the weight of the feces, and the weight of the larvae body were measured every day from the first day to the 12th.

153 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_{90}) 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_{90} values were calculated by using probit analysis. All data were calculated using SAS University Edition software 2.7 9.4
 M5.

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RESULTS AND DISCUSSION

161 Entomopathogenic fungi found in the survey

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. 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 to10 µm, while the mycelium was insulated.

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

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

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

176 Fungal pathogenicity againts *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 177 first to the fourth day showed that the consumed leaf area was not significantly different from those eaten by the untreated 178 (control) larvae. However, starting on the fifth day, the treated S. Frugiperda larvae began to significantly decrease its 179 appetite when compared to the untreated larvae (Table 3). On the following day, the leaf area eaten by the treated larvae 180 181 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 182 consistency in the larvae weight loss, yet on the third day of the observation the treated larvae body weight was 183 184 significantly lower than that of the untreated larvae weight (Table 4). From the 4th to the 6th days of the observations, all 185 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 186 treated and control larvae tended to show a significant difference, i.e. the feces weight produced by the treated larvae 187 188 tended to be lighter than that of the untreated (control) larvae (Table 5).

189 All Metahrizium isolates tested against the S. frugiperda larvae were pathogenic (70.67–78.67%); the most pathogenic 190 caused 78.67% of the mortality and suppressed the emergence of adults up to 81.2% (Table 6 and Figure 4) and they were 191 significantly higher than the untreated larvae (control) (13.33%). Although the mortality among the isolates did not show 192 any significant difference, LT_{50} (6.43 days) and LT_{90} (12.57 days) were found most briefly in PirOI isolates originating 193 from the soil near the rice roots in the lowlands of Pemulutan Ilir, Ogan Ilir District (Table 6). The treated larvae that were 194 still alive and turned into pupae were only around 21.3-29.3% and the larvae that successfully became into adults were 195 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 196

197 highlands of Pasai, Kota Pagar Alam with LT₅₀ for only 6.68 days. Consequently, the fungi in this study could reduce the 198 emergence of adults of S. frugiperda up to 81.2%.

199 The treated dead and dead larvae showed typical symptoms that distinguished them from the healthy larvae. The 200 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 201 pupae, while the untreated larvae could produce healthy normal pupae (Figure 6). The abnormal pupae were shorter in 202 size, bent, wrinkled wings to-be, darker color, not moving when touched with a brush, and unable to become adults. The 203 adults formed from the treated larvae had folded wings and were smaller than the untreated larvae (Figure 7). However, 204 205 this study did not measure the length of pupae and adults formed but only documented in the form of photographs. From 206 the data, the entomopathogenic fungi could kill S. frugiperda larvae and pupae, produce abnormal pupae and adults, and 207 reduce the percentage of appearance of their adults.





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210 Figure 2. Colonial (above) and conidial (below) morphology of Metarhizium isolates on culture SDA: CaBkOI (A), CaTtME (B), 211 TiCmLpOKI (C), LpTtME (D), TeSkME (E), CaGiPR (F), ToBkOI (G), PrSeStOI (H), TePsPGA (I), CaSPsPGA (J), CaSnPR (K), 212 PirOI (L), PdmOI (M), dan KtMtLH (N) 213

Taalataa	Conidial density	Conidial viability (%)			
Isolates	1x10 ⁸ (conidia mL ⁻¹)	24-hour culture	48-hour culture		
LpTtME	5.11±0.80	58.33±5.42	68.00±2.49		
PirOI	4.63±0.106	53.33±1.52	62.67±4.91		
TePsPGA	3.69±0.279	53.67±4.25	62.67±7.09		
ToBkOI	5.13±0.096	58.67±6.28	67.33±5.66		
CaGiPR	4.24 ± 0.078	49.67±4.06	59.67±3.34		
CaSnPR	4.60±0.590	51.33±3.54	57.67±2.88		
CaTtME	5.69±0.833	50.33±3.31	65.67±5.04		
TiCmLpOKI	5.06±0.141	56.33±1.78	61.33±4.84		
CaBkOI	4.52±0.590	59.33±1.91	61.33±5.19		
CasPsPGA	5.16±0.247	67.00 ± 2.05	62.33±6.28		
PrSeStOI	5.08 ± 0.268	56.67±3.93	63.00±4.78		
TeSkME	4.68±0.215	57.67±4.23	64.67±5.86		
PdmOI	5.29±0.318	53.67±5.19	68.67±4.38		
KtMtLH	4.79±0.107	48.67±4.23	69.33±3.03		
F-value	1.372640351 ^{ns}	1.01^{ns}	0.35 ^{ns}		
P value	0.233102731	0.47	0.98		
LICD vialua					

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 1×10^{6} conidia mL⁻¹

Isolatas	Leaf area eaten by larvae (cm ² larvae ⁻¹ day ⁻¹) on observation for 12 days											
isolates	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 ⁿ	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 $1 \ge 10^6$ conidia mL⁻¹IsolatesLarval weight (mg larvae⁻¹) on observation for 12 days

15014105	Laivar weight (ing faivae) on observation for 12 days											
	1	3	3	4	5	6	7	8	9	10	11	12
Control	3 00 h	6.03	17.03 h	23.68	26.85	3672	42.04 ab	52.00 ab	64.40	81.33	88.10	112.73
Control	5.90 0	0.93	17.950	23.08	20.85	30.72	42.94 ab	32.99 ab	ab	abc	ab	ab
L pTtMF	3 51 0	5 68	11 23 0	1734	24.14	38 53	40.08 sh	56.04 ab	74.63	84.17	98.83	120.38
LPTUME	5.51 a	5.08	11.23 a	17.54	24.14	36.55	49.08 ab	J0.94 a0	ab	abc	ab	ab
PirOI	3 17 2	6.14	10.02 a	21.35	27.84	41 72	54 95 h	71.65 h	95.01	117.68	132.59	159.13
1101	J.47 a	0.14	10. <i>12</i> a	21.55	27.04	41.72	54.75 0	/1.05 0	b	с	b	b
TePePGA	3.80 a	5.03	10.00 a	17.84	28 60	36.41	51.26 ab	61.08 ab	74.54	90.64	102.06	117.57
ICI SI UA	5.00 a	5.75	10.00 a	17.04	20.07	50.41	51.20 ab	01.00 ab	ab	abc	ab	ab
ToBLOI	2/10 2	5.04	0.35 a	18 16	26.40	35.81	46.20 ab	61 45 ab	67.81	91.95	103.89	120.03
TODKOT	2.47 a	5.04).55 a	10.10	20.40	55.61	40.20 ab	01.45 ab	ab	abc	ab	ab
CaGiPR	2 29 2	6.02	11 36 a	17.22	25.00	32 79	47 19 ah	60.45 ab	72.42	86.91	111.62	149.44
Caoli K	2.27 a	0.02	11.50 a	17.22	25.00	52.17	47.17 dð	00.45 ab	ab	abc	ab	ab
CaSnPR	3 33 9	5 89	10 47 a	20.48	27 30	3676	50.67 ab	63 37 ah	80.36	97.81	117.69	138.70
Cubiii K	5.55 u	5.07	10.47 u	20.40	27.50	50.70	50.07 db	05.57 40	ab	abc	ab	ab
CaTtME	2.69 a	8.23	11.50 a	17.91	23.22	32.69	46.86 ab	62.24 ab	76.13	88.41	105.22	125.29

									ab	abc	ab	ab
TiCmLpOKI	1.77 a	5.29	12.61 a	19.41	26.09	34.93	44.34 ab	55.16 ab	61.31	70.96	86.59	97.44
r				-,					ab	ab	ab	ab
CaBhOI	3 00 2	6 30	12/10 2	18 14	25.00	33 12	45.94 ab	57.60 ab	77.05	88.90	104.85	119.39
Cabkoi	5.07 a	0.50	12.47 a	10.14	25.07	55.12	43.74 d0	57.07 db	ab	abc	ab	ab
	1 5 0 a	6.09	11.56 a	17 45	26.11	24.02	16 99 ab	62.22 sh	85.10	97.56	122.99	129.71
Casrsrua	1.39 a	0.08	11.30 a	17.45	20.11	54.02	40.00 ab	03.22 ab	ab	abc	ab	ab
D.C.C.OI	2.06 h	6.07	10.25 -	17.04	22 75	22.00	42.90 -L	(1 (2 -h	76.11	83.97	97.10	120.61
Prsestor	3.96 D	6.07	12.35 a	17.24	23.75	33.08	43.89 ab	01.05 ab	ab	abc	ab	ab
	2.04	7.07	11.50	17.67	25.24	22.70	40.15.1	54.00 1	64.13	73.64	91.49	101.15
TeSKME	3.04 a	/.0/	11.50 a	1/.6/	25.34	33.78	40.15 ab	54.92 ab	ab	ab	ab	ab
D 1 0 T	• • •	101	0.10					10.04		58.46	76.93	94.76
PdmOI	2.48 a	4.86	9.69 a	15.47	22.23	29.91	35.64 a	42.26 a	52.69 a	a	a	а
									83 29	107 28	118 55	142.51
KtMtLH	2.59 a	6.99	11.60 a	20.93	27.86	39.60	54.88 b	65.89 ab	ab	hc	ah	ah
		1 4 1							uo	00	uo	uo
F-value	7 5/*	ns	1 37*	1 82 ^{ns}	0.78 ^{ns}	1 55 ^{ns}	2 60*	2.07*	2 28*	3 31*	2 50*	∩ ∩7*
D	7.54	0.21	4.52	1.02	0.78	0.15	2.09	2.07	2.28	0.00	2.30	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	48.91	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 10⁶ 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	1.07	5.05	7 73 h	11 15 h	16 14 c	20 56 d	23 50 c	27.23 0	28.32	30.60	34.39	36.40
Control	1.97	5.05	1.23 0	11.150	10.14 C	20.30 u	25.50 C	27.23 C	с	b	b	b
I nTtMF	1 78	3 63	670 h	631 a	8 06 ah	7 69 ah	14 18 ab	13 70 ab	14.37	12.38	16.52	17.18
LPIUML	1.70	5.05	0.700	0.51 a	0.00 ab	7.07 db	14.10 ab	15.70 db	ab	а	а	а
PirOI	1 59	2 71	6 08 ah	7 41 ah	0 50 ah	9.37 abc	11.92 ab	13 72 ah	24.78	18.57	24.70	22.07
1101	1.57	2.71	0.00 40	7.41 uo).5) uo	9.57 doe	11.92 uo	13.72 db	bc	а	ab	a
TePsPGA	1.02	2.38	4 80 ab	7 07 ah	6 49 ah	6 66 ab	783a	10 29 a	16.44	16.21	16.76	17.50
101 51 611	1.02	2.50	1.00 40	1.07 u 0	0.19 40	0.00 40	7.05 u	10.29 u	ab	а	a	а
ToBkOI	1.03	2 40	5 28 ah	6 95 a	5 67 a	8 79 abc	14 55 ab	10 78 a	15.07	15.17	19.41	20.61
TODKOT	1.05	2.40	5.20 uo	0.95 u	5.07 u	0.79 000	14.55 40	10.70 u	ab	а	а	а
CaGiPR	1.02	2.65	3 89 ah	8 01 ah	5 77 ah	10.88 bc	16 39 bc	11 53 ab	13.53	16.24	20.07	22.96
cuonin	1.02	2.00	5.07 u 0	0.01 40	5.77 u o	10.00 00	10.57 00	11.55 ub	ab	а	а	а
CaSnPR	1 10	2.67	7 07 b	8 02 ah	9 50 ah	8 53 abc	13.07 ab	9 97 a	16.93	16.38	21.31	23.49
Cubin K	1.10	2.07	1.07 0	0.02 do).50 ub	0.55 460	15.07 40).)/ u	abc	а	а	ab
CaTtME	1 39	3 44	4 11 ab	6 15 a	8 62 ah	10.58 bc	11 95 ab	17 16 ab	17.31	19.87	22.17	21.52
Curtuit	1.07	5.11		0.15 u	0.02 uo	10.50 00	11.95 40	17.10 40	abc	ab	a	а
TiCmI pOKI	0.99	3 04	5 87 ah	8 69 ah	10.23	12.90 c	11 29 ab	17.48	10.88	14.38	15.43	17.31
пешерокі	0.77	5.04	5.07 do	0.07 40	abc	12.90 €	11.27 dð	abc	а	а	а	а
CaBkOI	1 68	3 27	4 30 ah	8 19 ah	9 78 ah	8 25 ab	9 48 ah	18.24	21.18	20.91	20.19	21.89
Cubkor	1.00	5.27	4.50 ub	0.17 40).70 db	0.25 40	2.40 u 0	abc	abc	ab	а	а
CasPsPGA	1 88	2.74	4 19 ah	7 33 ah	10.83	9 10 abc	8 78 a	21.52 bc	20.82	19.77	18.90	21.62
Cust si ori	1.00	2.7 1	1.17 40	1.00 u 0	abc).10 uoc	0.70 u	21.52.00	abc	ab	а	а
PrSeStOI	1.77	3.14	4.09 ab	6.75 a	11.69	8.82 abc	15.04 ab	17.83	21.41	18.28	21.59	23.01
11505101		0.11		0170 u	abc	0.02 400	1010140	abc	abc	а	а	а
TeSkME	1 58	2.72	4 54 ab	5 73 a	10.45	5 91 a	11 92 ab	14 48 ab	15.48	17.51	17.58	18.80
TODRITE	1.00	2.72	1.5 1 40	5.75 u	abc	5.91 u	11.92 uo	11.10 40	ab	а	а	а
PdmOI	0.72	3.07	2.28 a	5.70 a	11.08	9.43 abc	9.33 ab	11.62 ab	12.96	15.00	14.69	16.07
1 unio 1	0	2.07	 o u	erre u	abc	<i>ye</i> uee)100 uc	11.02 40	ab	а	a	а
KtMtLH	1.61	2.75	6.10 ab	7.39 ab	11.42	10.84 bc	12.04 ab	17.28	19.87	18.91	22.29	23.64
			0110 40	, 109 uo	bc	1010100	1210 1 40	abc	abc	а	а	ab
F-value	1.24"	2.01 ⁿ										
 D 1	°	°	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	11.38	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.



Figure 3. The symptoms on maize leaves eaten by Spodoptera frugiperda larvae treated with entomopathogenic fungal isolates with 1 x 10⁶ 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).



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Figure 4. Mortality of Spodoptera frugiperda larvae treated with entomopathogenic fungal isolates with 1 x 10⁶ conidia mL⁻¹ on observation for 12 days

Table 6. Mean of larvae mortality, LT_{50} , and LT_{90} of *Spodoptera frugiperda* larvae treated with entomopathogenic fungal isolates with 1 x 10⁶ conidia mL⁻¹

Isolates	Mortality ± SE (%)	$LT_{50} \pm SE (days)$	$LT_{95} \pm SE (days)$	Regression equation
Control	13.33±2.17 a	-	-	
LpTtME	74.67±1.09 b	7.23±0.15	15.12±0.04	y = 0.04x + 7.1463
PirOI	78.67±1.09 b	6.43±0.20	14.31±0.13	y = 0.1915x + 6.0517
TePsPGA	76.00±0.00 b	7.14±0.08	15.01±0.18	y = 0.1485x + 6.839
ToBkOI	74.67±2.18 b	7.01±0.30	14.89 ± 0.41	y = -0.2405x + 7.4917
CaGiPR	77.33±2.88 b	7.09±0.43	14.97±0.41	y = 0.804x + 5.4857
CaSnPR	76.00±1.89 b	6.98±0.12	14.86±0.05	y = -0.1475x + 7.279
CaTtME	78.67±1.09 b	7.10±0.19	14.98±0.27	y = 0.3985x + 6.3077
TiCmLpOKI	70.67±2.18 b	7.18±0.16	15.06±0.24	y = 0.3355x + 6.5103
CaBkOI	76.00±1.89 b	6.82±0.18	14.69±0.31	y = 0.0015x + 6.812
CasPsPGA	78.67±1.09 b	6.68±0.32	14.55±0.44	y = -0.071x + 6.819
PrSeStOI	78.67±1.09 b	6.53±0.23	14.40 ± 0.20	y = -0.46x + 7.446
TeSkME	73.33±1.09 b	6.93±0.25	14.81±0.37	y = -0.053x + 7.0397

PdmOI	70 67+2 18 h	7 54+0 20	15 41+0 24	v = 0.42x + 6.6997
KtMtLH	78.67+2.18 b	6 74+0 40	14 61+0 38	y = 0.7495x + 5.2367
E-value	58 09*	0.96^{ns}	0.70^{ns}	y = 0.1495 x + 5.2507
D value	0.00	0.50	0.70	
I value	0.00	0.51	0.74	

246 Note: ns = not significantly different; * = significantly different; values within a column (the data of each isolate) followed by the same

247 letter were not significantly different at P < 0.05 according to Tukey's HSD test.



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

Table 7. Mean of percentage of *Spodoptera frugiperda* pupal formation and adults emerged after their larvae treated with entomopathogenic fungi 1×10^6 conidia mL⁻¹

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

254 Note: ns = not significantly different; * = significantly different; values within a column (the data of each isolate) followed by the same

255 letter were not significantly different at P < 0.05 according to Tukey's HSD test.



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



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

262 Discussion

263 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 264 fungal inoculums; the fungi can survive saprophytes in soil whose pH ranges from 4 to 6.7 (Safitri et al. 2018). In the low 265 266 acidity and moisture soil are 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 267 from 6 to 7 (Karthikeyan et al. 2008). In addition to pH, soil moisture content also influences the presence of 268 entomopathogenic fungi and in soil moisture 6-21%, Beauveria bassiana conidia is still found to be pathogenic and 269 capable of killing up to 80% of Anastrepha ludens adults (Wilson et al. 2020). The ideal in vitro medium moisture for the 270 entomopathogenic fungi to survive ranges from 15% to 35% and the optimum moisture is 35% (Chen et al. 2014). In this 271 272 study the soil moisture in the survey location ranged from 13% to 20.9% which is the range of moisture suitable for 273 saprofit mushroom habitat.

274 The fungi found in this study have morphological characteristics in accordance with the characteristics of the Genus, 275 Metarhizium which has been described by Thaochan and Sausa-Ard (2017), Lopes et al. (2018), and Chen et al. (2019). 276 The fungus colony form in the agar medium is initially colorless, then becomes light yellow after the colony is more than 5 277 days old, the conidia turns green which indicates that the conidia is 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. 278 2019). The Metarhizium has cylindrical, single and one-celled, and hyaline conidia, while the hyphae septate, smooth-279 walled, and hyaline (Lopes et al. 2018; Chen et al. 2019), the length of the Metarhizium conidia ranges from 7.3 to 14.4 280 281 μ m (Chen et al. 2019) and this measure of the fungal conidia in this study (8-10 μ m) falls into the above range.

282 In this study, the two most pathogenic isolates of S. frugiperda larvae and pupae characterized by the highest mortality 283 of larvae and pupae and able to kill them in the shortest amount of time were PirOI and CasPsPGA isolates. The PirOI 284 isolates originate from the soil near rice roots in the lowlands, while CasPsPGA isolates originate from the soil near chilli 285 roots in the highlands. The soil origin of the isolate did not determine the virulence of the fungus, in line with the results of 286 the study (Thaochan and Sausa-Ard 2017) stating that the original host or the geographic origin of the isolate has no 287 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 two most 288 289 pathogenic isolates originating from the lowlands and highlands of South Sumatra is a useful finding to develop local-290 specific mycoinsecticides to control pest insects in the highlands and/or lowlands. Because S. frugiperda is a maize insect 291 pest spreading from the lowlands to highlands in South Sumatra in particular and Indonesia in general, the location-292 specific mycoinsecticides are needed.

293 The data of area of the leaves eaten by the treated larvae showed the entomopathogenic fungus, *Metarhizium* sp. can 294 reduce larvae appetite and reduce larvae weight. On the fifth day of observation, the treated S. frugiperda larvae began to 295 significantly decrease their appetite because this is the period of which the process of infection by the fungus was taking 296 place. Initially, the conidia sticking on its cuticle or entering through the mouth of the larvae began to show its effect on 297 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 298 299 (Fernandes et al. 2007). Infection begins when germ tubes are able to penetrate the insect cuticle and the ability to infect is 300 a determining factor for the fungus virulence (Altre and Vandenberg 2001). After the germ tubes penetrate the cuticle and 301 reach haemocoel, they then produce specific infection hyphae originating at appressoria (El-Ghany 2015). Then the 302 hyphae spread to the haemolymph and develop to produce blastospores. After that, the blastospores produce secondary 303 metabolites and enzymes, for example *B. bassiana* produces enzymes of proteolytic and chitinolytic which can disrupt 304 normal cell metabolism (Mancillas-Paredes et al. 2019) which is seen from the decreased appetite that ultimately reduces 305 the weight of host insects. The next process, toxins from secondary metabolites begin to kill host insects, but the death of 306 these host insects is not only by the toxin but also due to the mechanical damage by fungal penetration into the body of an 307 insect (El-Ghany 2015).

308 The treated larvae were 78.67% dead and the surviving larvae were only able to become abnormal pupae and adults 309 and malformations. The larvae that were sick and dead caused by the fungi in this study showed the symptoms of his body 310 got dried, shriveled, smaller, and odorless, while the integument was covered by misellia and conidia resembling yellowish white flour mixed with dark green similar to the symptoms described by Thaochan and Sausa-Ard (Thaochan and Sausa-311 Ard 2017). The symptoms of sick insects due to the entomopathogenic fungal infections vary depending on favorable 312 environmental conditions, for example relative humidity higher than 62% (Gutiérrez-Cárdenas et al. 2019), then the 313 314 mycelia and mature conidia can grow well and cover the entire body of the insect, and the condition of unfavorable 315 mycelia and conidia does not appear (El-Ghany 2015).

Pupae getting sick 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, reduce fecundity, 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 most pathogenic causes of death reaching 78.67% and suppressing the emergence of adults up to 81.2%. Two of the most pathogenic isolates found from the lowlands (PirOI) and highlands (CasPsPGA) of South Sumatra both have the potential to be developed into localspecific mycoinsecticides to control pest insects in the highlands and/or lowlands in Indonesia.

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Exploring entomopathogenic fungi from South Sumatra (Indonesia) soil 443 and their pathogenicity againts a new invasive maize pest, Spodoptera 444 frugiperda 445

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452 Abstract. Fall armyworm (Spodoptera frugiperda) is a new invasive maize pest in Indonesia that can cause maize yield losses of 18 453 million tons/year. To overcome the pest, local-specific entomopathogenic fungi are needed. This study aimed to explore 454 entomopathogenic fungi from soil in South Sumatra and to determine their pathogenicity against S. frugiperda larvae. The fungi 455 exploration was carried out in the lowlands and highlands of South Sumatra and the pathogenicity of obtained isolates were tested 456 against the third instar larvae. The entomopathogenic fungi found were Metarhizium spp. and were successfully isolated as many as 14 457 isolates. All of the isolates were pathogenic to S. frugiperda larvae (70.67-78.67% mortality), the most pathogenic caused 78.67% 458 mortality and suppressed the emergence of adults up to 81.2%. Unhealthy larvae had a dry, shrunken, shrinking, odorless body, and its 459 integument was covered in mycelia and conidia like yellowish white powdery mixed with dark green. The unhealthy pupae and adults 460 were in the abnormal and malformation shape. The abnormal pupae were shorter in size, bent, the to-be wings got wrinkled, and darker 461 color, while the unhealthy adults had folded wings and were unable to fly. The two most pathogenic isolates were found from the 462 lowland (PirOI) and highland (CasPsPGA) soil of South Sumatra. In conclusion, both of these isolates had the potential to be developed 463 into local-specific mycoinsecticides to control pest insects in the highlands and/or lowlands in Indonesia.

464 Key words: Fall armyworm, isolate, larvae, *Metarhizium* sp., pupae

465 Abbreviations (if any): -

466 **Running title:** Entomopathogenic fungi againts *Spodoptera frugiperda*

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INTRODUCTION

468 Currently the maize (*Zea may*) in Indonesia is under severe invasion by a new invasive pest called the fall armyworm 469 (FAW) with the scientific name *Spodoptera frugiperda* (Lepidoptera: Noctuidae). The *S. frugiperda* originally comes from 470 South America (Nagoshi et al. 2017; Otim et al. 2018) and now this pest has spread to various continents. In 2016, the 471 FAW was reported to have entered Africa (Goergen et al. 2016). In 2017, the FAW spread into Europe (Early et al. 2018). 472 The pest began to enter Asia in 2018 and was first discovered in India (Ganiger et al. 2018). In April 2019, the FAW 473 reportedly began entering Indonesia and was first discovered in West Sumatra, after which it spread throughout Sumatra, 474 including Lampung (Lestari et al. 2020) and Palembang. Then, the pest crossed to Java and Kalimantan (IPPC 2019).

475 Maize attacked by S. frugiperda larvae generally suffered very heavy damage. In 2016, the maize yield loss in 12 476 African countries reached 18 million tons/year and the value of losses reached US \$ 13 millions (Harrison et al. 2019). In 477 2018, the FAW caused losses of about a third of the annual production maize in Kenya, estimated at about 1 million 478 tons/year (De Groote et al. 2020). The pest was also reported to attack rice, sugar cane, cotton, and ornamental plants 479 (IPPC 2019). In Brazil around 76 plant families were reported to be attacked by the pest (Montezano et al. 2018). The 480 outbreaks of FAW moth in maize was influenced by the growth stage of the crop, rainfall, and relative humidity (Nboyine 481 et al. 2020). From the initial survey in South Sumatra it was found that the S. frugiperda larvae attacked leaves, stems, 482 flowers, fruits, and growth points to the whole maize stalk. The S. frugiperda becomes a new invasive pest in Indonesia 483 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 the 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.

491 The soil which is a fungal habitat during the saprophytic phase has high entomopathogenic fungi inoculum potential, 492 especially those close to plant roots (Safitri et al. 2018) and is effective in killing the insect pests (Ayudya et al. 2019; 493 Sumikarsih et al. 2019; Gustianingtyas et al. 2020). Research results from other countries show the high pathogenicity of 494 entomopathogenic fungi from soil in controlling S. frugiperda population. In Mexico, Beauveria bassiana and 495 Metarhizium anisopliae are reported to be effective in killing S. frugiperda larvae (Rivero-Borja et al. 2018) and in 496 Tanzania, the B. bassiana and M. anisopliae can suppress the adult population of S. frugiperda (Ngangambe and 497 Mwatawala 2020). In India, the S. frugiperda were attacked by Nomuraea rileyi (Shylesha et al. 2018). In addition to 498 killing the larvae, the B. bassiana and M. anisopliae can kill the S. frugiperda eggs (Akutse et al. 2019) and adults 537 (Gutiérrez-Cárdenas et al. 2019). In Cuba, endophytic B. bassiana and M. anisopliae were found to be pathogenic to S. 538 frugiperda larvae (Ramos et al. 2020). However, the entomopathogenic fungi do not harm the egg and larval parasitoids of 539 S. frugiperda (Ngangambe and Mwatawala 2020) and the predatory arthropods (Prabawati et al. 2019). The 540 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 541 Sumatra soil need to be explored and tested for their pathogenicity against the S. frugiperda. The location-specific fungi 542 543 are needed because they tend to be more effective than exogenous ones (Thaochan and Sausa-Ard 2017). This study aimed 544 to explore entomopathogenic fungi from soil in South Sumatra and to determine their pathogenicity against S. frugiperda 545 larvae.

MATERIALS AND METHODS

547 Study area

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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, namely the Districts/Cities of Ogan Komering Ilir, Ogan Ilir, Prabumulih, Muara Enim, Lahat, Pagar Alam, Banyuasin, and East OKU (Figure 1).



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Figure 1. Locations of exploration for entomopathogenic fungi: District/City of Ogan Komering Ilir (1), Ogan Ilir (2), Prabumulih
 (C), Muara Enim (4), Lahat (5), Pagar Alam (6), and Banyuasin (7)

556 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 (\emptyset 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.

The soil sample was first cleaned from plant roots and sieved with 10 mesh sieves. Then, it was put into a plastic tray 564 $(32 \times 25 \times 5 \text{ cm}^3)$ each containing as much as 1000 g. It was then moistened with sterile distilled water with soil moisture 565 566 exceeding 20% according to the method of Chen et al. (2014). After that, the 30 newly molted larvae of the third instar T. 567 molitor were placed at the bottom of the tray and the bodies of the larva were sprinkled with a layer of sample soil whose 568 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 569 570 fungal conidia to infect them, after which the dead larvae infected with entomopathogenic fungi were grown in Sabouraud 571 Dextrose Agar (SDA, Merck) media.

572 Isolation and identification of the entomopathogenic fungi

573 The infected *Tenebrio* bait was then isolated and purified using the method of Safitri et al. (2018). The 574 entomopathogenic fungi infecting the *Tenebrio* bait and growing on the surface of the integument were isolated and 575 cultured in the SDA media. The surface of the larvae infected with the entomopathogenic fungi was first sterilized by 576 modifying the method of Sharma et al. (2018) by rinsing with 1% NaOCl for 1 minute, then rinsing with 100 mL of 577 distilled water for 3 times. The surface larvae sterilization was carried out to obtain the fungus that was in the haemocoel 578 or the one that already penetrated the larvae cuticle and prevented the presence of the air fungus. The sterilized larvae were 579 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 580 581 characteristics, for example the colory colors and shapes of culture on SDA, conidia shape and color using a taxonomic 582 book of Humber (2005) and El-Ghany (2015). The conidia density was calculated using the method of Sumikarsih et al. 583 (2019), while the viability was observed by growing 10 μ L of fungal conidia suspension (1 x 10⁶ conidia mL⁻¹) on 2% of 584 agar-water medium, containing 2 g of agar which was given 100 mL distilled water (w/v), then the culture was incubated 585 for 2 x 24 hours.

586 Pathogenicity test of the entomopathogenic fungi

Pathogenicity 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 29.30°C and 89%.

590 Preparation of test insect

591 The S. frugiperda larvae were collected from mainze fields in Indralaya Village which were not applied with synthetic 592 insecticides. These larvae were brought to the laboratory and kept in plastic cups (\emptyset 6.5 cm, height 4.6 cm) porous 593 separately between individuals because of their cannibal nature at room temperature. Into the containers were put the 594 maize leaves to feed S. frugiperda and the leaves were replaced daily with fresh new leaves. The last stage larvae entering 595 the pupae stage were transferred into a plastic container (Ø15 cm, height 25 cm) that was already provided with the soil (5 596 cm thickness) sterilized in the oven for 2 hours at 100°C. The containers containing pupae were placed in gauze cages (30 597 x 30 x 30 cm³), and the gauze cages were also provided with 10 pieces of water spinach (*Ipomoea aquatica*) for egg 598 placement for female adults. The water spinach was placed in a plastic bottle (Ø 5.5 cm, height 17 cm) containing tap 599 water height of 10 cm to maintain its freshness. The egg clutch placed by the adults on the surface of the water spinach 600 leaves were moved into the container containing the water spinach leaves. The feed for the first instar larvae was water 601 spinach leaves. After molting the skin, the second-instar larvae until the last instar were fed young maize leaves. The 602 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 603 the second instar and so on were cannibalistic. Mass-rearing was carried out to obtain a third generation of culture. The 604 second stage larvae aged 1 day were used for test insects in this study.

605 *Fungus application*

606 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 607 1 mL was dripped on maize leaves and topically also applied to the larvae, then the maize leaf was forced to be eaten by 608 the second instar larvae of S. frugiperda as much as 25 larvae per isolate for 6 hours and it was repeated three times. The 609 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 610 suspension, then they were transferred into a porous plastic cup (Ø 6.5 cm, height 4.6 cm) and each cup contained only 1 611 612 larvae and provided 2 x 5 cm² maize leaves per day per larvae. To measure the percentage of leaf area eaten (foliar damage 613 caused) by the larvae of S. frugiperda used bioleaf application by Machado et al. (2016). Every 1 x 24 hours the dead test 614 larvae were recorded and it was carried out for 12 days based on the previous research by Ayudya et al. (2019) and the 615 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_{90}). The area of the eaten maize 616 leaves, the weight of the feces, and the weight of the larvae body were measured every day from the first day to the 12th. 617

618 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_{90}) 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_{90} values were calculated by using probit analysis. All data were calculated using SAS University Edition software 2.7 9.4 M5.

RESULTS AND DISCUSSION

626 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 to10 µm, while the mycelium was insulated.

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

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	<i>Metarhizium</i> sp.	LpTtME	5.5	67.2
Ogan Ilir	Pemulutan Ilir	Paddy	Metarhizium sp.	PirOI	5.2	11.5
Pagar Alam	Pasai	Eggplant	<i>Metarhizium</i> 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

644

645 Fungal pathogenicity againts 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 646 647 first to the fourth day showed that the consumed leaf area was not significantly different from those eaten by the untreated 648 (control) larvae. However, starting on the fifth day, the treated S. frugiperda larvae began to significantly decrease its 649 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 650 651 larvae also showed differences (Figure 3). The decreased appetite in the treated larvae with the fungus did not show 652 consistency in the larvae weight loss, yet on the third day of the observation the treated larvae body weight was 653 significantly lower than that of the untreated larvae weight (Table 4). From the 4th to the 6th days of the observations, all 654 treatments of the larvae body weight showed no significant difference, but on the seventh to the twelfth days there was one 655 isolate (PdmOI) which consistently reduced the weight of S. frugiperda larvae. The weight of feces produced by the 656 treated and control larvae tended to show a significant difference, i.e. the feces weight produced by the treated larvae 657 tended to be lighter than that of the untreated (control) larvae (Table 5).

658 All Metahrizium isolates tested against the S. frugiperda larvae were pathogenic (70.67–78.67%); the most pathogenic 659 caused 78.67% of the mortality and 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 660 any significant difference, LT₅₀ (6.43 days) and LT₉₀ (12.57 days) were found most briefly in PirOI isolates originating 661 from the soil near the rice roots in the lowlands of Pemulutan Ilir, Ogan Ilir District (Table 6). The treated larvae that were 662 still alive and turned into pupae were only around 21.3-29.3% and the larvae that successfully became into adults were 663 664 only around 18.8-28%, while the untreated larvae managed to become adults as many as 86.67% (Table 7). The lowest 665 emergence of adults (18.8%) was found in CasPsPGA isolates originating from the soil near the roots of chili in the 666 highlands of Pasai, Kota Pagar Alam with LT_{50} for only 6.68 days. Consequently, the fungi in this study could reduce the 667 emergence of S. frugiperda adults up to 81.2%.

668 The treated dead larvae showed typical symptoms that distinguished them from the healthy larvae. The treated larvae's 669 body was dry, contracted, shrinking, odorless, and the integument was covered by mycelia and conidia like yellowish 670 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, 671 wrinkled wings to-be, darker color, not moving when touched with a brush, and unable to become adults. The adults 672 formed from the treated larvae had folded wings and were smaller than the untreated larvae (Figure 7). However, this 673 study did not measure the length of pupae and adults formed but only documented in the form of photographs. From the 674 data, the entomopathogenic fungi could kill S. frugiperda larvae and pupae, produce abnormal pupae and adults, and 675 676 reduce the percentage of f their adults emergence.





Figure 2. Colonial (above) and conidial (below) morphology of Metarhizium isolates 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)

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

Taala4aa		Conidial viability (%)			
Isolates	Conidial density 1x10 ⁸ (conidia mL ⁻¹)	24-hour culture	48-hour culture		
LpTtME	5.11±0.80	58.33±5.42	68.00±2.49		
PirOI	4.63±0.106	53.33±1.52	62.67±4.91		
TePsPGA	3.69±0.279	53.67±4.25	62.67±7.09		
ToBkOI	5.13±0.096	58.67±6.28	67.33±5.66		
CaGiPR	4.24 ± 0.078	49.67±4.06	59.67±3.34		
CaSnPR	4.60 ± 0.590	51.33±3.54	57.67±2.88		
CaTtME	5.69±0.833	50.33±3.31	65.67±5.04		
TiCmLpOKI	5.06±0.141	56.33±1.78	61.33±4.84		
CaBkOI	4.52±0.590	59.33±1.91	61.33±5.19		
CasPsPGA	5.16±0.247	67.00±2.05	62.33±6.28		
PrSeStOI	5.08 ± 0.268	56.67±3.93	63.00±4.78		
TeSkME	4.68±0.215	57.67±4.23	64.67±5.86		
PdmOI	5.29±0.318	53.67±5.19	68.67±4.38		
KtMtLH	4.79±0.107	48.67±4.23	69.33±3.03		
F-value	1.372640351 ^{ns}	1.01 ^{ns}	0.35 ^{ns}		
P value	0.233102731	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 1×10^6 conidia mL⁻¹

Table 4 a m	Leaf area eaten by larvae (cm ² larvae ⁻¹ day ⁻¹) on observation for 12 days											
Isolates	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 ⁿ	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

693 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.

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 00 h	6.03	17 03 h	23 68	26.85	3672	42.04 ab	52.00 ab	64.40	81.33	88.10	112.73
Control	5.90 0	0.95	17.950	23.08	20.85	30.72	42.94 aU	52.99 aU	ab	abc	ab	ab
I nTtMF	3 51 a	5 68	11 23 a	17 34	24 14	38 53	49.08 ab	56 94 ah	74.63	84.17	98.83	120.38
LPIUNL	5.51 a	5.00	11.25 a	17.54	24.14	50.55	49.00 ab	50.74 ab	ab	abc	ab	ab
PirOI	3 47 a	6 14	10.92 a	21 35	27.84	41 72	54 95 h	71.65 h	95.01	117.68	132.59	159.13
1101	5.47 u	0.14	10.92 u	21.55	27.04	41.72	54.95 0	/1.05 0	b	с	b	b
ΤeΡsPGΔ	3.80 a	5 93	10.00 a	17.84	28 69	36.41	51.26 ab	61.08 ab	74.54	90.64	102.06	117.57
101 51 071	5.00 a	5.75	10.00 a	17.04	20.07	50.41	51.20 db	01.00 ab	ab	abc	ab	ab
ToBkOI	2.49 a	5.04	935a	18 16	26.40	35.81	46.20 ab	61 45 ah	67.81	91.95	103.89	120.03
TODKOT	2.47 a	5.04).55 a	10.10	20.40	55.01	40.20 40	01.45 40	ab	abc	ab	ab
CaGiPR	2 29 a	6.02	11 36 a	17 22	25.00	32 79	47 19 ah	60.45 ab	72.42	86.91	111.62	149.44
Cuon K	2.2) u	0.02	11.50 u	17.22	23.00	52.17	47.17 40	00.45 ub	ab	abc	ab	ab
CaSnPR	3 33 a	5 89	10 47 a	20.48	27 30	3676	50 67 ab	63 37 ah	80.36	97.81	117.69	138.70
Cubiii R	5.55 u	5.07	10.17 u	20.10	27.50	20.70	20.07 40	05.57 40	ab	abc	ab	ab
CaTtME	2 69 a	8 23	11 50 a	17 91	23.22	32 69	46 86 ah	62 24 ah	76.13	88.41	105.22	125.29
Curtuin	2.07 u	0.23	11.50 u	17.71	23.22	52.07	40.00 40	02.24 ub	ab	abc	ab	ab
TiCmLnOKI	1 77 a	5 29	12.61 a	1941	26.09	34 93	44 34 ah	55 16 ab	61.31	70.96	86.59	97.44
menilipoiti	1. <i>/ /</i> u	0.2	12.01 u	17.11	20.07	51.75	11.51 40	55.10 ub	ab	ab	ab	ab
CaBkOI	3 09 a	6 30	12.49 a	18 14	25.09	33 12	45 94 ab	57 69 ab	77.05	88.90	104.85	119.39
Cubkor	5.07 u	0.00	12.17 u	10.11	23.07	55.12	15.51 40	57.07 40	ab	abc	ab	ab
CasPsPGA	159a	6.08	11 56 a	17 45	26.11	34.02	46 88 ah	63 22 ah	85.10	97.56	122.99	129.71
Cust SI OII	1.57 u	0.00	11.50 u	17.10	20.11	51.02	10.00 40	03.22 do	ab	abc	ab	ab
PrSeStOI	3 96 h	6.07	12 35 a	17 24	23 75	33.08	43 89 ah	61 63 ab	76.11	83.97	97.10	120.61
11505101	0.000	0.07	12.00 u	17.21	20110	22100	10107 40	01100 40	ab	abc	ab	ab
TeSkME	3.04 a	7.07	11.50 a	17.67	25.34	33.78	40.15 ab	54.92 ab	64.13	73.64	91.49	101.15
100mm	010 T u		11100 u	17107	20101	00110	ionie uo	0 11) <u>-</u> 40	ab	ab	ab	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	76.93	94.76
rumor	2.10 u	1.00).0) u	10.17	22.23	27.71	55.01 u	12.20 u	52.09 u	а	а	а
KtMtLH	2.59 a	6.99	11.60 a	20.93	27.86	39.60	54.88 b	65.89 ab	83.29	107.28	118.55	142.51
intititi	2.57 u	0.77	11.00 u	20.75	27.00	57.00	51.000	00.07 40	ab	bc	ab	ab
F-value		1.41		P ⁰	n°	P °						
	7.54*	115	4.32*	1.82	0.78^{ms}	1.55	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	48.91	63.10

Table 4. Mean of weight of *Spodoptera frugiperda* larvae treated with entomopathogenic fungal isolates with 1×10^6 conidia mL⁻¹

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 1×10^6 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 07	5.05	7 23 h	11 15 h	16.14 c	20 56 d	23.50 c	27.23 c	28.32	30.60	34.39	36.40
Control	1.97	5.05	1.23 0	11.150	10.14 C	20.30 u	23.30 C	27.25 C	с	b	b	b
I nTtMF	1 78	3 63	6 70 h	631a	8 06 ah	7 69 ah	14 18 ah	13 70 ab	14.37	12.38	16.52	17.18
LpitiviL	1.70	5.05	0.700	0.51 a	0.00 a0	7.07 40	14.10 ab	15.70 40	ab	а	а	а
PirOI	1 59	2 71	6 08 ah	7 41 ah	9 59 ah	9 37 abc	11 92 ab	13 72 ab	24.78	18.57	24.70	22.07
1101	1.57	2.71	0.00 40	7.41 uo).5) uo	9.57 abe	11.92 do	15.72 d0	bc	а	ab	а
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	16.21	16.76	17.50
	1.02	2.00		/10/ uc	0115 40	0.00 40	,100 u	10.29 u	ab	а	а	а
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	15.17	19.41	20.61
									ab	а	a	а
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	16.24	20.07	22.96
									ab	a	a	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	16.38	21.31	23.49
									aDC	a 10.97	a 22.17	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	19.8/	22.17	21.52
					10.22			17 49	10.99	aD 1429	a 15.42	a 1721
TiCmLpOKI	0.99	3.04	5.87 ab	8.69 ab	10.25 aba	12.90 c	11.29 ab	17.40	10.00	14.50	15.45	17.51
					abe			18 24	a 21.18	a 20.01	a 2010	a 21.80
CaBkOI	1.68	3.27	4.30 ab	8.19 ab	9.78 ab	8.25 ab	9.48 ab	10.24	21.10	20.91 ah	20.19	21.09
								abe	abe	aU	a	d

CasPsPGA	1.88	2 74	/ 10 ah	7 33 ah	10.83	0.10 abc	878 2	21.52 bc	20.82	19.77	18.90	21.62
	1.00	2.74	4.17 au	7.55 ab	abc).10 abc	0.70 a	21.52.00	abc	ab	а	а
DrSoStOI	1 77	3 14	4.00 ab	675 0	11.69	8 82 abc	15.04 ab	17.83	21.41	18.28	21.59	23.01
11565101	1.//	5.14	4.07 ab	0.75 a	abc	0.02 abc	15.04 a0	abc	abc	а	а	а
TestME	1 58	2 72	4.54 ah	5 73 9	10.45	5.01 a	11.02 ah	14 48 ab	15.48	17.51	17.58	18.80
ICONIVIL 1.5	1.50	50 2.72	4.94 au	5.15 a	abc	5.71 a	11.92 do	14.40 ab	ab	а	а	а
PdmOI	0.72	3.07	2 28 2	5 70 a	11.08	9.43 abc	0 33 ah	11.62 ab	12.96	15.00	14.69	16.07
1 unio1	0.72	5.07	2.20 a	5.70 a	abc	7.45 abc	7.55 au	11.02 d0	ab	а	а	а
KtMtI H	1.61	2 75	6 10 ah	7 30 ah	11.42	10.84 bc	12.04 ab	17.28	19.87	18.91	22.29	23.64
KUVILLII	1.01	2.15	0.10 ab	7.39 ab	bc	10.84 00	12.04 a0	abc	abc	а	a	ab
E volvo	1.24 ⁿ	2.01 ⁿ										
r-value	s	s	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	11.38	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.



Figure 3. The symptoms on maize leaves eaten by *Spodoptera frugiperda* larvae treated with entomopathogenic fungal isolates with 1 x 10⁶ 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 1×10^6 conidia mL⁻¹ on observation for 12 days

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with 1 x 10^6 conidia mL⁻¹**Table 6.** Mean of larvae mortality, LT₅₀, and LT₉₀ of *Spodoptera frugiperda* larvae treated with entomopathogenic fungal isolates
with 1 x 10^6 conidia mL⁻¹

Isolates	Mortality ± SE (%)	$LT_{50} \pm SE$ (days)	$LT_{95} \pm SE$ (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	-	-

725 Note: ns = not significantly different; * = significantly different; values within a column (the data of each isolate) followed by the same 726 letter were not significantly different at P < 0.05 according to Tukey's HSD test.



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



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

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 Table 7. Mean of percentage of Spodoptera frugiperda pupal formation and adults emerged after their larvae treated with entomopathogenic fungi $1 \ge 10^6$ conidia mL⁻¹

 Isolates
 Pupal formation (%)
 Adults emerged (%)

Isolates	Pupal formation (%)	Adults emerged (%)	
Control	86.7 b	86.7 b	
LpTtME	25.3 a	24.0 a	
PirOI	21.3 a	21.3 а	
TePsPGA	24.0 a	21.3 а	
ToBkOI	25.3 a	22.7 а	
CaGiPR	22.7 a	21.3 а	
CaSnPR	24.0 a	21.3 а	
CaTtME	21.3 а	18.7 a	
TiCmLpOKI	29.3 a	28.0 a	
CaBkOI	24.0 a	22.7 а	
CasPsPGA	21.3 а	18.7 a	
PrSeStOI	21.3 а	21.3 а	
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.



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

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 fungal 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 are 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 is 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 saprofit 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 is 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 μ m (Chen et al. 2019) and this measure of the fungal conidia in this study (8-10 μ m) falls into the above range.

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 in South Sumatra in particular and Indonesia in general, the location-specific mycoinsecticides 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 (Fernandes 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 haemocoel, they produce specific infection hyphae originating at appressoria (El-Ghany 2015). Then, the hyphae spread to the haemolymph 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 sick and dead 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 misellia and conidia resembling yellowish white flour mixed with dark green similar to the symptoms described by Thaochan and Sausa-Ard (Thaochan and Sausa-Ard 2017). The symptoms of sick 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).

Pupae getting sick 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, reduce fecundity, 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 most pathogenic causes of death reaching 78.67% and suppressing the emergence of adults up to 81.2%. Two of the most pathogenic isolates found from the lowlands (PirOI) and highlands (CasPsPGA) of South Sumatra both have the potential to be developed into local-specific mycoinsecticides to control pest insects in the highlands and/or lowlands in Indonesia.

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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: xxxx. 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

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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 the 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

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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 rilevi (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 (Ramos al. 2020). However, larvae et 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 locationspecific 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, Banyuasin, and East OKU (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.

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Figure 1. Locations of exploration for entomopathogenic fungi: District/City of Ogan Komering Ilir (1), Ogan Ilir (2), Prabumulih (C), Muara Enim (4), Lahat (5), Pagar Alam (6), and Banyuasin (7)

The soil sample was first cleaned from plant roots and sieved with 10 mesh sieves. Then, it was put into a plastic tray $(32 \times 25 \times 5 \text{ cm}^3)$ 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% NaOCI 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 haemocoel 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 $(1 \times 10^6 \text{ conidia mL}^{-1})$ 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

Pathogenicity 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 29.30°C and 89%. This experiment used the randomized block design with isolates as treatments and was replicated three times.

Preparation of test insect

The 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 100°C. The containers containing pupae were placed in gauze cages (30 x 30 x 30 cm³), 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 (\emptyset 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 (\emptyset 6.5 cm, height 4.6 cm) because the second instar and so on were cannibalistic. Mass-rearing was carried out to obtain a 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° 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 (\emptyset 6.5 cm, height 4.6 cm) and each cup contained only 1 larvae and provided $2 \times 5 \text{ cm}^2$ 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 to10 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 againts the *Spodoptera frugiperda* larvae

The measurement of leaf area eaten by the treated larvae with entomopathogenic fungi 1×10^6 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).

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)

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, LT₅₀ (6.43 days) and LT₉₅ (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 LT₅₀ for only 6.68 days. Consequently, the fungi in this study could reduce the emergence of S. frugiperda adults up to 81.2%.

The treated dead larvae showed typical symptoms that distinguished them from the 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 f their adults 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 _ are 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 is 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 saprofit fungal habitat.

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

	Conidial density	Conidial vi	ability (%)
Isolates	1x10 ⁸	24-hour	48-hour
	(conidia mL ⁻¹)	culture	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 1×10^6 conidia mL⁻¹

Isolatos		Leaf area eaten by larvae (cm ² larvae ⁻¹ day ⁻¹) on observation for 12 days										
Isolates	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 10⁶ 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	48.91	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 1×10^{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	11.38	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

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 is 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 um (Chen et al. 2019) and this measure of the fungal conidia in this study (8-10 μ m) falls into the above range.

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.

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Figure 3. The symptoms on maize leaves eaten by *Spodoptera frugiperda* larvae treated with entomopathogenic fungal isolates with 1 x 10⁶ 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 1×10^6 conidia mL⁻¹ on observation for 12 days

Isolotos	Mortality	LT ₅₀	LT ₉₅
Isolates	± SE (%)	\pm SE (days)	\pm 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	-	

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

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 Spod	optera frugiperda pupae :	formation and adults	emerged after their	larvae treated with
entomopathogenic fungi 1 x 106 conidi	a mL ⁻¹			

Isolates	Pupae formation (%)	Adults emerged (%)
Control	86.7 b	86.7 b
LpTtME	25.3 а	24.0 a
PirOI	21.3 а	21.3 а
TePsPGA	24.0 a	21.3 а
ToBkOI	25.3 а	22.7 a
CaGiPR	22.7 а	21.3 а
CaSnPR	24.0 a	21.3 a
CaTtME	21.3 а	18.7 a
TiCmLpOKI	29.3 a	28.0 a
CaBkOI	24.0 a	22.7 a
CasPsPGA	21.3 а	18.7 a
PrSeStOI	21.3 а	21.3 a
TeSkME	26.7 a	18.7 a
PdmOI	29.3 a	28.0 a
KtMtLH	21.3 а	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.



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



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

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 (Fernandes 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 haemocoel, they produce specific infection hyphae originating at appressoria (El-Ghany 2015). Then, the hyphae spread to the haemolymph 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 dead 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 misellia and conidia resembling yellowish white flour mixed with dark green similar to 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 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, reduce fecundity, 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 from the lowlands (PirOI) and highlands (CasPsPGA) of South Sumatra both have the potential to be developed into local-specific mycoinsecticides to control pest insects in the highlands and/or lowlands in Indonesia.

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